International Conference of Life Sciences

The COINS 2023

Book of Abstracts

Contents:

CASZYME

Caszyme is a biotech company, founded in 2017, based in Vilnius, Lithuania. The company specializes in development and applications of CRISPR Cas technology and aims to develop new discoveries, innovative applications, and top-quality research in the field of CRISPR-based molecular tools. Since different industries and applications that use CRISPR-Cas technology require Cas proteins with different characteristics. Caszyme offers a platform of different CRISPR-based Cas proteins. The company identifies, characterises, and develops these proteins, which are then offered to company clients for applications in human therapeutics, diagnostics, agriculture, research tools, and industrial biotech.

Caszyme activities:

- Exploring new Cas Proteins for various applications.
- Providing research services for CRISPR applications.
- Developing and characterizing new CRISPR based Molecular Tools.

April 24th

Microbiology and Biotechnology

A.1. ETHANOLIC EXTRACTS OF CIRSIUM VULGARE SAVI TEN. ANTIOXIDANT ACTIVITY

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Cirsium vulgare (Savi) Ten. is also known as a spear thistle, bull thistle or common thistle. It is a species of the Asteraceae, genus Cirsium [1]. Studies shown, that *Cirsium vulgare* is a plant which contains great diversity active compounds such as flavonoids and amino acids. These compounds especially phenolics is characterized by strong antioxidant effect. Due to this, our research was to examine those active compounds diversity in bull thistle herb extracts made with 50 percent ethanol/wather solvent and to investigate those extracts antioxidant effect,

Antioxidant effect was investigated by CUPRAC method. The CUPRAC spectrophotometric method is a simple, low-cost detection and reducing power method that does not require sophisticated equipment. Compared to other methods for the determination of antioxidant activity based on the electron transfer reaction, several advantages are distinguished: the iron ion, due to the high spin and semi-d - orbitals of this method, is classified as a chemically inert compound, while the electronic structure of copper determines a fast chemical reaction. Due to rate differences, the method for determining the reducing power of copper may oxidize thiol-type antioxidants. CUPRAC reagent is more stable and easier to produce than chromogenic radical reagents (ABTS, DPPH). The CUPRAC method tests are performed under neutral conditions (pH 7.0), which are close to physiological conditions, while the FRAP method requires acidic conditions (pH 3.6). The method can simultaneously measure hydrophilic and lipophilic antioxidants (for example, carotene and alpha-tocopherol). The CUPRAC study includes most of the research conducted in the practice of biochemistry and food chemistry, and the results obtained show a clear advantage over other induced antioxidant methods.

When determining the antioxidant effect by the CUPRAC method, a relationship was established between the antioxidant activity and the amount of compounds isolated from the raw part of the plant ($p=0.010$). The averages obtained are statistically significantly different ($p<0.05$). It was found that the biologically active compounds isolated from the flowers of the plant, whose antioxidant activity was determined to be 0.355±0.018 mg/g, have the highest antioxidant activity tested. Ethanol extracts made from a part of the plant - leaves (0.261± 0.015 mg/g) are characterized by lower antioxidant activity. It was found that there is a statistically significant difference in the antioxidant activity depending on the growth period of the plant raw material ($p=0.047$). It was found that ethanolic extracts of the plant raw material collected at the end of August - beginning of September, i.e. at seed maturity 0.211 ± 0.013 mg/ml and at the end of flowering (0.355 ± 0.018 mg/g), have the highest antioxidant effect. Ethanol extracts of the plant, the raw material of which was collected during the mass flowering of the plant (in July (0.208 ± 0.013 mg/g)) are slightly less active.

Due to results, it is known that antioxidant activity of extracts for the further investigation, for example, investigations with cancer cell in vitro, can be done by taking the most active mass flowering phenological stage extracts made of plant flowers.

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A.2. ANTIMICROBIAL PROPERTIES OF BLACK SOLDIER FLY FAT

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Globalization leads to the fast and wide spreading of living organisms, and simultaneously, various pathogens, thus the fight against pathogenic microorganisms is an important global aim.

The larvae of the black soldier fly (*Hermetia illucens*) are among the most lipid-rich compared to other industrially farmed insects. Fat makes up about half of the larva's mass. One of the advantages of cultivating these larvae is that they feed on a variety of feeds, recycle various food waste very efficiently, and the biomass converted by the larvae becomes a sustainable source of high-quality fat, protein, and chitin.

Black soldier fly larvae (BSFL) are a source of antimicrobial agents. In the fat of BSFL, the dominant saturated fatty acids are lauric and palmitic, and the dominant unsaturated fatty acid is oleic [1]. Lauric acid has inherent antibacterial activity [2]. Fats from BSFL can become alternatives in many sectors: feed production (in animal, fish, and poultry feed, food industry (e.g.: butter/margarine), bioactive compounds, cosmetics industry. The fatty acid composition of these larvae is similar to that of coconut or palm kernel oil. Only a few scientific studies have successfully demonstrated the antibacterial properties of BSFL fat extracts. Fats of BSFL and their extracts effectively inhibit several species of bacteria, including pathogenic bacteria, e.g., antibiotic resistant *Klebsiella pneumoniae* [3]. However, the effectiveness of BSFL fat extracts in inhibiting the growth of fungal microorganisms has been poorly investigated.

The aim of this work is to identify BSFL-associated microorganisms, produce BSFL fat extracts, and evaluate their antibacterial and antifungal properties. Cultivable microorganisms were isolated from BSFL by microbiological methods, genera/species identification was performed by molecular methods (PCR with primers specific for bacterial ribosomal RNA regions, restriction fragment length polymorphism analysis, DNA sequencing). Several BSFL fat extracts were produced. Microbiological methods were used to study the inhibitory effects of fat extracts on yeast and bacterial cultures.

This work was supported by Research Council of Lithuania (project No. P-ST-22-85).

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A.3. IDENTIFICATION AND BIOCIDAL PROPERTIES OF YEASTS ISOLATED FROM PASTURES SOIL

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Yeasts found in the environment are among the most abundant and diverse forms of life on Earth, and their biodiversity is linked to human and animal health. In the last few decades, fungi are becoming frequently recognized as causative agents of various infections. However, there is still a lack of yeast research in pasture soil samples. The present work's objective was to identify yeasts in soil samples from livestock pastures and evaluate their biocidal properties.

In the course of our research, samples were collected from horses, sheep, and cattle pastures. Soil samples were washed out with distilled water, concentrated, and applied for direct isolation of microorganisms. It was found that most microorganisms were detected in the samples collected from the horse pasture. Nineteen strains of yeast-like morphology, representing each of mentioned above habitats, were used for molecular species identification and killer assay. Predominated yeasts were representatives of the genera Rhodothurula (6/19 strains) and Cryptococcus (3/19 strains). The cultivable yeast isolated from the soil was grown at temperatures of 4, 12, 30, and 37°C. Most of the yeasts were found to grow best at 30°C degrees, but 3 yeast strains were thermotolerant and formed colonies at 37°C. Four biocidal strains were isolated from pastures, they belonged to Williopsis, Pichia, Candida and Cystofilobasidium genera. The killer assay was performed on MB media at pH 4.0, 4.8, and 5.6. The strongest biocidal properties of identified killer yeasts were observed at pH 4.8. None of the killer yeasts were found to be infected with dsRNA viruses, which means that the biocidal properties of those yeasts are encoded in the plasmid or chromosomal DNA.

This study, focusing on the biodiversity of yeasts in the soil of pastures, is important to predict the condition of food-producing animals, for maintaining healthy livestock and having a high potential in the food industry.

A.4. IDENTIFICATION AND HETEROLOGOUS BIOSYNTHESIS OFNOVEL BACTERIOCINS WITH THE ANTI-PHYTOPATHOGENIC BACTERIAL ACTIVITY

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An increasing antimicrobial resistance among phytopathogenic bacteria has shown significant problems in the soil ecosystem, since these pathogens can cause a wide range of plant diseases as well as economic damage to various plant crops on a local, national, and global scale. In 2019, losses from phytopathogen-caused illnesses in five major crops, wheat, rice, maize, potato, and soybean, were 10.2 %, 10.8 %, 8.5 %, and 8.9 %, respectively [1]. Chemicals are currently being used to control these plant infections, but these methods are often no longer as effective and could be dangerous for both, human health and the environment [2]. In order to solve this problem, it may be feasible to use bacteriocins, a heterogeneous group of ribosomally synthesized antimicrobial peptides that have the ability to kill closely related or a diverse range of bacteria species [3]. Bacteriocins are much safer to use than some chemicals, and because they are specific to targets, they have no negative side effects.

The aim of this study was to identify bacteriocins that are secreted by microorganisms that have been isolated from the soil and may have an impact on phytopathogenic bacteria. 63 isolates were isolated from the soil and screened in order to evaluate their antibacterial activity against 9 phytopathogenic bacteria starins, such as *Pectobacterium carotovorum*, *Xanthomonas vesicatoria*, etc. We successfully identified two bacteria strains: *Streptomyces* sp. AB3 and *Bacillus* sp. DM1.10 that produce antibacterial substances. The DM1.10 genome analysis revealed 14 gene clusters, three of which are associated with bacteriocin synthesis and others with antibiotics, polyketide and non-ribosomally synthesized peptide synthesis. Four of 41 gene clusters identified by analysis in the AB3 genome are involved in the production of bacteriocins, while other gene clusters are involved in the synthesis of polyketides and non-ribosomally synthesized peptides. One of them is presumably responsible for biosynthesis of a lasso peptide type bacteriocin. In this study, we describe heterologous expression of genes responsible for the lasso peptide bacteriocin biosynthesis in *Escherichia coli* cells.

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A.5. INVESTIGATION OF PLANT-DERIVED PROTEIN HYDROLYSIS VIABIO-CATALYSIS

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The increasingly growing global population indicates that protein demand in food and feed systems will continue to rise in the coming years. Proteins are one of the most essential nutrients required for critical functions in cells, tissues, organs, and all systems. Popularity of plant-based proteins has grown in recent years. The global plant-based protein market is projected to reach 17.4 bln USD by the end of 2027[1]. Plants provide high levels of proteins without the high content of fats, which is often related to animal proteins. Furthermore, it can provide a range of vitamins, minerals, and antioxidants. Plant-based proteins are more environmentally friendly than animal-based ones, requiring less energy and water to produce. Current policy directives and initiatives (EU Green Deal, EU Bioeconomy Strategy, etc.) are directed to boost plant use for protein production. Plants are sustainable and environmentally feasible protein sources [2].

Protein hydrolysate is the most nutritious form of proteins as it contains a mixture of short-chain peptides and free amino acids, which are more readily absorbed by the body. The traditional chemical methods of plant protein hydrolysis are usually performed under hazardous conditions, influencing protein structure and other functional properties [3]. One of the alternative ways to get protein hydrolysates is enzymatic hydrolysis. Enzymatic hydrolysis is an easy controllable process due to the specificity of enzymes. It eliminates antinutritional components, increases protein solubility and improves the functional and nutritional properties of proteins. Protein hydrolysates can be applied in the food industry as a functional food and in pharmaceutical industries because of formed bioactive peptides. Bioactive peptides could exhibit various beneficial features such as antioxidant activity, antihypertensive activity, and antimicrobial activity, etc. [4].

The aim of this study is to develop an enzymatically based process for the hydrolysis of plant proteins. An investigation object is the alfalfa plant. In this study the alfalfa hydrolysis process will be evaluated and performed in two ways. The first uses commercial proteases, the second – uses recombinant protease. The obtained results will be presented during the poster session in more detail.

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A.6. INVESTIGATION OF CELLULASE THERMAL STABILITY IN DEEPEUTECTIC SOLVENTS

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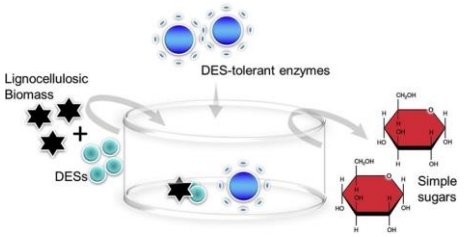
Fluctuating fuel prices, climate change, soil and ecosystem degradation, and a growing worldpopulation are motivating researchers to explore new technologies that lessen the reliance on Earth's ecological capacity. Lignocellulosic biomass is recognized as one of the largest sources of sustainableand renewable organic material on Earth that could be used to produce fuels and bio-based products [1]. Effective fractionation of biomass into its main components (cellulose, hemicellulose and lignin) is essential to efficiently utilize lignocellulosic materials. Pre-treatment is considered the most essentialstep for obtaining an efficient conversion of biomass into value-added products; nevertheless, it isalso one of the most complex steps in a biorefinery process [2].

Recently, Deep Eutectic Solvents (DES), new alternatives of ionic liquids (ILs) derived fromnatural and renewable components, have been identified as potential green solvents for the pretreatment of lignocellulosic biomass [3]. DES are a mixture of two compounds with a significantlylower melting point than the starting materials. They are made by combining an appropriate hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD) [4]. DES exhibit similar physicochemical properties to classical Ils but has additional advantages, such as that they are easy to prepare, simplerpurification processes and utilization of renewable materials. However, most of the currentpublications on the application of DESs for biomass processing mainly discuss the capability and role of DESs in lignocellulose solubilization and decrystallization, neglecting the suitability of DESs for the enzymatic hydrolysis thus leaving the applicability of the whole system uncertain [3].

Figure 1. Production of sugars by DES-cellulase system[5].



In this project, the system consisting of DES and cellulase was used as a new environmentally- friendly biocatalytic approach for the pretreatment and hydrolysis of lignocellulose. The compatibility between two different choline chloride-based DES and a cellulase was investigated by monitoring the stability of cellulase at 20, 40 and 60 °C, up to 24 h.

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A.7. APPLICATION OF A NON-SPECIFIC SACCHARIDES BIOSENSOR FOR A-AMYLASE ACTIVITY DETERMINATION

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Acute pancreatitis (AP) is an inflammatory injury with symptoms including edema, hemorrhage and necrosis in pancreas caused by tissue autodigestion in relation to various pathogens. It can range from mild discomfort to a severe, life-threatening illness. Most people with acute pancreatitis recover completely after getting the right treatment when the disease is diagnosed in the early stages. In severe cases, acute pancreatitis can cause bleeding, serious tissue damage, infection, cysts and can also harm other vital organs such as the heart, lungs, and kidneys. One of the criteria for determining AP is the measurement of α-Amylase levels in the blood or urine [1]. The main method for the determination of α-Amylases is colorimetric, but these methods are relatively slow and require expensive equipment and trained staff. α-Amylase (E.C.3.2.1.1) is the enzyme that causes the degradation of starch molecules and hydrolyzes them into small-chain dextrins by acting upon the α-1,4 glycosidic bonds present in the starch polysaccharide. Theoretically, acute pancreatitis correlates with α-Amylase activity in blood and urine, so α-Amylase activity could be determined by the amount of products formed during the reaction catalyzed by this enzyme.[2]

Our work discusses a bioelectrocatalytic system that would allow the rapid, easy and relatively inexpensive determination of α-Amylase activity in water-based solutions. In our work we used a PQQ-depended glucose dehydrogenase from a microorganism, which was immobilized onto a terylene membrane using thermally reduced graphene oxide and a mixture of glutaraldehyde and bovine serum albumin. The selectivity of PQQ glucose dehydrogenase was tested (maltodextrins up to 20 monomers were used) and it was found that this enzyme can oxidize all sugars with reducing ends. The α-Amylase catalyzed reaction produces products that are oxidized by PQQ glucose dehydrogenase in a reagentless system. It means that according to the strength of the generated signal, we can determine α-Amylase activity because the amount of reducing sugars (which are oxidized by PQQ glucose dehydrogenase) depends on this enzyme. This work demonstrated how the non-specific PQQ glucose dehydrogenase can be used in the construction of a carbohydrate biosensor and how it can be applied in the rapid and simple diagnosis of α-Amylase activity in water based solutions. In this work, we investigated the accuracy of the biosensor, its selectivity for substrates, optimal conditions for operation, and analyte detection limits. The results determined by the biosensor were compared to the analogous colorimetric method.

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A.8. ELECTROCHEMICAL METHOD FOR HYBRIDISATION SIGNAL AMPLIFICATION OF METHYLENE BLUE-LABELLED DNA OLIGONUCLEOTIDES

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Nucleic acid detection and quantification are crucial steps in many biotechnological and clinical applications, including disease diagnosis, genetic engineering, and personalized medicine. Current nucleic acid detection methods, such as polymerase chain reaction (PCR) and quantitative PCR (qPCR), usually employ optical signal readout methods [1]. Electrochemical readout could provide comparable or better sensitivity, specificity, cost-effectiveness, and simplicity, possibly faster sample-to-answer times. However, electrochemical nucleic acid sensors have limitations. One of the challenges – achieving clinically or technologically relevant detection limits – often requires complicated designs. The designs may involve surface damaging voltametric techniques (usually suitable only for end-point measurements) and the use of expensive nanomaterials.

In this study, we developed an electrochemical system for real-time detection of nucleic acid hybridisation. The target, single-stranded DNA (ssDNA) labelled with methylene blue (MB), was captured at a gold electrode by an immobilized complementary probe, resulting in an increase in oxidation current during constant potential amperometry measurements. The detection was enabled by the use of ascorbic acid (AA) as a freely diffusing signal amplifier [2]. AA reduced MB to leucomethylene blue (LB), which was oxidized back to MB at the electrode by applying an appropriate potential, creating a cyclical electrocatalytic amplification of the signal (Fig. 1). The resulting biosensor provides real-time hybridisation measurements with 1-second temporal resolution, enabling electrochemical readout of a redox-tagged oligonucleotide hybridisation signal. When compared to previously used hybridisation evaluation technique that employed cyclic voltammetry without signal amplification [3], the new method offers several advantages: (1) temporal resolution was improved from 20 s to 1 s; (2) assay time was reduced from 15 min to 5 min; (3) LOD was reduced by half to 5 nM.

In summary, the development of this electrochemical hybridisation biosensor brings a new level of simplicity, sensitivity, and speed to nucleic acid detection and quantification. Real-time signal readout allows using this tool for studying heterogenous hybridisation. Such biosensors have the potential to greatly improve biotechnological applications by providing more efficient and accurate results cost-effectively.

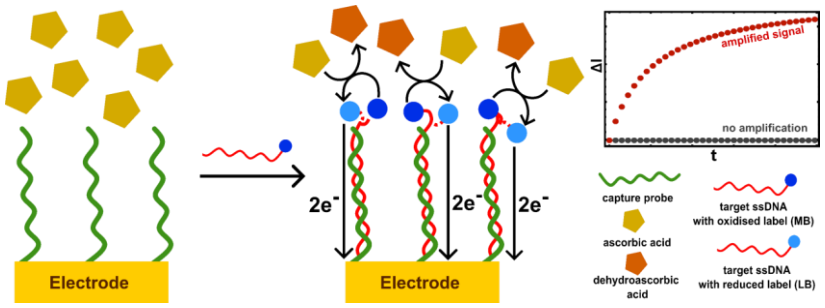

Figure 1. Amperometric nucleic acid hybridisation monitoring enabled by signal amplification. Left – target oligonucleotide hybridisation and signal amplification scheme, legend below. Right – illustration of recorded current in case no signal amplification is employed, and in case the signal is amplified by ascorbic acid.

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A.9. ANTIMICROBIAL EFFICACY OF PLANT HYDROLATES

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The currently ongoing spreading of drug-resistant microorganism strains causes serious concern and dictates the need for researching alternative antimicrobial agents. In the last few decades, various plant-based extracts have gathered a fair amount of attention due to their wide spectra of bioactive properties and "generally recognized as safe" status. While a significant amount of research has been done with essential oils (EOs), plant hydrolates are still relatively poorly studied.

Hydrolates are aqueous solutions of secondary plant metabolites, mostly different terpenes and phenolic compounds, and are generated as a by-product of the plant matter distillation process during EO production. The composition of hydrolates – the variety of different compounds and their relative amounts - can differ greatly from that of their corresponding EOs. The total concentration of biomolecules in hydrolates is much lower than that found in EOs and does not exceed 1 g/mL [1]. However, the hydrophilicity of hydrolates makes the compounds in them more accessible to and diffuse more easily into other water-based systems, while the hydrophobicity of EOs thwarts these processes. Due to these reasons, the antimicrobial efficacy and primary mechanisms of action of hydrolates can be different than that of their corresponding EOs and need to be investigated. Hydrolates also tend to be less toxic to human cells than undiluted EOs and are therefore more appealing for potential oral or topical use.

The aim of this work was to study the antimicrobial efficacy of four different plant hydrolates (wild lavender, lemon verbena, oregano and cistus). Two model organisms were chosen for antibacterial (*Escherichia coli*, *Bacillus subtilis*) and antifungal (*Saccharomyces cerevisiae*, *Candida albicans*) tests each. Antibacterial and antifungal properties of hydrolates were assessed by evaluating the reduction of viable microbial counts. Bacteria were found to be more sensitive than yeast to all of the hydrolates tested,
B. subtilis being the most sensitive microorganism to the majority of them. Cistus hydrolate possessed the strongest antibacterial and antifungal properties. Using fluorescence microscopy, it was determined, that hydrolates, unlike their corresponding EOs, do not damage the cellular envelope integrity of *S. cerevisiae* yeast. Altering the properties of lipid membranes is considered to be one of the main antimicrobial mechanisms of action of EOs [2], therefore our results affirm that the cytotoxicity of hydrolates takes effect in a different manner.

The findings of this study are important for elucidating the antimicrobial efficacy of plant hydrolates and their potential for use in healthcare, surface disinfection and food safety.

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A.10. MICRO RNA AS BIOMARKERS IN NEURODEGENERATIVE DISEASES

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Neurodegenerative diseases (NDs) occur due to brain or spinal cord neuron abnormalities that gradually lose their function and ultimately die. Pathological study of these tissues indicates the loss of synapses, misfolded protein tangles, and immune cell activation even in the initial stages of the disease, before the appearance of severe clinical symptoms. If NDs are detected early, targeted medications can slow disease progression. Treating early neuron damage and loss or maintaining neuron functions may be effective and prevent worse damage. Due to their complex pathophysiological characteristics and varied clinical symptoms, NDs lack sensitive and effective diagnostic methods.

Moreover, no sensitive biomarkers are available to track ND progression, offer prognostic predictions, or measure therapeutic effectiveness. Recent studies indicate microRNAs (miRNAs) are potential biomarkers in diagnostics and therapy for NDs.MiRNAs are small, non-coding RNA molecules (about 18–25 nucleotides in length) that attach to mRNA and inhibit its expression. There are already over 1000 identified human miRNAs, and miRNAs regulate more than 50% of mammalian protein-coding genes. In specific diseases, miRNAs may be overexpressed or repressed, and the inhibition or replacement of miRNAs is a potential field of research for developing treatments. Research on miRNAs has primarily focused on two aspects: 1) miRNAs as possible disease biomarkers and 2) miRNAs as a molecular target of disease therapies. We searched PubMed and found 350 articles. PubMed systematically searched for articles about miRNA levels in the blood, brain tissue, or cerebrospinal fluid (CSF) of ND patients vs. Healthy controls (HC). Eighty-three eligible studies were included for further analysis. We found that the most common microRNAs are let-7b, miR-124, miR-34a, and miR-29a. These microRNAs show up repeatedly in scientific papers about different ND diseases. These miRNAs show the importance of miRNA biomarkers in clinical diagnostics and treatments of neurodegenerative diseases.

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A.11. MOLECULAR CHARACTERIZATION OF NEISSERIA MENINGITIDIS ISOLATES RECOVERED FROM PATIENTS WITH INVASIVE MENINGOCOCCAL DISEASE IN LITHUANIA

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Neisseria meningitidis is a gram-negative bacterium responsible for life-threatening invasive infections known as invasive meningococcal disease (IMD). The number of IMD cases reported in Lithuania during the period of 2009–2020 was higher than that reported in other European countries. Meningococcal disease in Lithuania is a public health concern because of high mortality rate.

The aim of the current study was to evaluate the possibility for using a simple, rapid, and relatively inexpensive method for N. meningitidis strain characterization, thus to improve a national molecular surveillance of invasive meningococcal disease.

In total 321 N. meningitidis isolates were collected in Lithuania during the period of 2009–2021 and analysed applying a multilocus restriction typing (MLRT). MLRT was carried out following the technique described by Benett et al. [1]. MLRT involves restriction fragment length polymorphism analysis of PCR products obtained from the seven loci of housekeeping genes (abcZ, adk, aroE, fumC, gdh, pdhC and pgm) used in MLST [2]. PCR products were digested with restriction endonucleases MspI and MnlI (Thermo Fisher Scientific Inc.) according to the manufacturer's instructions. The digested fragments were analysed by 1 % agarose gel electrophoresis. Several different restriction patterns (alleles) were observed for each of the seven loci examined. The alleles at each of the seven loci were combined to give an allelic profile or restriction type (RT). The study revealed polymorphism of the examined loci. The number of alleles varied between four in adk, the most conserved locus, and fourteen in pgm.

Amplification of penA gene and restriction fragment length polymorphism analysis was carried out to identify modified penA genes. An analysis to detect an altered penA allele was made as previously described by Antignac et al. [3]. After digestion with restriction enzyme TaqI (Thermo Fisher Scientific Inc.) and separation on a 1 % agarose gel, six different profiles were obtained.

The data matrix was created, which was further used to construct the dendrogram using the Hierarchical Clustering (UPGMA—Unweighted Pair-Group Method with Arithmetic Averages). Visualization of dendrograms was done using an online tool for phylogenetic tree preparation Tree Of Life [4].

We identified 83 strains based on MLRT genotyping, and 87 strains based on MLRT and penA allelic profile relationship established among 321 N. meningitidis isolates. UPGMA dendrogram for genetic variation among the isolates demonstrated that according to MLRT results all isolates fell into four major clusters and into three major clusters considering the relationship between MLRT and penA.

We also have identified certain groups of N. meningitidis that were isolated in different periods of time, sharing similar genotype profile.

MLRT method can contribute to the monitoring and molecular epidemiology of N. meningitidis in the middle- or low-income countries and can be used by laboratories with limited resources, it does not require highly trained laboratory personnel or sophisticated equipment. The MLRT use allows grouping the isolates into broad clusters, however, it is not sufficiently discriminatory for differentiating among the closely related isolates.

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A.12. THE SPREAD OF AMINOGLYCOSIDE RESISTANCE GENES IN *ACINETOBACTER BAUMANNII* CLINICAL ISOLATES FROM THENATIONAL CANCER INSTITUTE

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Acinetobacter baumannii (Ab) has been recognized over the last two decades as an important opportunistic hospital pathogen [1]. *Ab* is capable of causing a wide spectrum of disease including urinary tract infections, ventilator-associated pneumonia and meningitis to immunocompro- mised patients, and is associated with high mortality rates [2]. Nosocomial *Ab* infections are difficult to treat due to multidrug-resistant (MDR) phenotype, which includes resistance to β-lactams, aminoglycosides, fluoroquinolones, cephalosporins and carbapenems [3].

The present study aimed to phenotypically characterize carbapenem and aminoglycosides resistance phenotypes of clinical isolates of *Ab* and verify the occurrence of selected aminoglycoside resistance genes. A total of 63 isolates of *Ab* were collected and microbiologically characterized in National cancer institute (2013-2019) and confirmed by the detection of *blaOXA-51-like* gene in our laboratory. Quantitative antibiotic susceptibility testing (MIC determination for meropenem and gentamicin) was performed using broth microdilution technique. Results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [4].The presence of selected *Ab* genes, implicated to aminoglycoside resistance, was assessed by PCR.

The findings of this study show that 75% of *A. baumannii* isolates were resistant against meropenem (carbapenem), 77% - resistant to gentamicin (aminoglycoside). The presence of the *armA* gene encoding a 16S ribosomal RNA methyltransferase, conferring broad-spectrum resistance to aminoglycosides was detected in 28,6% of *Ab* isolates, while the *rmtB* gene was not found in any isolate. Methylase production (genes *armA, rmtB*) decreases the affinity of the aminoglycosides for 30S ribosomal subunits and therefore inhibit the bacterial protein synthesis. In addition, 41.3% of *Ab* isolates possessed *strA* and *strB,* which are the most common streptomycin resistance genes, encoding aminoglycoside phosphotransferases.

Knowledge of *Ab*, including its antibiotic resistance mechanisms is crucial for preventing this pathogen from further disseminating, that's why targeted surveillance must be carried out.

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A.13. ENGINEERING NEW *GEOBACILLUS* SP. 95 ESTERASE GDEST-95 VARIANTS VIADIRECTED EVOLUTION

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Thermozymes are enzymes, synthesized by thermophiles and hyperthermophiles. These enzymes are thermostable and have the highest activity at temperatures ranging between 50 and 125 °C [1]. One of the bacterial sources of thermozymes, belong to the *Geobacillus* genus. In this work, we focused on *Geobacillus* sp. 95 synthesized carboxylesterase GDEst-95. Esterases catalyse various reactions, including hydrolysis, esterification and acidolysis. Due to their properties that are often associated with stability in solvents and detergents as well as regio-selectivity and stereo-specificity, esterases are widely used in many industrial applications [2]. However, naturally found enzymes typically have low yield and poor adaptability, which makes it difficult to apply them for large-scale technological processes. Directed evolution has emerged as a powerful approach for improving enzyme properties and has been widely used in engineering many enzymes.

In this research, after performing error-prone PCR on a gene encoding GDEst-95, new mutant gene variants named Est-1 and Est-3 were generated. Vector pET-21c(+) was used for recombinant protein expression in *E. coli* BL21 (DE3). Protein synthesis was induced using milk permeate [3]. After DNA sequencing data analysis new mutations Gly20Ser and Arg75Gln were identified. The aim of the study was to evaluate if these mutations have any impact on enzyme thermostability, substrate specificity and ability to catalyze reactions at different temperatures.

Our results indicate that mutant Est-1 and Est-3 esterases are more stable after exposure to different temperatures (30-90 °C) compared with parental GDEst-95 esterase. Mutant variants retained more than 70
% of their activity after incubation at 30-70 °C. The optimal temperature for the activity of Est-1 and Est-3 was 70-75 °C, using *p*-NP dodecanoate (C12). In contrast, GDEst-95 esterase reaches its maximum activity at a temperature of 60 °C. The substrate specificity of recombinant esterases was determined using various *p*-NP esters. The significant difference was for *p*-NP dodecanoate hydrolysis. Mutant esterases show 35% increased activity towards *p*-NP dodecanoate. Kinetic analysis shows that mutant esterases have K_m value of 2.26-2.59 mM which is lower than GDEst-95 esterase and increased maximum reaction velocity (Vmax) from 5.88 to 16.08-24.37 (μmol min^{-1} mg^{-1}).

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A.14. COMPARISON OF THE PROTEOLYTIC RESISTANCE OF FREE AND ENCAPSULATED NISIN AGAINST PROTEINASE K

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The preservation of food is crucial in the modern food economy. The globe is still plagued by food contamination brought on by bacteria, such as Listeria, which is linked to numerous ready-to-eat meals, or Vibrio cholerae, which is found in a variety of seafood. Food manufacturers use artificial preservatives, excessive amounts of salt or sugar, or both to address the problem. Studies, however, demonstrate that this type of conservation can cause human health related problems. Therefore, new, natural antibacterial agents are promising alternatives to old preservation methods. One promising substance is nisin.

Nisin is a polycyclic antibacterial peptide produced by the bacterium *Lactococcus lactis* that is used as a food preservative. It has antimicrobial activity against a broad range of Gram-positive bacteria, including many foodborne pathogens and spoilage bacteria [1]. Nevertheless, nisin can be harmed by food-derived enzymes, which would result in a loss of antimicrobial activity. To ensure the stability of nisin, it could be coated with biopolymers [2]. Biopolymers, also known as polysaccharides, are often obtained from plants, animals, and microorganisms and consist of subunits connected by glycosidic linkages.

The aim of this study is to prepare nisin-loaded particles and compare their proteolytic stability with free nisin (Fig. 1). To encapsulate nisin five different types of biopolymers, i.e., fucoidan, ulvan, and three structurally different pectins (high methoxyl pectin, low methoxyl pectin, and pectic acid) were used. In all cases, the final concentration of nisin and biopolymer in the sample was 0.4 mg/ml. To test the proteolytic stability of nisin samples proteinase K was used. Proteinase K is a non-specific, serine endopeptidase with maximal activity in the pH range of 7.5 – 12.0 [3].

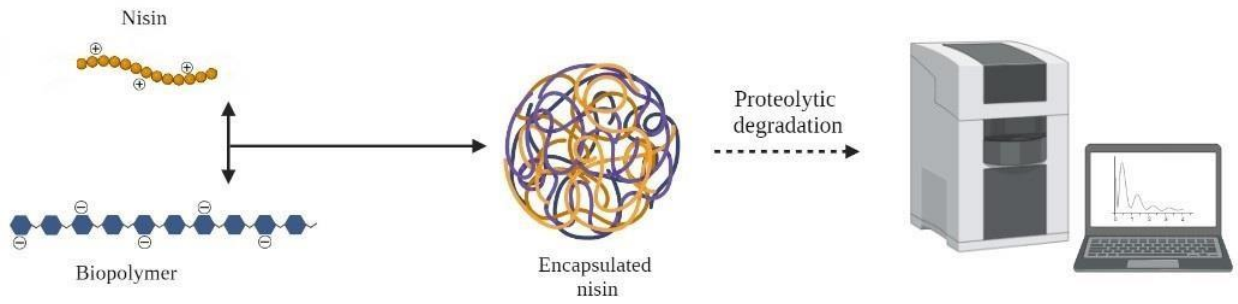

Figure 1. Schematic view of the study.

First, studies were conducted to determine the suitable conditions for proteolysis, i.e., enzyme concentration and duration of proteolysis. After that, nisin-loaded and free nisin samples at pH 7 were affected by proteinase K. Proteolysis was performed for 24 h at 37 °C. Finally, samples with proteolytic degradation products were analyzed using the capillary zone electrophoresis method using the 7100 Capillary Electrophoresis unit (Agilent Technologies). The obtained results show that regardless of the biopolymer used, encapsulation of nisin significantly improves its resistance to proteolytic degradation.

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A.15. PRODUCTION OF RECOMBINANT LIPOLYTIC ENZYMES USING MILK PERMEATE AS AN INDUCER AS WELL AS REDUCED AMOUNTS OF AMPICILLIN IN THE CULTIVATION MEDIA

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Biotechnology is a rapidly evolving field that offers tremendous opportunities for innovation and growth. The development of advanced technologies has greatly improved the efficiency and accuracy of recombinant enzyme-based applications. This market is growing and presents a strong opportunity for the development of novel products and services. While all these factors have resulted in a dynamic and exciting recombinant enzyme market, the production price of recombinant enzymes is still a limiting factor for their application in many areas [1].

Microorganisms, particularly *Escherichia coli*, remain the preferred host to produce a variety of recombinant enzymes. Isopropyl β–D–1–thiogalactopyranoside (IPTG) is the most used inducer for the synthesis of recombinant proteins in *E. coli*, due to its inability to be metabolized by *E. coli*; however, it is known that the use of IPTG has a variety of disadvantages such as toxicity, increased cell stress, and expensive costs. This study therefore examines the use of milk permeate (MP) as an alternative means to IPTG for the induction of the expression of recombinant proteins. Additionally, it is speculated that utilizing milk permeate, similarlyto lactose, will reduce the level of cellular stress from the introduction of a non-native inducer. The proliferating issue regarding the emergence of antibiotic-resistant bacteria has called into question the utilization of antibiotics in the biotechnology sector. The selection process of many cells that have undergone gene engineering depends on antibiotic resistance genes, and antibiotics are still broadly used in industrial applications. The use of antibiotics is not only harmful, but also costly. Therefore, this study investigated the effects of lower concentrations of ampicillin in the cultivation medium for the production of recombinant enzymes.

Induction of synthesis experiments were performed for a variety of recombinant proteins, their length varied from 23 kDa to 99 kDa. The proteins, namely GD-95RM lipase, GDEst-Lip lipolytic enzyme, GDEst-95 esterase, and Cut+SP cutinase were chosen because of their features and applicability to the current enzyme market. All proteins were shown to have been synthesized in *E. coli* BL21 (DE3) cells under lower antibiotic concentrations (25, 5 µg/mL) as well as using milk permeate instead of IPTG.

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A.16. MOLECULAR CHARACTERIZATION OF DRUG-RESISTANT CLINICAL KLEBSIELLA PNEUMONIAE ISOLATES

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Klebsiella pneumoniae is a Gram-negative, rod-shaped, facultative anaerobic bacterium. These bacteria can cause severe diseases such as pneumonia, meningitis, urinary tract infections [1]. Genotyping allows us to make assumptions about the *K. pneumoniae* spread in the community, healthcare institutions, inter- and intra- hospital transmissions among hospital personnel and patients.

The aim of the current study was to characterize drug-resistant clinical *K. pneumoniae* isolates using three different repetitive DNA elements sequence-based genotyping techniques: BOX-, REP-, and (GTG)5-PCR. Clinical isolates were collected from Republican Vilnius University Hospital. PCR reactions were performed in a 50 µL reaction mixture containing DreamTaq Green PCR Master Mix (Thermo Fisher Scientific). Standard primers were used for the PCR reactions: BOXA1R primer (5‘- CTACGGCAAGGCGACGCTGACG-3‘) [2] was used for BOX-PCR, primers REP1R (5-IIIICGICGICATCIGGC-3) and REP2I (5-ICGICTTATCIGGCCTAC-3) [3] were used for REP-PCR, and (GTG)5 primer (5'-GTGGTGGTGGTGGTG-3') [4] was used for (GTG)5-PCR. Electrophoretic profiles were analyzed by electrophoresis through 1% agarose gel and compared visually. To compare isolates, the dendrogram was constructed by the unweighted-pair group method with arithmetic mean (UPGMA) grouping method.

First, the BOX-PCR fingerprinting was used to genotype 20 isolates. BOX-PCR experiments resulted in a total of 11 different electrophoretic profiles. Groups U1, U2, U3, U4, U6, U7, U8, and U9 were represented by a single unique isolate each. Groups U5 and U10 contained two isolates each. Group U11 included seven isolates. As different genotyping methods are known to have different resolution in various taxa, two other methods were used, but only BOX-identical isolates were analyzed. REP-PCR resulted in a total of 6 different electrophoretic profiles. Group B2 had a single isolate. Groups B1, B3, B4, and B5 contained two isolates each. Three isolates have been found to belong to the group B6. (GTG)5-PCR resulted in only 4 unique electrophoretic profiles. Groups A1, A2, and A3 included two isolates each. Group A4 contained 5 isolates. After summarizing genotyping results, UPGMA dendrogram was constructed, allowing the identification of related groups of the isolates. It is generally accepted that the isolates with the different electrophoretic profiles belong to the different strains. Based on the obtained results, it was concluded that 20 drug-resistant isolates represent 18 different strains.

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A.17. ANTIBIOTIC SUSCEPTIBILITY AND RESISTANCE GENES IN CLINICAL ISOLATES OF OPPORTUNISTIC PATHOGEN STENOTROPHOMONAS MALTOPHILIA

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Stenotrophomonas maltophilia is Gram-negative multidrug resistant opportunistic pathogen mostly causing bacteremia, pneumonia and urinary tract infections associated with especially high mortality rates.

S. maltophilia ability to cause infections and survive in clinical settings is generally attributed to its natural multidrug resistance [1]. Due to efflux pumps, antibiotic inactivating enzymes as well as reduced membrane permeability S. maltophilia is known to be intrinsically resistant to multiple classes of antibiotics (β-lactams, fluoroquinolones, aminoglycosides). Trimethoprim-sulfamethoxazole (TMP-SMX) targeting folic acid synthesis remains the main agent for treatment of S. maltophilia infections [2], however, resistance to this antibiotic is also on a rise. Interestingly, S. maltophilia isolates resistance to TMP-SMX rates highly varies depending on the world region, reaching from 10.25 % in Iran [3], to 39 % in Taiwan [4]. Although S. maltophilia has become an important opportunistic multidrug-resistant pathogen worldwide, studies analyzing resistance of S. maltophilia isolates in Lithuania have not yet been performed.

The aim of this study was to evaluate the antibiotic resistance genes' distribution as well as susceptibility to antimicrobial agents of 33 clinical isolates of S. maltophilia collected during 2017–2021. Sensitivity to TMP-SMX, ciprofloxacin, tigecycline, gentamicin, chloramphenicol, ceftazidime was evaluated using disc diffusion method. The prevalence of genes conferring resistance to β-lactams, aminoglycosides, quinolones and sulfonamides antibiotic classes were assessed using PCR.

Susceptibility analysis showed that roughly half of the S. maltophilia clinical isolates are resistant to gentamicin. Almost all isolates were resistant to ceftazidime, while chloramphenicol, TMP-SMX, tigecycline and ciprofloxacin showed an inhibitory or intermediate effect against majority of isolates. The detection of antibiotic resistance genes showed that the blaL1 and blaL2 genes encoding β-lactamases were the most commonly detected in clinical isolates. The high prevalence of these genes in clinical isolates of S. maltophilia could explain the established resistance to ceftazidime. Genes conferring resistance to aminoglycosides and quinolones were also detected in almost all tested isolates.

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A.18. MOLECULAR CHARACTERIZATION OF LISTERIA MONOCYTOGENES ISOLATES

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Listeria monocytogenes is a gram-positive pathogenic bacterium distributed in the environment. It can cause serious diseases such as listeriosis or meningoencephalitis in humans with a mortality rate of 20-30 percent [1]. Immunocompromised patients, pregnant women, newborns, and the elderly are at the most risk of being infected with this foodborne pathogen.

The aim of this study was to identify *Listeria monocytogenes* and to determine its lineages and serovars. Seventy-seven isolates were collected from Lithuanian hospitals during the period of 2016–2021. Most of the isolates were cultured from clinical samples - patients' blood or cerebrospinal fluid. PCR-based methods were used for the characterization of invasive bacterial isolates. The results were visualized using 1% agarose gel electrophoresis. Amplification of the *prs* gene was used to identify genus *Listeria* and that of the *isp* gene was used for species identification. *L. monocytogenes* bacteria were detected in all collected clinical samples. Amplification of lineage-specific L1, L2, and L3 DNA fragments was used to discriminate *L. monocytogenes* major lineages (LI, LII, LIII). Out of seventy-seven isolates, there were eighteen isolates of lineage LI and fifty-nine isolates of lineage LII. Lineage LIII was not detected in any of the samples. Afterward, the main serovars (1/2a, 1/2b, 1/2c, and 4b) of *L. monocytogenes* were differentiated. The marker genes selected for the PCR were ORF2100, ORF2819, lmo0737, and lmo1118. Genes ORF2819 and ORF2100 were used to identify serovars of LI lineage of *L. monocytogenes*. Gene targets lmo0737 and lmo1118 were used to determine serovars of LII lineage. Four serovars were identified: 1/2a (amplification of only the lmo0737 DNA gene), 1/2b (amplification of only the ORF2819 gene), 1/2c (amplification of both lmo0737 and lmo1118 genes), and 4b (amplification of both ORF2819 and ORF2110 genes).

This study demonstrates that the PCR-based methods provide fast and accurate information which is necessary for further research or testing samples during outbreaks. Hereafter, more studies may be continued to understand how antimicrobial resistance occurs in *L. monocytogenes* and how it varies between hospitals. In the future, the results can be used to prevent infections and outbreaks of this pathogenic bacteria in Lithuanian healthcare institutions.

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A.19. IDENTIFICATION OF POLYURETHANE DEGRADING ENZYMES IN WILD-TYPE BACTERIA

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Plastic pollution is a major environmental concern – plastic waste accumulates in oceans, landfills, and other natural habitats and harms natural wildlife. One of the most used plastics in the world is polyurethane (PU), with almost 25 million metric tons produced annually [1]. Exceptional resistance to tearing and abrasion, elasticity, impact resistance and other properties make PU a perfect choice for various applications. Despite its many consumer oriented benefits, the disposal of PU waste remains a significant ecological challenge. Traditionally, PU waste is disposed of by incineration or landfilling, but such methods are not sustainable. PU is also recycled by mechanical and chemical means, but the use cases of mechanically recycled PU are very limited and chemical recycling methods require high temperatures and solvents. Development of enzymatic recycling methods could provide more sustainable alternatives. Identifying new enzymes that can efficiently degrade PU has become an urgent need. These enzymes could potentially break down PU waste into compounds, e.g., diethylene glycol, adipic acid and polyols, used by various chemical industries [2]. Furthermore, discovering new enzymes can provide insights into molecular mechanisms of PU degradation, leading to the development of more effective solutions to the plastic pollution problem.

This research aims to identify PU-degrading enzymes from bacteria capable of degrading PU. This degradation was observed by a formation of translucent halos around the microorganism colonies grown on PU-containing media. It was determined that microorganisms of interest belong to the *Bacillus* genus using 16S rRNA gene sequencing. The two different approaches for enzyme identification were being implemented:

- *Construction of a genomic library and its functional analysis.* Library is being prepared using pUC19 and modified pBR322 plasmid vectors. The library clones, coding the gene for PU degrading enzyme, form a translucent halo around their colonies when grown on PU-containing media.
- *Separation and isolation of PU degrading enzyme from PU degrading bacterium's proteome and itsidentification using mass spectrometry*. Bacterium of interest is grown in minimal salt media with orwithout PU. Proteins of both cell cultures are separated using SDS-PAGE and compared.

Obtained results will be presented in more detail during the poster session.

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A.20. SURFACE DISPLAY OF *ACINETOBACTER BAUMANNII* BLP1 PROTEIN C-TERMINAL FRAGMENT BY *SACCHAROMYCES CEREVISIAE*

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The gram-negative opportunistic pathogen *Acinetobacter baumannii* is one of the leading causes of nosocomial infections worldwide. Infections such as pneumonia, sepsis, and meningitis, increase mortality among immunosuppressed patients. Antibiotic therapy has become less effective due to the global emergence of multidrug-resistant *A. baumannii* strains. In 2017, reduced antibiotic efficacy led the World Health Organization to designate *A. baumannii* a top priority pathogen for which new therapeutic strategies are urgently needed [1]. An endemic situation has already developed in Lithuania as of 2019 [2].

Vaccines open an alternative path for effective control of *A. baumannii* infections. Unfortunately, the development of vaccine remains in preclinical stage. The progress in vaccine development is hampered by bacterial capsular polysaccharides that shield most potential vaccine targets from immune recognition. Importantly, some proteins penetrate the capsule layer and are involved in biofilm formation and adhesion to the host cell [3]. One such example is Blp1, which has a surface-exposed C-terminal 711 amino acid fragment. It possesses a conservative 163 amino acid sequence known to be universal for the most prevalent *A. baumannii* clinical strains [4].

An attractive method for vaccine development is yeast surface display. When using *Saccharomyces cerevisiae,* its most important properties are (1) GRAS status that enables oral human consumption, (2) the induction of mucosal immune response in addition to systemic one, and (3) the immunostimulatory function of cell wall components. The display of antigens occurs through yeast cell wall proteins, such as a-agglutinin. The system is based on two subunits, Aga1 and Aga2. The antigen is expressed as a fusion protein with Aga2 subunit, linked by two disulfide bonds to cell wall-anchored Aga1 subunit [5].

The aim of this study was to display the conservative C-terminal fragment of *A. baumannii* Blp1 protein on the surface of *S. cerevisiae*. The 163 amino acid fragment was immobilized on yeast cell wall. Western blot and immunofluorescence assays were performed to confirm antigen expression and location. To quantify the fraction of yeast cells with immobilized antigen, flow cytometry was employed. Analysis indicated that 48.5% of singlets displayed the antigen of interest. These initial findings suggest that this yeast surface display technology is a promising platform for development of oral vaccine against *A. baumannii*.

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A.21. STANDARDIZATION OF ALKALINE PHOSPHATASE ACTIVITY AND DAPI MEASUREMENT METHODS

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Standardization of methods for the measurement of alkaline phosphatase (ALP) activity and DAPI-DNA complexes fluorescence is one of the solutions to the problems arising from methods used in the bioengineering of artificial tissues. *In vitro* assays are used in the development of artificial bone tissue to monitor cell differentiation processes and measure changes in cell number.

One of the main biomarkers found in bone-differentiating cells is the enzymatic activity of ALP. ALP of the cell membrane degrades ATP to individual phosphate residues, which, together with calcium, are stored as hydroxyapatite within the cell itself [1]. That occurs when cells are grown in an osteogenic differentiation medium. Calcification and ALP expression are therefore mutually interdependent biomarkers when it comes to the identification of differentiating osteoblasts or some cancer cell lines. However, when ALP activity is measured quantitatively, a direct dependence of the signal of the resulting colorimetric enzymatic reaction product on the number of cells in the sample is observed.

A highly efficient, sensitive, and less expensive DAPI method is used to study stem cell differentiation on polymer scaffolds [2]. This method based on DNA-binding and fluorescent DAPI molecules allows reliable measurement and assessment of cell proliferation in 2D, 2.5D, and 3D environments. Although the DAPI method cannot distinguish between viable and dead cells, it can help to reliably track the number of cells at a given time point in 2.5D and 3D cultures.

Two cell samples are currently required for the quantification of ALP activity, as one cell sample is only required for the colorimetric assessment of ALP activity and the other cell sample is required for the determination of the cell number by DAPI. Thus, two different samples of cultured cells are needed to track both cell differentiation and cell number for the development of artificial bone tissue. Furthermore, since ALP and DAPI are measured separately in different samples, this leads to inaccuracies as the cell samples are not identical, even though they have been grown under the same conditions. Therefore, a combination of the two methods is needed to increase sensitivity and reduce the sample costs. There are many methods to determine the number of cells in the sample, however, DAPI method is the most suitable because differentiation is characterized by a particular change in the molecular mechanisms of cells and the DAPI method is resistant to them. To evaluate the same cell sample using different methods, it is necessary to demonstrate that the ALP activity measurement is compatible with DAPI method used for cell count quantification. This work aims to modify ALP activity measurement method and align ALP activity and DAPI fluorescence measurements, and thus show that we can obtain more accurate results from a single cell sample based on two different methods: measurement of the alkaline phosphatase activity characterizing cell differentiation and cell number determination by DAPI.

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A.22.*PANTOEA* HOST - PHAGE INTERACTIONS AT THE INITIAL STAGES OFINFECTION

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Bacteriophages (phages) are bacterial viruses specifically infecting their bacterial hosts. The host spectrum of phages in the very early stages of infection is primarily determined by interactions between bacterial surface structures (receptors for phage adsorption) and phage anti-receptors. Anti-receptors are the phage virion proteins that selectively recognize cell surface proteins, polysaccharides, or even more complex structures like pili and flagella [1-3].

The small and flexible fibrils and spicules of phage virion are often responsible for the host specificity, and often have enzymatic activity that helps the phage to break down the bacteria's defensive layers. Phages from Gram-negative bacteria usually have lipopolysaccharide or capsule polysaccharide disrupting enzymes on the virion surface [4]. Both phage fibrils and spicules often act in a coordinated manner, i.e. they are adapted to recognize similar receptors in order to maximize the efficiency of the initial phase of infection.

The aim of this work is to identify the host range proteins of lytic bacteriophages infecting *Pantoea* bacteria. For a long time, very few phages infecting *Pantoea* have been known, and therefore very little research has been carried out. Meanwhile, research on this genus of bacteria is becoming increasingly important due to its applications in agriculture and biotechnology [5, 6].

Two genetically similar *Pantoea* phages vB_PagM_AAM37 and vB_PagM_PSKM infecting different hosts were chosen to study the mechanisms of recognition selectivity in this work. For this, the adsorption kinetics of phages and the nature of receptors were determined. Also, the putative tail fiber recombinant proteins were constructed, and the self-assembled protein nanostructures were analyzed with transmission electron microscope. In addition, protein-cell interaction experiments were performed seeking to find out if these proteins may be responsible for host recognition.

The results show that the recombinant proteins of vB_PagM_AAM37 virions adopt fibrillar structures resembling those of native phages. Therefore, this methodology confirms the predicted structural function of studied proteins. However, according to protein-cell interaction experiments, the fiber proteins do not completely determine the phage's host spectrum, which may be influenced by other factors.

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A.23. APPLICATION OF GDEST-LIP FOR HYDROLYSIS OF PCL FILMS

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Plastics are ubiquitous polymers used in all fields of science, industry, and commerce. The production of plastics is steadily increasing and has reached 390,7Mt in 2022 [1]. Therefore, the environmental pollution caused by plastic waste is becoming an increasingly urgent problem. One way to combat this issue is to employ biocatalysts like lipases, esterases or other carboxyl ester hydrolases for the degradation of polyester plastics.

GDEst-lip is a fused chimeric carboxyl ester hydrolase made up of an esterase GDEst-95 and a lipase GD-95 from *Geobacillus* sp. 95 [2]. This protein can catalyse the hydrolysis of ester linkages which are crucial for the integrity of polyester plastics like PET, PLA or PCL. Therefore, GDEst-lip is a possible candidate for the degradation of these polymers. On top of this, GDEst-lip remains active and stable in a broad temperature and pH range which makes it an appealing protein for industrial application [2]. GDEst- lip is also resistant to various organic solvents, which not only opens up the possibility to increase the effectiveness of polyester degradation but might also allow the formation of useful esters as a reaction product.

The aim of this study is to evaluate GDEst-lip ability to degrade PCL films and to optimize reaction conditions using organic solvents. Degradation assay was performed by making PCL films and submerging them completely in protein – solvent solutions for 24 hours with constant mixing. After treatment, PCL films were removed from flasks, thoroughly washed three times with deionized water and dried until a constant weight was obtained. The weight losses after the incubation were calculated. The effectiveness of PCL hydrolysis was calculated using the following equation [3]:

Weight loss of PCL films (%) = 100 x ((Wi– Wpd)/ Wi)

Wi – weight of pre-degraded PCL films, Wpd – weight of post-degraded PCL films

Preliminary results show that 0.05 mg/mL of this protein is able to degrade around 40% of a PCL film
at 30°C over 24 hours. The use of organic solvents significantly enhanced the degradation process. With addition of 50% methanol in solution, at the same reaction conditions 100% degradation was achieved.

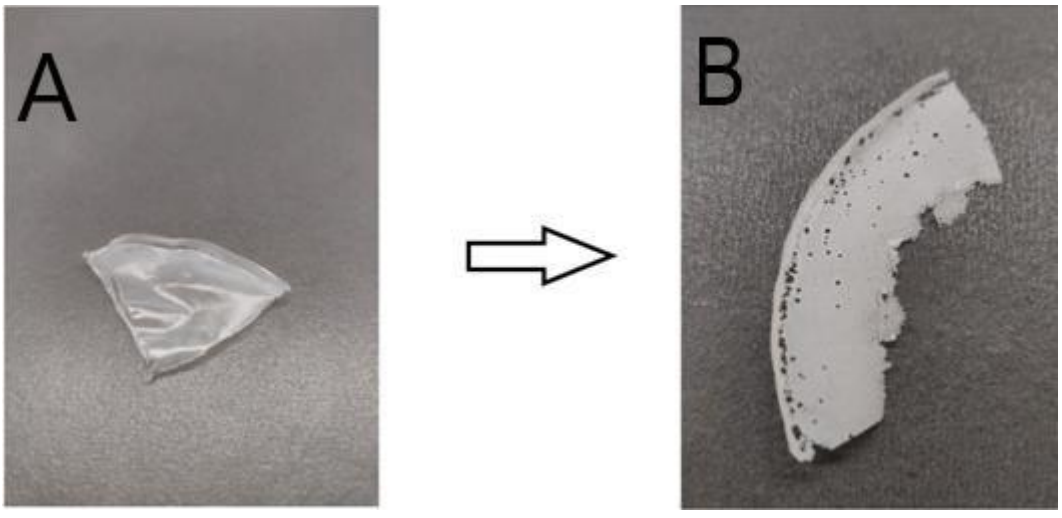

Figure 1. A PCL film before (A) and after degradation (B) caused by GDEst-lip.

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A.24. BACTERIAL MEMBRANE MIMICKING MODEL AND ITS INTERACTION WITH ANTIMICROBIAL PEPTIDES

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Bacteria are ubiquitous in nature, and some strains have the potential to colonize human hosts and cause infectious diseases. Although the discovery and widespread use of antibiotics in the past century has greatly reduced the morbidity and mortality associated with bacterial infections, the emergence of antibiotic-resistant strains poses a significant threat to public health [1]. As a result, the development of alternative antimicrobial therapies, such as antimicrobial peptides (AMPs), is of critical importance. To enable rapid and efficient screening of potential AMP candidates for preclinical and clinical evaluation, innovative screening methods are needed [2,3].

In this context, tethered bilayer lipid membranes (tBLMs), which mimic bacterial membranes, have been constructed and quantitatively evaluated using electrochemical impedance spectroscopy (EIS) [4]. Experimental results showed that bacterial membrane-mimicking tBLMs exposed to colistin exhibited decreased systems current conductivity, indicating that the membrane became stiffer and more tightly packed with lipids, which resulted in the elimination of naturally occurring membrane defects. Conversely, bacterial membrane-mimicking tBLMs exposed to gramicidin and melittin, a well-known AMPs, displayed rapidly increasing conductivity due to the formation of ion-permeable pores in the lipid bilayer.

In addition to EIS, we also used atomic force microscopy (AFM) to visualize the changes occurring in the bilayer structure induced by the AMPs. We were able to successfully image bacterial membrane-mimicking tBLMs, and got the images of partially destroyed tBLMs when incubating with melittin for prolonged periods of time, however visualization of pores is still ongoing. These results provide valuable insights into the mechanisms underlying the action of AMPs against bacterial membranes.

Taken together, our findings demonstrate that tBLMs coupled with EIS and AFM can be a powerful tool for screening and characterizing the activity of AMPs against bacterial membranes.

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A.25. DESIGN AND SYNTHESIS OF A HYPOALLERGENIC *ARTEMISIA VULGARIS* ALLERGEN COMPONENT ART V 3 VARIANTS

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During the pollination season, airborne pollen and related allergies are significant public health issues and climate change has exacerbated their consequences. The World Health Organization has predicted that by the year 2050, one out of two people will suffer from allergy [1]. Allergen sources may vary: pollen, dust mites, food and pharmaceuticals, but the most common allergens are proteins. An allergen is described as any molecule that causes allergic responses and production of allergen-specific imunoglobulin E (IgE). Strong immune response caused by an allergen can contribute to the development of diseases such as asthma, allergic rhinitis and eczema [2]. In Germany, people had a lifetime prevalence of allergic diseases at 3.5% for atopic dermatitis, 8.1% for contact dermatitis, 14.8% for allergic rhinitis, and 8.6% for allergic asthma, according to the Environmental Medicine Commission of the Robert Koch Institute. Around the globe, allergies are a common health issue, although exact numbers are not accessible, estimates range from 20% to 40%. [3]. Anti-inflammatory drugs and allergen avoidance are the most common recommendations to suppress allergic symptoms, but have no long-term effect and do not modulate the immune responses to allergens. In the future, the development and the application of hypoallergens in the personalized allergen immunotherapy could help to prevent adverse immune reactions. Currently, allergen-specific immunotherapy (AIT) is the only treatment that aims to induce allergen tolerance by altering the innate and adaptive immune response. Modified allergen derivatives and hypoallergenic peptides are less likely to elicit IgE modulated response than natural whole-allergen extracts or recombinant wild type-like allergen components. Molecules known as hypoallergens are less likely to induce allergen-specific IgE response but has the ability to elicit the T-cell response, without causing allergic symptoms in the patient. Hypoallergens may eventually lead to more specialized, "tailor-made" allergen-specific immunotherapy [4].

In the present study, mutants of *Artemisia vulgaris* allergen component Art v 3 were generated and analyzed. Eleven different variants of Art v 3 protein mutants were selected for the analysis. Two of them were generated and experiments of their synthesis in *E. coli* were performed during this work. Amino acids that may be involved in IgE-binding epitope formation were identified based on the literature and bioinformatic analysis. Mutagenesis of these amino acids is important for the identification of IgE-binding epitopes and synthesis of hypoallergens.

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A.26. SYNTHESIS OF ART V 3 ALLERGEN COMPONENT FROM ARTEMISIA VULGARIS IN YEAST S. CEREVISIAE

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Allergy affects many people constantly. For instance, in Germany about 20 % of the population suffer from this chronic disease. The prevalence vary between countries from 20 % to 40 % [1]. There are different types of allergies – inhalant, food, contact, drug and insect allergies. The immune system of allergic people reacts to allergens and produces antigen-specific immunoglobulin E (IgE). People, who suffer from allergy, can feel many symptoms like itchy eyes, swollen tongue and etc. It could also cause chronic diseases like eczema and asthma. Allergens are described as a material that can trigger allergic reactions. It can be proteins, carbohydrates and drugs but most often it is protein. Some examples of allergens can be dust, animal fur, pollen, food products and etc. [2]. One source can contain more than one different allergen component, for example, *Artemisia vulgaris* (common mugwort) has 6 different components (Art v 1-6) [3]. The same allergic person can be sensitized to only one or more different allergen components. Whole- allergen extracts, produced from natural allergen sources, are still widely used for allergy diagnostics and immunotherapy. The usage of natural extracts, which are difficult to standardize, is limited by many factors. Variable amounts of different allergen components and the absence of some of them in the extract, the different biological activity of those components, undefined materials and contaminants can lead to false positive/negative results and ineffective immunotherapy [4]. Recombinant single allergen molecules could help to improve diagnosis and treatment.

In this study, recombinant allergen component Art v 3 from *Artemisia vulgaris* was analyzed. This plant releases pollen and causes most pollinosis in late summer and fall. Art v 3 protein belongs to nsLTP protein family and has IgE cross-reactivity with many other allergen components, for example, Pru p 3 from peach, Cor a 8 from hazelnut, *Parietaria judaica* (Pellitory of the wall) allergen component Par j 2 and *Ambrosia artemisiifolia* (Short ragweed) allergen component Amb a 6 [3]. Two constructs of recombinant Art v 3 protein were analyzed –single allergen component Art v 3 and Art v 3, fused with maltose binding protein (MBP). Genes of these two protein variants were inserted in a protein expression vector and experiments of recombinant protein synthesis were performed in the yeast expression system.

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A.27. PREVALENCE OF GR1-GR34 TYPE REPLICONS IN ACINETOBACTER BAUMANNII: GENBANK METADATA ANALYSIS

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Introduction: *Acinetobacter baumannii* is an opportunistic pathogen that causes hospital-acquired infections. A critical problem with this pathogen is its ability to rapidly gain resistance to antibiotics. Antibiotic resistance is usually acquired through mobile genetic elements, such as plasmids. Due to plasmids' capability to accumulate multiple resistance genes, it is important to study them in greater detail. For this reason, in 2010, Bertini et al. proposed a scheme for plasmid classification called PCR-based replicon typing, which is based on the similarity of *rep* genes between different plasmids. The genes within the same replicon group must be >=74% identical and have >=90% coverage. The aim of this study was to investigate the global distribution of GR1-GR34-type replicons and find correlations between different replicon types, isolation source, and isolation countries based on metadata.

Methods: Plasmid *rep* gene sequences were searched according to the parameters defined by Bertini *et al.*, using reference genes for the GR1-GR34 groups that have been collected beforehand by R. A. Moran, creating local *rep* gene database. From resulting replicons, metadata was extracted. The distribution of GR1-GR34 replicons was then assessed, followed by a detailed analysis of the distribution of the different replicon types in the clinical and natural environments. Finally, the qualitative distribution across continents was assessed in relation to the year of collection of the isolates.

Results: Out of all collected data (n = 1142), the most frequent replicon types found were GR2 and GR6. While the percentage of clinical isolates in the entire data was 78.1% (n = 981), it greatly increased to 92.2% when only the prevailing replicon types (GR2 and GR6, n = 295) were examined. Moreover, GR2 and GR6 types were only dominant in clinical *A. baumannii* isolates, while in non-*baumannii* clinical isolates and in environmental isolates the prevailing replicon types differed. *A. baumannii* were also the dominant species, accounting for 71.7% of all clinical isolates with GR1-GR34 replicons (n = 549). Considering the replicon type distribution by continent, it appears that most of the replicons in the GenBank database belong to the continents of Asia, Europe, and North America. Some replicon types were found to be unique to only two of the three continents, however, only GR25-type replicons (n = 23) were found to be unique to a single continent and country - Asia (China). The most common replicon types on all three continents were GR2 and GR6. In regards to age, the oldest isolates found are *A. baumannii* with GR2 and GR6 replicon types, first collected in North America in 1948. Meanwhile, the first replicons discovered in Europe were in 1951. In Asia, the collection of isolates started much later, in 1996.

Conclusions: *A. baumannii* with GR2 and GR6-type replicons were the most common among clinical *Acinetobacter* spp. GR1-GR34-type replicon isolates. However, GR2 and GR6 types were not predominant in non-*baumannii* clinical isolates and *Acinetobacter* spp. environmental isolates. While most GR1-GR34-type replicons are globally widespread, the GR25-type seems to be local to China, however, further epidemiological significance is to be determined.

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A.28 OVARIAN CANCER CELL LINES EXHIBIT DIFFERENTIAL STEMNESS-RELATED PROTEIN AND GENE EXPRESSION IN 2DAND 3D MODELS

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Epithelial ovarian cancer (EOC) in most developed countries is the leading cause of death among gynecological malignancies. The high level of heterogeneity of this cancer plays a big role in the lack of successful treatments [1]. To improve our understanding of epithelial ovarian tumor biology and behavior itis important to adopt a suitable culture system. Usually, the majority of *in vitro* experiments are performedin two-dimensional (2D) cancer cell line monolayer cultures. Even though the 2D model is commonly used, it does not fully reflect the tumor complexity therefore more sophisticated models should be adopted. Cancer cell microstructures formed as three-dimensional (3D) spheroids closer represent the main characteristics of *in vivo* tumors, such as cell-cell interactions, hypoxia and pH rate, exposure to nutrients and metabolites, and gene expression profiles [2].

In our study, we aimed to adopt and optimize the 3D culture conditions of four different EOC cell lines: A2780, SKOV3, COV362, and OV7. Next, we compared the gene and protein expression profiles between 3D and widely used 2D cell culture models by performing flow-cytometry (FACS) and analyzing the expression of genes related to stemness properties, epithelial-mesenchymal transformation and interaction with the immunesystem by quantitive real-time PCR (qPCR).

EOC spheroids were measured and observed in different growth conditions and at a different seeding density as a starting point. We noticed that the A2780 cell line formed no spheroids in our culture conditions and as such this cell line was excluded from further analysis. After optimization when the best cell count and conditions were determined, protein and gene expression profile evaluation was performed. We found out that the OV7 cell line had the upregulated TGF-β pathway and epithelial-mesenchymal transformation-related genes. Next, we compared the gene and protein expression in respective 2D and 3D cultures, where we found substantial differences.

In conclusion, for each cell line, we characterized its gene and protein expression profile in 2D and 3D culture conditions.

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A.29. INTRODUCTION OF SURFACE CYSTEINE AND ITS EFFECT ON BACTERIOPHAGE vB_EcoM_FV3 SELF-ASSEMBLING NANOTUBE

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Biological self-assembling nanosystems have relevant applications in nanomedicine and nanobiotechnology, including the development of vaccines, imaging, drug, and gene therapy delivery systems, and *in vitro* diagnostic methods. A great example of biological nanosystems is viruses and viral nanoparticles, composed of robust, stable proteins with a defined geometry and the ability to self-assemble into precise and stable nanostructures. Viral proteins' ability to gather in spontaneous nanostructures in combination with a surface modification can be applied to deliver specific signals (tags, ligands, or drug molecules) to a definite tissue or cell without damaging their biological activity. Cysteine, due to its rarity in proteins, the reactivity of the sulfhydryl group, and the availability of different methods is one of the most popular amino acids used for the modification of nanostructures. Cysteine modifications with advanced functionalization technics will help to develop next-generation targeted delivery systems and vaccines with optimal drug loading capacity, tunable immunogenicity and stimuli of the correct type of polarized immune response, and selectivity to the target cell [1,2].

Our research focuses on the development of modifiable nanostructured material based on a self- assembling tail sheath protein gp053 from *Escherichia coli* infecting bacteriophage vB_EcoM_FV3 [3]. Based on the analysis of the gp053 tertiary AlphaFold structure model, we have designed modification sites oriented toward the outer surface of the tubular structures. By creating a mutant protein in which all four native cysteines have been replaced by serines, we have verified that the mutant protein forms tubular structures indistinguishable from the nanotubes formed by a wild-type protein. Thereafter, serine amino acids located in the loop at the selected positions were replaced by cysteine (two mutant proteins with cysteine at positions 120 and 232). The transmission electron microscopy analysis indicated that the mutant proteins with newly introduced cysteine self-assembled into tubular nanostructures. Moreover, the formation of 2-nitro-5- thiobenzoate (NTB) after mutant proteins reaction with 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) under a non-reductive environment indicated the presence of free sulfhydryl groups on the protein-formed nanotube surface. The constructed mutants maintained their tubular nanostructures in 10 mM pH 8 Tris-HCl buffer, at 4 ℃ for one month. The observed stability is essential for the subsequent application of nanostructures. The resulting nanotubes with free cysteine groups will be further bioconjugated to various molecules, such as tags with fluorescent dyes, catalytic proteins, or drug molecules, and used as delivery nanosystems.

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A.30. ANTIMICROBIAL ACTIVITY OF PIGMENTS EXTRACTED FROM PIGMENT-PRODUCING MICROORGANISMS ISOLATED FROM THE ENVIRONMENT

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Microorganisms are one of the possible solutions not just to change synthetic colors, but also, they have attracted the attention of the industry, as the production of new, easily degradable, safe, environmentally friendly pigments with no adverse effects, which is becoming more and more relevant.

The aim of this work is to isolate pigmented microorganisms from environmental samples, select fermentation conditions, isolate pigments from microorganisms and test their antimicrobial activity.

Microbial pigments have been isolated from various sources such as food waste, soil, flour, etc. Fermentation parameters such as temperature, pH, tryptone and NaCl salt concentration were optimized to improve the growth of microorganisms and pigment production. After fermentation, cells were lysed in an ultrasonic bath and then the pigments were extracted with methanol. During research five pigments were successfully isolated from six investigated microorganisms. The dried microbial pigments were diluted with analytical grade ethanol [1, 2].

To evaluate the antibacterial properties of the investigated pigments, 8 strains of pathogenic bacteria were used in the study: Escherichia coli ATCC 8739, Listeria monocytogenes ATCC 13932, Staphylococcus aureus ATCC 25923, Pseudomonas aeruginosa ATCC 13525, Salmonella typhimurium ATCC 14028, Enterococcus faecalis ATCC 19433, Citrobacter freundii ATCC 43864, Micrococcus luteus ATCC 9341.

The tested bacteria were dissolved in physiological salt solution (NaCl 8.5 g/L) to form suspension. Adjustment of washed cell bacteria was standardized to the density of about 106 CFU/mL according to McFarland standard. Bacterial strains were grown on the plate count agar (PCA, Liofilchem, Italy), which were incubated at 37°C for 24 h. [3]. The suspension of bacteria cells was introduced into the dissolved and cooled to 47 °C PCA media, 10-12 ml of which were pipetted into a 90 mm diameter Petri plate.

The antibacterial activities of the microbial pigments were determined by using filter disk diffusion method. For direct contact agar diffusion tests, sterile 6-mm diameter filter discs (Hahnemuehle, Germany) were placed on the surface of the solidified inoculated PCA medium surface. A 10 µL volume of ethanolic solutions of microbial pigments were pipetted on the filter discs using an automatic pipettor Eppendorf Research plus. Ethanol was used as a negative control preparation.

The plates were then incubated at 37°C for 24 hours. The antibacterial activity was evaluated by measuring the diameter of clear inhibitory zones in millimeters using digital calliper. The experiments were conducted in triplicate. The average values ± standard deviations were calculated. Analysis of variance was performed on the basis of mean values to determine the significant difference between pigments at $P \leq 0.05$ [4].

The results of the study showed that among the tested pathogenic bacteria, P. aeruginosa was the most sensitive to the effects of pigments. Pink – red pigment showed the most antimicrobial activity against tested pathogenic bacteria. So, the investigated pigments showed bactericidal effects against certain human pathogens, but bacteriostatic effects against pathogens were also observed in the obtained results.

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A.31. TRICHODERMA REESEI AND PRODUCTION OF CELLULASES

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Currently, most of the world's energy needs are met by fossil fuel resources. Several different technologies can be used to transition to more sustainable energy sources. Cellulose is the most abundant organic material in the biosphere. Therefore, the isolation and industrial use of cellulases could be used in the production of biofuels from organic cellulose sources.

Trichoderma reesei is a mesophilic, filamentous fungus, known for its efficient production of cellulase enzymes, which are necessary for breaking down crystalline cellulose into glucose. In 1950 it was isolated in the Solomon Islands from decaying textiles. *T. reesei* produces three main types of cellulolytic enzymes: endogluconase, cellobiohydrolase, and b-glucosidase. Endogluconase shears internal connections in cellulose, cellobiohydrolase cleaves cellobiosyl units from the non-reducing end of cellulose, and beta- glucosidase cleaves glycosyl units from the non-reducing end of cellulose oligosaccharides. These enzymes work synergistically to depolymerize cellulose and convert it into glucose [1]. However, the close relationship between lignin and cellulosic biomass can create barriers to the enzymatic hydrolysis of cellulose into fermentable sugars. Hemicellulose and lignin prevent cellulosic enzymes from functioning, inhibiting enzyme structure and reducing matrix pore size. The crystalline structure of cellulose with a glucan chain stops the hydrolysis process. Different pretreatment methods have specific effects on different lignocellulosic components. However, a significant number of them can create some kind of obstacles to the action of cellulases [2].

In our study we used RTS-1C bioreactor for cultivation of *T. reesei* ATCC 26921 cells. When growing *T. reesei* preculture in the bioreactor, it was important to create a constant aeration, to maintain optimal pH and to ensure the optimum temperature. After ensuring the optimal preculture growth conditions, the developed methodology was applied to the cultivation of cellulase-producing cultures. In the further cultivation of enzyme-producing cultures, it is planned to study the influence of the processing of cellulose raw materials on the final yield of enzymes, also to investigate different additives that can increase the excreted amount of the enzyme or the increase enzyme activity. It is expected to optimize the cultivation, enzyme production and purification technologies to provide convenient, cost-effective, and efficient conditions, as well as to investigate the specificities of the culturing of the culture in the bioreactor.

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A.32. STUDY OF THE GENES OF MICROORGANISMS FOUND IN THE ENVIRONMENT AND INVOLVED IN THE BIODEGRADATION OF MICROPLASTICS

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Plastic pollution is a global problem caused by the increasing production of synthetic plastics. Their annual production was about 350 to 400 million tons in 2020 and is expected to triple by 2050 [1]. Unfortunately, a lot of waste of it is released into the environment. Despite plastic recycling technologies, plastic waste is mostly disposed of in landfills, where it degrades into small pieces. Microplastic pieces (MPs) (<5 mm in diameter) have potential dangerous impacts on aquatic and terrestrial ecosystems, as well as human health, and are resistant to natural degradation [2], so finding solutions for its removal from the environment is key.

Microorganisms are considered to be the best solution to utilize microplastic because they can use MPs as a carbon source and release just H_2O and CO_2 during the aerobic process. The degradation process is possible because they produce extracellular enzymes that attack C-H and C-C bonds to cut long chains into small monomers and oligomers that microbial cells can take up. Several studies have reported MPs degrading microorganisms from landfills [2, 3]. Thus, finding the species (or genes) responsible for biodegrading artificial polymers is crucial for studying the mechanism and applying it to microplastic utilization. Unfortunately, not much is known about enzymes involved in microplastic degradation. However, some common enzymes from microorganisms like cutinases, alkane hydroxylases, styrene oxygenases, laccases, lipases, carboxylesterases, and proteases can also attack nonstandard substrates such as petro- polymers due to structural similarity to the original target of the enzyme [3].

This study analyzes samples from different environmental sources for known microorganism enzymes capable of degrading microplastics. 23 samples were taken from different spots such as clean soil from Molėtai, the operating Kazokiškės landfill, and the closed Kariotiškės landfill. Target genes were used to identify the potential of microorganisms found in the samples to biodegrade MPs and metagenomic analysis was used to verify whether microorganisms that have these genes based on their origin can be found.

It was found that the diversity of enzymes in samples from landfills is more various than from clean soil. This can be expounded by the fact that in landfills microbiota is surrounded by a lot of substances including various plastics. Based on the results, microorganisms found in a closed landfill site have a greater potential for the biodegradation of plastics due to a higher number of lipases, cutinases genes (associated with PET utilization [1]), polyvinylalcohol dehydrogenases, polyethylene glycol dehydrogenases genes (associated with PPG utilization [4]), and styrene monooxygenase, alkane hydroxylases genes (associated with PS utilization [1]). This can be explained by the fact that an active landfill site has a markedly different, fluctuating, and unstable environment compared to a closed landfill site, which has a more consistent environment but still has different types of carbon sources. This consistency in a closed landfill site is conducive to the growth of microorganisms and the formation of diversity, which could explain the higher biodegradation potential.

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A.33. GRANULAR STARCH DEGRADATION BY ALPHA-AMYLASE FROM MICROBACTERIUM SP. SIN02

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Starch is a renewable energy source and raw material used in various industries such as food, paper, pharmaceutical, etc. Industrial processes involving starch hydrolysis employ α-amylases, β-amylases, and glucoamylases. However, enzymatic raw starch degradation is complex due to poor solubility and semi- crystalline structure, distinct for each starch source. These issues are currently resolved using physical factors such as high-temperature and high-pressure conditions in the starch-based products manufacturing process. Heat and pressure break down the semi-crystalline structure of starch and cause starch solubilisation and gelatinisation, making it accessible to various starch-degrading enzymes. Nevertheless, these processes increase the cost of starch-based product manufacturing. Moreover, they are not sustainable. An alternative sustainable solution is to exploit raw starch-degrading amylases. These enzymes can degrade the semi-crystalline starch without the gelatinization step as a prerequisite. Isolation and characterisation of these enzymes are also essential to broaden the common knowledge about raw starch degradation mechanisms and factors determining the enzyme's ability to break down semi-crystalline starch. These enzymes are found in bacteria genus such as *Bacillus*, *Microbacterium*, and others.

Here, we present *Microbacterium* sp. SIN02 based on its ability to degrade soluble starch and different starch granules. We also investigate an enzyme AM1.3 - a multidomain α-amylase from *Microbacterium* sp. SIN02. Bioinformatic analysis revealed that AM1.3 comprises one catalytic domain, four fibronectin type 3 domains, two adjacent CBM25 domains, and one CMB74 domain. According to the CAZy database, AM1.3 is a member of the GH13_32 subfamily, known for producing maltotriose, and domains CBM25 and CBM74 are identified as carbohydrate-binding domains known for their ability to bind to granular starch. After recombinant expression and purification, we yielded amylase capable of degrading granular starch via pore formation. We confirmed pore formation using scanning electron microscopy and imaging of various starch granules. Moreover, using TLC and HPLC-ELSD analysis, we identified the major products formed during starch hydrolysis by AM1.3.

A.34. FURTHERING THE UNDERSTANDING OF *GEOBACILLUS* SP. 95 ESTERASES(GDEST-95) USING SITE-DIRECTED AND RANDOM MUTAGENESIS

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As industrial processes require higher and higher efficiency to meet both the standards of production speed as well as managing the wellbeing of the planet, new innovative solutions will have to be implemented. Enzymes can be incredible tools in the industry, as they are environmentally friendly, biodegradable and can perform reactions at greater speeds than chemical catalysts. Lipolytic enzymes (lipases and esterases) are some of the most important enzymes used in industrial processes, since they can perform various types of reactions (ester bond hydrolysis, synthesis, transesterification etc.). However, like any tool, enzymes have to be improved to provide better catalytic efficiency and be less susceptible to various physical and chemical effects. As a consequence, further research is needed in enzyme structure-function relationships, especially in thermophilic proteins, which have the inherent benefit of being more active and stable in higher temperatures.

Our work centers around thermophilic esterase from *Geobacillus* sp. 95 GDEst-95. Based on *in silico* analysis[1], several amino acids were identified as potentially important for substrate binding, or catalytic residue positioning: Gly108, Ala410, Thr317, Leu226 and Leu411. Using site directed mutagenesis (in this case overlap extension) the gene encoding GDEst-95 was modified with these amino acid substitutions. Modified genes were first ligated to cloning vectors (pJet1.2 blunt) that were transformed into competent *Escherichia coli* DH5α cells. After selection, plasmids were extracted, excised with restriction enzymes, and ligated into expression vectors (pET-21c(+)) and transformed to competent *E. coli* BL21 (DE3) cells. Synthesis of the modified proteins was expressed using milk permeate as an inducer[2] and recombinant proteins were purified by IMAC. Enzyme activity, temperature activity, thermal stability, substrate specificity and othercharacteristics were evaluated using spectrophotometric measurements. Our results show that the suspected amino acids do affect the characteristics of GDEst-95, and can provide useful insights when designing other thermophilic proteins.

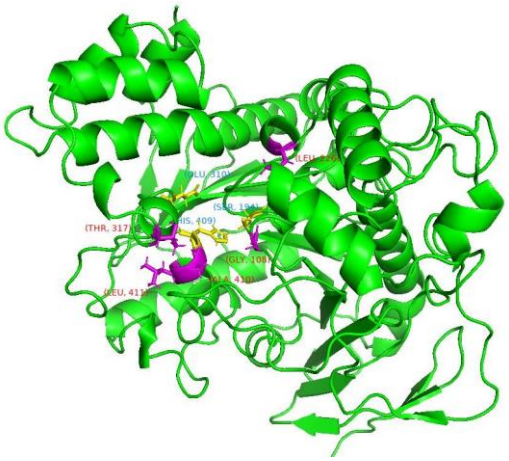

Figure 1. Predicted structure of GDEst-95. Protein model was generated using Robetta server (RoseTTAFold method)[3]. Protein backbone is displayed in green cartoon representation, catalytic residues are displayed as yellow sticks (labeled in marine), residues that were selected for mutagenesis are displayed in magenta (labeled in red).

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A.35. QUANTUM-DOT-BASED MULTIPLEXED BIOMARKER IMMUNODETECTION AND SIGNAL AMPLIFICATION USING MICRO-VOLUMETRIC ANALYTE QUANTIFICATION METHOD

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Quantum dot-linked immunosorbent assay (QLISA) is an approach similar to the enzyme-linked immunosorbent assay (ELISA), except QDs are used instead of enzymes [1]. Quantum dots (QDs) are fluorophores, absorbing photons and re-emitting them at different wavelengths. QD optical and chemical properties allow the detection to be more sensitive than with absorbance-based assays [2]. One of the best advantages is that different wavelength QD combinations can be used to detect multiple analytes in the same sample. It is key to choose QDs whose photoluminescence spectra don't overlap in order to distinguish fluorescence signal between different biomarkers.

The aim of this study was to develop an immunodetection method for at least two analytes in the immune-complex using fluorescence spectroscopy and quantitative measurement of QDs in micro- volumetric format.

The system was demonstrated by using two different analytes – cartilage oligomeric protein (COMP) and human growth hormone (hGH). 17C10 anti-COMP biotinylated antibody and anti-hGH biotinylated antibody were used. For multiplexed analysis of two biomarkers a combination of streptavidin-coated QD525 and QD605 was chosen. To compare fluorescence signal intensity, after we measured in two formats – 96-well plate and after the transfer to micro-volumetric format on a reiterative glass slide. The transfer was made possible by adding an immune-complex degrading solution which detaches QD-Ab complexes from the bottom of the well and can be transferred to micro-volumetric format and measure QD fluorescence. Singleplex detection of each analyte was done as a comparison of fluorescence intensity.

The results indicate that with our proposed method can be used for at least two analyte detection in the same sample using different wavelength QDs. In 96-well format, different analyte concentrations were hardly distinguishable, but the use of the immune-complex degrading solution and transfer to micro- volumetric detection increases the signal strength and can be used to detect different analyte concentrations.

The conclusion. This micro-volumetric format of analysis can be used not only for single analyte detection (singleplex), but also quantification of two or more analytes (multiplex) in the same well, using different wavelength QDs.

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A.36. DIGESTIBILITY PROPERTIES OF SINGLE AND DUALLY CHEMICALLY MODIFIED POTATO STARCH

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Starch is an easily available, low-cost, biodegradable, biocompatible, non-toxic natural polymer; however, granular native starch has limited solubility in water, doesn't contain lipophilic groups and doesn't form emulsions [1]. Modified starches, when used as food ingredients, are effective in preparation of oil-in-water emulsions and suitable as carriers for bioactive food components encapsulation [2], particularly for their controlled release. Potato starch was dually chemically modified for developing food-grade ingredients of lower digestibility and their properties were compared to those of single modified and native starches. The modified starches were produced in the Department of Polymer Chemistry and Technology at Kaunas University of Technology. The objective was comparison of dual octenylsuccinic anhydride modified hydroxypropylated potato starches (HP/OSA) digestibility properties with single OSA-modified and native potato (P-native) starches.

Hydroxypropylation with propylene oxide (HP) followed by esterification with octenylsuccinic anhydride (HP/OSA) of potato starch produced derivatives with lower digestibility than esterification solely with OSA. The selected enzymes and *in vitro* digestion method applied in our study was originally developed for assessing the release of antioxidants [3]; however, it simulated digestion conditions and may be applied for the *in vitro* simulation of digestion of other nutrients such as starch derivatives. The dextrose equivalent (DE) and the contents of maltose and glucose were the main indicators for *in vitro* digestion. DE was expressed as reducing sugars; the content of glucose and maltose in the soluble digestion fractions was analysed with UPLC-TQ-S-MS.

It was obtained that the addition of OSA groups into P-native did not reduce DE, while inclusion of OSA groups in HP starch resulted in a significant decrease in DE indicating increase in resistance of derivative to enzymatic hydrolysis. HP starch without OSA groups was less digestible ($P<0.05$) than P-native. Although the differences in the DE obtained in our study for the tested starch derivatives were not remarkable, the tendency of lower digestibility of modified starch derivatives may be clearly observed – single OSA-modification and particularly dual HP/OSA-modification reduced the digestibility of P-native [4].

Further the effect of modification on maltose and glucose content was evaluated after *in vitro* digestion. The amounts of maltose and glucose after the final (3^{rd}) step of digestion were 174.4–301.4 and 54.3–75.8 mg/g dw, respectively. The consistent dependence between maltose content changes during digestion and the type of modification was not clearly observed; however gradual reduction in maltose content was observed for HP/OSA with the highest food grade OSA level modified starch: 309.3 ± 3.5 (1^{st}) → 234.7 ± 9.8 (2^{nd}) → 174.48 ± 2.7 (3^{rd}) mg/g dw. The highest content of glucose after the 3^{rd} step was from P-native, indicating that it was most digestible starch. In general, it could be concluded that dual HP/OSA-modification was the most effective in terms of resistance to digestion: dually HP/OSA modified with the highest food grade OSA level starch derivative was the least digestible, the glucose and maltose content were lower by 28.3% and 42.1% compared to P-native.

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A.37. HYDROGEL CAPSULES WITH TUNABLE PERMEABILITY

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Microfluidics (MS) has multiple benefits for bioanalytical procedures where thousands of separate reaction micro-compartments are created, and which can be utilized for high-throughput screenings in the fields of drug discovery, targeted evolution of proteins, or single-cell analysis [1]. The miniaturized devices facilitate the processing and analyzing of small samples in extremely small reaction volumes. However, analytical tests with micro-droplets can also present it's technical limitations. One of these challenges is the ability to introduce or remove chemical compounds from/to micro-droplets. Therefore, various ways are being sought to extend the benefits of MS for multistage reactions based on analytical studies. The idea of this study is to employ microfluidics technique, to form and examine gelatin microspheres with tunable permeability variations.

Micro-capsules having a liquid core surrounded by the hydrogel shell were produced using a co-flow microfluidic device. An aqueous two-phase system (ATPS) composed of gelatin methacrylamide and dextran was used to generate core/shell capsules [2]. The physically cross-linked capsules were further stabilized by enzymatic crosslinking at two different temperatures 22 °C or 37 °C degrees. The resulting capsules contained a thin shell enriched in enzymatically crosslinked gelatin methacrylamide and a liquid core enriched in high molecular weight dextran (500kDa). After polymerization process, gelatin-based capsule permeability was evaluated using confocal laser scanning microscopy images. 5 different molecular weights (20kDa, 40kDa, 70kDa, 150kDa, 250kDa) fluorescein-dextran dyes were chosen to investigate each dextran permeability to gelatin capsule core. Average gelatin capsule permeability was evaluated by capsule core and background fluorescence intensity mean value ratio. Experimental results of gelatin-based capsule permeability of various molecular weight fluorescein-dextran dyes are shown in Fig. 1.

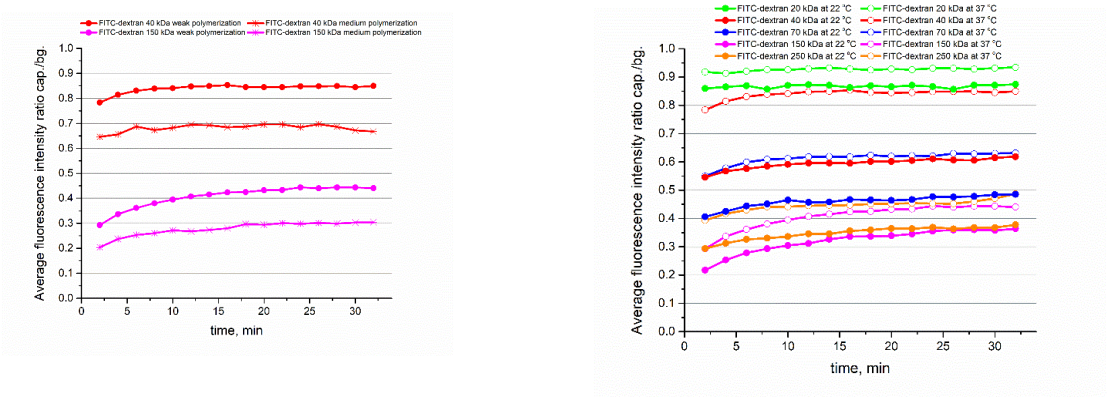

Figure 1. Gelatin-based capsule permeability dependence using different molecular weight FITC-dextran dyes: with weak and medium polymerization process (left side); with cross-linking temperatures at 22 °C and 37 °C (right side).

Results indicate that hydrogel capsule permeability can be tuned depending on enzyme concentrations during cross-linking process and by enzymatic polymerization temperature alterations. Thus, the ability to control hydrogel capsule shell permeability can open the gates for further applications in the fields of microbiology, life sciences, and chemical sciences.

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A.38. EVALUATION OF PARP GENE FAMILY EXPRESSION IN TUMOR AND NORMAL RECTAL TISSUE SAMPLES

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Colorectal cancer is a prevalent malignancy, ranking as the third most frequently diagnosed cancer and the second leading cause of cancer-related deaths globally. Rectal cancers account for approximately 38% of all colorectal cancer cases [1]. The early-stage disease can be treated with radiotherapy or surgical removal of the tumor, sometimes with preoperative treatment. However, in advanced stages, neoadjuvant chemoradiotherapy (CRT) followed by surgery is usually required [2]. CRT aims to shrink the tumor before surgery by inducing DNA damage in cancer cells, leading to their death [3]. Despite advances in CRT, issues such as resistance to therapy, tumor heterogeneity, and damage to adjacent normal tissues remain major challenges that limit its effectiveness. Therefore, current research is focused on identifying molecular targets within cancer cells to achieve more effective treatment.

The poly (ADP-ribose) polymerase (PARP) family, comprising 17 members, represents a potential therapeutic target for the treatment of cancer. These proteins catalyze the addition of ADP-ribose units to target molecules such as proteins, DNA, and RNA. Overall, PARPs are involved in a variety of cellular processes, including DNA damage repair, antiviral response, transcriptional regulation, and chromatin organization [4]. PARP1, which plays a critical role in homologous recombination repair, is currently targeted by several inhibitors in the treatment of various cancers with deficient homologous recombination. This leads to the accumulation of DNA damage in cancer cells, ultimately causing their death. [3]. Further research may reveal whether other members of the PARP family are potential targets for cancer therapy.

This study aimed to evaluate the expression levels of *PARP9, PARP12, PARP13*, and *PARP14* in tumor and normal rectal tissue samples from patients before and after chemoradiotherapy. A total of 67 patients' tissue samples were analyzed. First, samples of both potential tumor tissue and adjacent normal tissue were collected during a biopsy. After the presence of rectal cancer was confirmed, patients underwent 5 weeks of CRT. Subsequently, additional samples of the tumor and adjacent normal rectal tissue were collected during surgery 8–12 weeks after completion of CRT. Total RNA was extracted from the samples and analyzed for integrity by capillary electrophoresis. RT-qPCR was performed using *GADPH* as a reference for expression normalization. Finally, statistical analysis was performed comparing *PARP* expression levels in tumor and normal tissue samples before and after CRT using ΔCt and ΔΔCt values.

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A.39. EFFECT OF SILYMARIN FLAVONOLIGNANS AND THEIR DERIVATIVES ONGLIOBLASTOMA, PANCREATIC AND BREAST CANCER CELL LINES

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Despite broader and more specialized treatment options and great concern in research, cancer remains the leading cause of death worldwide. In 2020, there were 19.3 million new cases and 10 million deaths [1]. One of the treatment options is chemotherapy. Silymarin is a naturally occurring polyphenolic extract from the thistle *Silybum marianum* (L.). It is known for its antioxidant, anti–inflammatory, and antiviral properties, and also antiproliferative activity [2,3]. Furthermore, silymarin does not show cytotoxic effects on normal cells [4]. For this reason, it is possible, that newly synthesized silymarin flavonolignan derivatives could have similar effects not only in 2D but also in 3D models. The aim of our research was to evaluate the activity of silymarin flavonolignans and their new derivatives in both 2D and 3D cancer cell models.

Three types of cancer cell lines were used as model systems: glioblastoma cell line U–87, pancreatic cancer cell line Panc–1 and triple–negative breast cancer cell line MDA–MB–231. The MTT method was used to determine the compound effect on cell viability and the half-maximal effective concentration (*EC50*) was calculated. The most active compounds were tested in the clonogenic assay, which shows the effectiveness of the compound in inhibiting cell colony growth when the cells are seeded at low concentrations [5]. Cancer cell spheroids (3D cultures) were formed using the magnetic 3D Bioprinting method. The compound activity on the growth of the spheroids was evaluated by taking photos of spheroids every other day and analyzing them by ImageJ software.

Of the tested compounds, 2,3–dehydrosilybin A (3), anhydrodehydrosilychristin (5), 8–phenylluteolin (6), 8–(4–F–phenyl)luteolin (7), and 7–benzylsilybin (8) were the most active against all tested cell lines. Moreover, these compounds were 1.1 to 7.7 times more selective against cancer cells than against fibroblasts. Therefore, these five compounds were selected for further experiments. The formation of Panc–1 cell colonies was inhibited by compound 7 at 5 µM. Compound 8 at 10 µM reduced the capability to form the MDA–MB–231 cell colonies. In the 3D model, the most compact spheroids were formed from U–87 cells. Compounds 5, 6 and 7 increased the area of spheroids compared to the control group after 10 days of incubation. The growth of Panc–1 spheroids was inhibited by compounds 5, 6 and 7 at 20 µM. Compounds 3 and 5 increased the spheroid area after 10 days of incubation, while compounds 6, 7 and 8 inhibited the growth of MDA–MB–231 cell spheroids. This can be explained by different mechanisms of action of these compounds but needs further investigation.

In conclusion, silymarin flavonolignans and their derivatives show anticancer activity on glioblastoma, pancreatic, and breast cancer cells. Furthermore, they inhibit not only cell viability in 2D models, but also inhibit spheroid growth.

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A.40. EXPLORING THE INTERACTION BETWEEN TRIPLE-NEGATIVE BREAST CANCER CELL LINE MDA-MB-231 SUBLINES IN 2D AND 3D MODELS

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Background. The tumor microenvironment (TME) consists of cancerous cells, also immune and endothelial cells, fibroblasts, extracellular matrix, and other cells. Numerous studies underlined the contribution of cell–cell interaction for tumor progression and resistance to chemotherapy [1]. Cell–cell interaction studies show that cancer cell interaction with fibroblasts due to secreted fibroblast factors promotes cell growth, survival and stimulate the migration of malignant cells [2]. Moreover, combining two different breast cancer cell lines (MDA-MB-231 and MCF-7) resultsin the cell growth rate increase [3]. Under certain conditions, cancer cell interaction between immune cells can induce tumor-suppressive or tumor-promoting effect [4]. Nevertheless, there is limited knowledge of how the cancer cells interact with each other, so we aimed to explore the impact of the interaction between phenotypically diverse sublines isolated from the MDA-MB-231 cell line on their response to doxorubicin and paclitaxel treatment.

Methods. Three distinct MDA-MB-231 sublines were isolated and characterized. Our selection of the MDA-MB-231 sublines was based on their characteristics: the F5 subline corresponds to cancer stem cell phenotype, the D8 – faster-migrating, and the H2 subline exhibits DOX/PTX-resistant phenotype. We divided the MDA-MB-231 sublines into three groups: 1) cancer cells combined with human fibroblasts (HF), 2) cancer cells with parental cell line and HF, and 3) combinations between cancer cells and HF, respectively. Cell interaction research on chemosensitivity to doxorubicin (DOX) and paclitaxel (PTX) was performed in 2D and 3D cell cultures. Cell viability was assessed using the MTT assay. The spheroids were formed by the magnetic 3D bioprinting method. The effects of DOX and PTX on 3D cell cultures were determined by measuring the size change of spheroids and quantifying the results using *ImageJ* software.

Results. Combining the MDA-MB-231 sublines with HF, parental cell line, or between each other resulted in decreased resistance to DOX. In cell subline combinations between each other, resistance to PTX increased, while viability was from 18% to 34% higher compared to the viability of the separate sublines. When including H2 and D8 or F5 sublines, the formed spheroids were smaller than in other groups. The spheroids, formed from the H2 subline, were found to be more sensitive to DOX or PTX compared to the control, with a decrease in size from 15% to 29%. Cell combinations of HF and D8/F5 in monolayer were 2 times more resistant to PTX than DOX, while the same combinations in spheroids were 1-2 times more resistant to both drugs. In 2D cell cultures, combinations of H2 with HF, or H2 with HF and parental cell line were twice more resistant to DOX and PTX. Meanwhile, in spheroids, combinations including HF, H2, and D8 or F5 sublines displayed a 1-3 times greater sensitivity to DOX and PTX compared to the control.

Conclusion. The interaction of F5 and D8 increased resistance to PTX in monolayer and resistance to DOX and PTX in spheroids. The H2 interaction with other phenotypes decreased resistance to DOX in monolayer and in spheroids.

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A.41. EFFECTS OF ADDITIONAL EXERCISES COMBINED WITH CARDIAC PHYSICAL TRAINING PROGRAM ON STRENGTH PARAMETERS IN PEOPLE AFTER OPEN HEART SURGERY WITH FRAILTY

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Introduction. World population is ageing and people over 65 years old will be increasing about 1,5 billion in 2050 causing sarcopenia and frailty [1]. Frailty can be defined as a phenomenon of increased vulnerability to stressors due to decreased physiological reserves on older patients and thus leads to poor clinical outcomes after cardiovascular diseases [2]. One of the most important impacts on mobility arises from changes in muscle strength which is decreasing to 2,5 % per year after 60 years old [3].

Purpose. To evaluate and compare the effects of additional exercises combined with cardiac physical training program on strength parameters in people after open heart surgery with frailty.

Methods. All patients over 65, after open heart surgery and with Frailty (Edmonton frail scale ≥4) were invited to study. Patients who agreed to participate in study were randomly assigned in 2 groups: control (CG) and intervention group (IG). Both groups were assessed 2 times: on admission to rehabilitation (T1) and after the rehabilitation (T2). Grip strength was measured using a hand dynamometer and leg press strength using one repetition maximum (1RM) with HUR. All patients completed a conventional 20-day cardiac rehabilitation program which included aerobic cycle ergometer training (30 minutes 5-6 days/week, 30-60% watt maximum), stretching exercises (15 minutes after each session with ergometer), breathing exercises (15 minutes 7 days/week). IG underwent program as CG and additional individualized program for frailty which included balance (with unstable balance platforms) and resistance exercises (using elastic resistance bands and weights) (3 days/week 45 minutes). Mann – Whitney and Wilcoxon tests were used to access a significant difference ($p<0,05$), the results were expressed as medians with min and max ranges.

Results. Results showed that age, gender and frailty did not differ significantly between the groups. However, statistically significant difference was observed during grip strength T1 testing therefore we could not identify the impact of additional training on grip strength between groups. In CG we had 73,3 % of males and this could be the reason for the data separation (statistical analysis showed strong correlation between grip strength and gender). After T2 1RM leg press results showed that there are statistically changes in both and between groups. CG showed 8% and IG 41 % improvement in 1RM leg press test. After rehabilitation leg press strength in IG was 58% bigger than in CG.

Conclusion. We could not measure the impact of additional training on grip strength therefore more detailed studies should be conducted to measure the influence of gender. Results showed that additional exercises combined with cardiac physical training program appeared to be an effective way to improve lower legs (leg press) strength in people after open heart surgery with frailty.

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A.42. SYNTHESIS AND BIOLOGICAL EVALUATION OF 2-(1*H*-INDOL-3-YL)-5-(SUBSTITUTED)-1,3,4-OXADIAZOLES IN *CAENORHABDITIS ELEGANS*

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Development of novel pharmaceutically active ingredients is time consuming process. A number of novel compounds has to be synthesized, screened, then modified and screened again. Therefore, choice of a proper synthesis direction and optimal biotesting procedure is at the core of successful research.

Caenorhabditis elegans is a free-living soil nematode that has been successfully used in many research fields for decades. Due to the worms' short life cycle, small size and the possibility to be cultivated in liquid-based media, *Caenorhabditis elegans* enables whole-organism *in vivo* high-throughput screenings [1]. Significant portion of its genes have homologs in humans, making it a useful model organism to study various aspects of human diseases [2]. On the other hand, it shares many anatomical, physiological and molecular traits with parasitic nematodes, making it applicable for the search for new anthelmintic drugs.

In our previous research we presented synthesis of 2-((1*H*-indol-3-yl)methyl)-5-(alkylthio)-1,3,4-oxadiazoles [3]. Three of them protected Friedreich ataxia-fibroblasts against glutathione depletion induced by γ-glutamylcysteine synthetase inhibitor buthionine sulfoximine (BSO). Moreover, two active compounds increased survival of *Caenorhabditis elegans* exposed to juglone-induced oxidative stress. To further elucidate protective potential of 1,3,4-oxadiazole-indole hybrids in this project we ought to synthesize novel 2-(1*H*-indol-3-yl)-5-(substituted)-1,3,4-oxadiazoles. In this way we could further define the oxadiazole- indole substitution pattern and its efficiency for protective activity against oxidative stress. At the same time the influence of methylene bridge between indole and oxadiazole heterocycles for biological activity of this class of compounds can be defined.

The two-step synthesis was carried out starting from 3-metoxycarbamoylindole seeking to prepare a series of compounds. In the first step 3-methoxycarbamoylindole was treated with hydrazine hydrate to get an intermediate hydrazide. The 1,3,4-oxadiazole structural unit is formed either by treating crude hydrazide with alkyl isothiocyanate followed by cyclization or with CS_2 followed by cyclization and S-alkylation. These two synthesis directions afforded either *N*-alkyl or *S*-alkyl substituents in oxadiazole ring. The structures of obtained compounds were elucidated and confirmed by thorough NMR, IR and mass spectrometry.

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A.43. NOVEL *N*–HETEROCYCLE–FUSED TETRAHYDRO–1,4–DIAZEPINONES: SYNTHESIS AND CHARACTERISATION

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Seven-membered heterocyclic ring fragment can be found in various bioactive compounds. It is known that heterocycles with two 1,4–distanced heteroatoms exhibit various biological activities [1]. Among them, 1,4–diazepine heterocycles with two 1,4–distanced nitrogen atoms possess a variety of biological properties such as antiviral [2], antitumor [3] and antimalarial [4] activity. For example, a 5,6,7,8-tetrahydro-4*H*- pyrazolo[1,5–*a*][1,4]diazepine–2–carboxamide derivative inhibits the respiratory syncytial virus (RSV) polymerase complex [2], whereas 8,9–dihydro–7*H*–pyrimido[4,5–*b*][1,4]diazepine derivative inhibits the growth of the cell line IGROV1 responsible for ovarian cancer [3]. Another example includes 1,6,7,8– tetrahydropyrazolo[3,4–*b*][1,4]diazepine derivative which inhibits the growth of *P. falciparum* and has the potential for treating malaria [4].

In this work, we reported the synthesis of novel pyrazole and indole–fused tetrahydro–1,4–diazepinones. For the optimization of the reaction conditions, reactivity of easily accessible corresponding 1*H*–pyrazole–5(3)–carboxylates towards alkylation with epichlorohydrin was investigated. *N*–Alkylation of pyrazole derivatives was carried out using NaH as a base in DMF. The reaction resulted in the formation of a mixture of *N*–substituted regioisomers 1*H*–pyrazole–5–carboxylates and 1*H*–pyrazole–3–carboxylates. Obtained 1–(oxiran–2–ylmethyl)–1*H*–pyrazole–5–carboxylates were further treated with ammonia resulting in oxirane ring–opening and direct cyclisation affording target tetrahydro–4*H*–pyrazolo[1,5–*a*][1,4]diazepin–4–ones. 1–(Oxiran–2–ylmethyl)–1*H*–pyrazole–3–carboxylates were also treated with ammonia which resulted only in oxirane ring-opening without subsequent cyclisation. Obtained cyclisation products were further functionalized by treatment with *N*–chloro–, bromo– and iodosuccinimides to obtain 2(3)–halogenated tetrahydro–4*H*–pyrazolo[1,5–*a*][1,4]diazepin–4–ones.

For the expansion of the study, an analogous two-step synthesis approach was applied to obtain tetrahydro[1,4]diazepino[1,2–*a*]indol–1–one derivatives from corresponding indole–2–carboxylates. To increase the yield of ethyl 1–(oxiran–2–ylmethyl)–1*H*–indole–2–carboxylates, *N*–alkylation of indole–2–carboxylates with 2–(chloromethyl)oxirane was carried out using KOH as a base in DMF. Obtained ethyl 1–(oxiran–2–ylmethyl)–1*H*–indole–2–carboxylates were further treated with ammonia affording target tetrahydro[1,4]diazepino[1,2–*a*]indol–1–ones [5]. Obtained 4–hydroxy–2,3,4,5–tetrahydro–1*H*–[1,4]diazepino[1,2–*a*]indol–1–one was further alkylated with methyl iodide. It was determined that *O*–alkylation only occurs when the compound is already *N*–alkylated.

The structures of novel synthesized heterocyclic compounds were confirmed by ^{1}H, ^{13}C, and ^{15}N-NMR spectroscopy.

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A.44. CHANGES IN OFFSPRING GUT MICROBIOTA AND BEHAVIOR LINKED WITH MATERNAL HIGH-FAT DIET

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Aims: A typical Western diet is excessively fatty, leading to a rapid increase in obesity in human population, including the women of reproductive age. There is growing evidence that maternal high-fat diet (mHFD) increases the risk of neurodevelopmental disorders in the offspring [1, 2, 3]. mHFD affects offspring neurodevelopment through various pathways, including changes in maternal metabolic status, increased chronic inflammation, or alterations in maternal and offspring gut microbiota [4, 5, 6]. Our aim was to evaluate whether mHFD increases maternal chronic inflammation and alters maternal metabolic status and gut microbiota, leading to the changes of offspring gut microbiota and abnormal offspring behavior.

Methods: Female C57Bl/6J mice were fed a control diet (CD, 10% fat) or high-fat diet (HFD, 60%) from weaning to lactation. Before mating, the metabolic status of the dams was evaluated by the body mass as well as glucose and insulin tolerance tests. The offspring were weaned to CD. After lactation, the concentration of maternal plasma insulin and proinflammatory cytokines were measured by ELISA tests. We investigated the behavioral phenotype of the offspring in three-chamber sociability, reciprocal social interaction, open field, marble burying, novel object recognition, and Barnes maze tests. To investigate gut microbiota, dam and offspring cecums were collected. Bacterial communities were determined by 16S rRNA amplicon sequencing.

Results: We determined that the consumption of HFD caused metabolic dysfunction but did not increase inflammation in the dams. mHFD changed the relative abundance of different gut bacteria genera in both dams and offspring. Decreased sociability was determined in both mHFD males and females although only males showed increased activity and only females showed decreased repetitive behavior.

Conclusions: Our findings demonstrated, that mHFD altered the composition of the offspring gut microbiota which may contribute to abnormal behavior in a sex-specific manner.

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A.45. SYNTHESIS OF NEW PHENYLPYRAZOLE-BASED DERIVATIVES AND THEIR BIOLOGICAL EVALUATION

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Pyrazole compounds are major in the fields of organic and medicinal chemistry since they exhibit numerous properties such as antimicrobial, anticancer, anti-inflammatory, antioxidant, antitubercular, etc [1]. Both pyrano[4,3-*c*]isoxazole and chalcones and flavanones are typified by biological properties. These compounds are used in agriculture and in the pharmaceuticals industry as antibacterial, antiviral, and antifungal agents [2, 3].

Firstly, the 1-phenyl-1*H*-pyrazol-3-ol is alkylated and then the formylation reaction is performed by the Vilsmeier-Haack reaction. The obtained different aldehydes were used in the synthesis of pyrano[4,3-*c*]isoxazole, chalcone and flavanone derivatives.

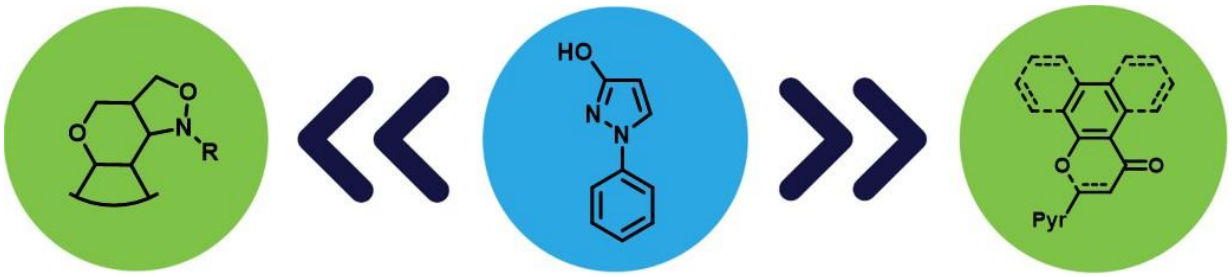

Figure 1. General synthetic approaches of 1-phenyl-1*H*-pyrazol-3-ol

Biological studies of some synthesized compounds have been studied. Antibacterial study and DPPH inhibition were estimated. The DPPH antioxidant method showed that observed compounds did not transcend ascorbic acid. 1-(1-Hydroxynaphthalen-2-yl)-3-(1-phenyl-3-methoxy-1*H*-pyrazol-4-yl)prop-2-en-1-one performed best - 10.52%. The antibacterial activity was tested against *Escherichia coli*, *Rhizobium radiobacter*, *Bacillus subtilis* and *Xanthomonas campestris*. 3-(Allyloxy)-1-phenyl-1*H*-pyrazole-4-carbaldehyde was found to inhibit *Escherichia coli*, *Rhizobium radiobacter*, and *Bacillus subtilis* bacteria growth at various concentrations. Several other compounds inhibited the growth of *Rhizobium radiobacter* best: 1-phenyl-3- methoxy-1*H* -pyrazole-4-carbaldehyde, 3-(1-phenyl-3-methoxy-1*H*-pyrazol-4-yl)-2,3-dihydro-1*H*-benzo[*f*]chromen-1-one and 1-(2-hydroxy-4-nitrophenyl) -3-(1-phenyl-3-methoxy-1*H*-pyrazole-4-yl)prop-2-en-1-one. *Xanthomonas campestris* was best inhibited by 1-phenyl-3-methoxy-1*H*-pyrazole-4- carbaldehyde at a concentration of 1 mg/ml.

The obtained chalcone and flavanone derivatives were not characterized by strong activity, but it is worth investigating other biological properties, as well as conducting research with pyrano[4,3-*c*]isoxazole compounds.

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A.46. HUMAN MESENCHYMAL STEM CELLS DERIVED FROM VARIOUS SOURCES AS NANOPARTICLE DELIVERY VECTORS

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Lanthanide-doped multilayered upconverting nanoparticles (UCNPs) and their complex with photodrug chlorin e_6 (UCNPs-Ce_6) could improve cancer diagnostics and treatment. These nanoparticles can be excited with near-infrared light (λ_{ex} = 980 nm), that falls in the so-called biological imaging windows (750–1300 nm) where light can reach deeper tissues [1]. However, the nanoparticles alone do not provide sufficient selectivity for tumours and tend to accumulate non-specifically in certain organs. It has been shown that less than 1 % of intravenously injected nanoparticles reach the tumour site [2].

Mesenchymal stem cells (MSCs) have been proposed as an alternative way of delivering nanoparticles to the tumour. MSCs are multipotent stem cells that have attracted significant interest due to their regenerative and immunomodulatory properties, their anti-tumour effect and their ability to migrate to thelesion site, including the tumour. Besides, these cells can be easily isolated and are found in almost all tissues in specific niches. One of the main sources of MSCs is bone marrow, but much less is known about MSCs isolated from skin or menstrual blood. Therefore, in order to use MSCs as Trojan horses due to their oncotropic properties, it is necessary to select the most suitable source of MSCs for nanoparticle delivery. This is important because cells from various sources may have different specific properties [3].

The aim of our study was to investigate the differences and similarities between MSCs isolated from skin, bone marrow and menstrual blood in order to use them as cellular multilayered nanoparticle carriers. The intracellular accumulation of UCNPs-Ce_6 in a monolayer of MSCs from skin, bone marrow and menstrual blood was demonstrated using scanning confocal microscopy. Accumulation study has shown that nanoparticles accumulate similarly in all MSCs from various sources and are concentrated around the nucleus after 24h of incubation. Next, a lactate dehydrogenase cytotoxicity assay was used to evaluate the dark toxicity of UCNPs and UCNPs-Ce_6. We showed that these nanoparticles are non-toxic after 24h of incubation in the dark. To examine the ability of MSCs to migrate towards cancer cells, a three-dimensional model system was chosen, i. e. breast cancer cell spheroids were formed. The cellular spheroids represent a simplified tumour microenvironment and bring the study closer to natural biological structures. MSCs from different sources migrated towards cancer cell spheroids with and without nanoparticles.

Overall, MSCs from different sources show great potential as carriers of multilayered nanoparticles towards cancer cells.

Acknowledgment
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A.47. STIMULATION OF CHONDROGENIC DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELLS USING POLYPYRROLE NANOPARTICLES IN VITRO

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Articular cartilage is an avascular tissue, which contains primary cells – chondrocytes, responsible for tissue integrity. Once damaged, cartilage is challenging to repair and regenerate itself, which often results in the development of osteoarthritis (OA) [1]. One of the ways to restore and protect cartilage tissue from degradation is cell therapy using human mesenchymal stem cells (MSCs). Due to MSC ability to differentiate into cartilaginous tissue, these cells have become an excellent tool for *in vitro* regenerative technologies [2]. It is known that electrical stimulation (ES) activates cells, depolarizes the plasma membrane, and improves the flow of calcium (Ca^{2+}) ions important for chondrogenic differentiation, which stimulates the production of further cartilage tissue proteins [3]. One of the most popular and widely used electrically conductive polymers in biomedicine is polypyrrole (Ppy). This polymer can be obtained by electrochemical and chemical synthesis in the form of layers or micro- and nanoparticles at neutral pH of aqueous solutions. It has excellent biocompatibility, flexibility, and stability. Ppy is a potential tool in the delivery of drugs or target proteins. Ppy structures are known to improve the rate of charge transfer across the cell membrane and stimulate the proliferation and differentiation of stem cells [4]. According to our knowledge, chondrogenic differentiation of MSCs using Ppy nanoparticles has never been studied before. Thus, the aim of this study was to evaluate the chondrogenic potential of human bone marrow MSCs (BMMSCs) after stimulation with Ppy nanoparticles and compare them to cartilage-derived chondrocytes. In this study, we tested two mostly used Ppy nanoparticles – Ppy and Ppy combined with golden nanoparticles (Ppy/Au) for BMMSCs and chondrocyte functions, including proliferation, viability, and chondrogenic differentiation, without the use of ES. For proliferation and viability tests, we used Alamar blue fluorescent dye and Live/Dead kit, which revealed that both, BMMSCs and chondrocytes remained viable after cultivation with Ppy and Ppy/Au for 21 days. For chondrogenic differentiation we stimulated cells for 21 days in 2D and 3D models, using a specific chondrogenic medium and Ppy, Ppy/Au nanoparticles. After differentiation ended, we measured cartilage oligomeric matrix protein (COMP) (ELISA) from cell supernatants, analyzed chondrogenic gene expression (RT-qPCR) and evaluated cartilage extracellular matrix (ECM) formation by histology/immunohistochemistry. The results demonstrated significantly higher amounts of COMP in BMMSCs samples stimulated with Ppy and Ppy/Au. Also, gene expression revealed upregulated chondrogenic genes (SOX9, ACAN, COL2A1) in samples stimulated with Ppy and Ppy/Au, as compared to the control. Histological analysis indicated similar tendencies of ECM production, after staining histological sections with toluidine blue and safranin O in Ppy and Ppy/Au samples, as compared to controls. In conclusion, Ppy and Ppy/Au nanoparticles are promising for application in the chondrogenic differentiation model and can be considered for further technological approaches using ES.

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A.48. COMPARISON OF MYOCARDIAL INFARCTION AND PATHOANATOMICAL FINDINGS OF THE HEART BETWEEN DEATHS RESULTING FROM ISCHEMIC HEART DISEASE AND OTHER CAUSES

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Introduction. Myocardial infarction (MI) is caused by decreased or complete cessation of blood flow to the myocardium. MI may go undetected, or it could lead to sudden death. Further research is needed on whether cardiac characteristics differ between deaths of cardiac-related causes and non-cardiac causes. According to the literature, the mean left ventricular wall thickness could be thicker in acute MI cases than in non-cardiac deaths and have a statistically significant difference in the degree of obstruction of the coronary arteries between the groups [1]. Another research, claims that cardiac-related cases have a higher prevalence of cardiomegaly, left ventricular hypertrophy, and severe coronary artery stenosis than non-cardiac trauma-related causes of death [2]. Our study aims to investigate whether MI scar area differs between different causes of death, how it depends on MI localization and whether general cardiac characteristics differ between genders.

Methods. We included data from 323 autopsy cases with existing MI scars that were investigated at the State Forensic Medicine Service from 2013 to 2022. We divided the entire cohort into two groups by the cause of death to ischemic heart disease-related causes n=211, and other causes n=112. General cardiac characteristics from autopsy protocols were retrospectively collected.

Results. The mean age of the cohort was 66.78 years (range 32-97). The gender ratio was close to 3:1 with male predominance (238 men and 85 women). Men died significantly younger than women ($p<0.001$). There were statistically significant differences in heart characteristics between men and women: the mean heart mass, the mean of the left and right ventricular inflow and outflow tracts, and the average weight of the left and right ventricle in the men's group were greater (all with $p<0.05$). The two most common localizations of MI were found to be in the left ventricle - the anterior and lateral walls (37% and 22%, respectively). The size of the myocardial infarction scar was found to have statistically significant differences comparing different MI localizations (lateral vs anterior wall $p<0.001$, anterolateral vs anterior wall $p<0.001$, posterior vs anterior wall $p=0.014$). When dividing the study cohort by the cause of death, patients who died from ischemic heart disease were significantly younger than those who died from other causes ($p=0.011$). When the cause of death was heart pathology, the average area of myocardial infarction was higher, but it did not show a statistically significant difference.

Conclusions. We conclude that the most common localizations of MI scars were the anterior and the lateral wall of the left ventricle and the size of the MI scar has statistically significant differences comparing different MI localizations. The mean heart mass and general cardiac measurements were greater in men than in women. Patients who died from ischemic heart disease were significantly younger than those who died from other causes and the same cases with heart pathology had a higher average area of MI scar, but it did not show a statistically significant difference.

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A.49. BIBLIOMETRIC ANALYSIS OF RESEARCH TRENDS ON ROLE OF MYOKINES IN THE CONTEXT OF PHYSICAL PERFORMANCE

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The relationship between physical performance and myokines has attracted an increasing number of researchers in recent years. Myokines are cytokines that are produced and released by skeletal muscle cells in response to muscular contractions [1]. Myokines are possibly essential components of whole-body homeostasis and may directly impact some variables associated with physical performance (especially in elite athletes) [2]. However, the relationship among myokines and physical performance were still not fully understood. Our study aimed to identify the current research hotspot, status and trend in this field by using bibliometric analysis.

Articles published in the PubMed database in the period 2013-2023 were filtered using relevant keywords 'physical performance', 'elite athletes' and 'myokines'. The bibliometric analysis was done using VOSviewer tool (version 1.6.19). Two standard weight parameters were applied - "link attribute" and "total link strength attribute" (TLS) to find out the most common keywords and its links, trending themes, organizations and authors who research these topics the most.

In total 86,213 scientific articles on the topic of 'physical performance' were published between 2013 and 2023. Most of them were descriptive studies and 7,148 (8,3%) clinical trials. The 'physical performance' had 883 occurences (TLS=8,810, associated with 'aging', 'muscle strength', 'athletes'). The 'myokines' with a total link strength (TLS) of 2,593 had a link to 'cytokines' (TLS=1,878, and associated with 'interleukin-6', 'muscle proteins'), 'exercise' (TLS=4,539, associated with 'myostatin', 'c-reactive protein', 'fibronectins', 'biomarkers') and 'muscle, skeletal' (TLS=6,300, associated with 'irisin', 'interleukin-15', 'fndc5', 'gene expression'). Overall, 1,734 publications with keyword 'myokines' were identified between 2013 and 2023. Globally, 77 publications of authors who studied the connection between 'physical performance' and 'myokines' were published in the last decade with TLS of 4,027. The 10 of all studies were clinical trials (13%). J. Jensen was the most productive author in this field (number of publications = 7) and was cited 52 times (TLS=76).

In the past 10 years, the relationship between physical performance and myokines has gained attention and number of publications grows exponentially (Fig. 1). Our bibliometric analysis showed that the study in this field mainly focused on physical performance and the discovery of valuable molecular biomarkers has become a focal point of current research. Myokines, such as interleukin-6, interleukin-15, irisin, myostatin, may act as candidate biomarkers because of their gene expression in response to muscle contraction. The latest studies of genetics and genomics has become "big data science" and provide new insights into biological processes or systems at molecular through physiological levels [3].

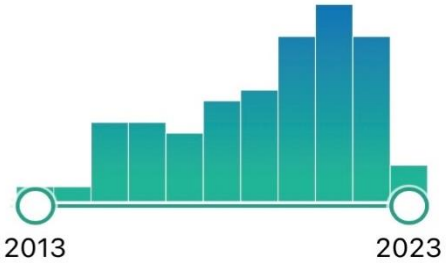

Figure 1. Increasing number of publications with keywords 'physical performance' and 'myokines' in PubMed.

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A.50. MOLECULAR AND COGNITIVE ASPECTS OF COVID-19 IMPACT ONHUMAN BRAIN: INTERMEDIATE RESULTS OF THE PROSPECTIVE STUDY

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It was thought at first that COVID-19 is exclusively a respiratory disease, however ever-growing clinical data now suggests, that the virus also affect central nervous system (CNS). SARS-CoV-2 is capable of infecting neurons, brain endothelial cells and neuroglia directly. Indirect injury can be caused by cytokine storm, autoimmune response, or hypoxia. Inflammatory reaction disrupts function of endothelial cells of brain vessels thus increasing paracellular permeability and allowing toxic components of plasma to affect brain [1]. This and other pathophysiological mechanisms of COVID-19 can lead to neurocognitive impairment [2]. The aim of our study was to determine what changes, regarding cognitive functions and cytokines in blood plasma, does COVID-19 cause. Therefore, we evaluated cognitive functions and measured blood plasma markers between three patient groups: healthy, COVID-19 and recovered.

Cognitive functions were evaluated using Mini-Mental State Examination, 2nd Edition™, Lithuanian language adaptation. We analyzed a total of 64 individuals: 21 in healthy group, 26 COVID-19 and 17 recovered groups. Median of age was 45 (36,25 – 58), youngest individual was 22, oldest – 65. 51.6 percent of patients were men. Impaired cognitive function (i. e. MMSE score < 24 points) was found in two individuals: mild cognitive impairment in one healthy individual (MMSE score of 23 points) and marked cognitive impairment in COVID-19 patient (MMSE score of 15 points). Statistically significant differences were found in median sum of score (29 (29 - 30) vs. 30 (30 - 30) points between healthy and recovered groups, $p=0.011$; and 28 (27 - 30) vs. 30 (30 - 30) points between COVID-19 and recovered groups, $p<0.001$), memory subtest score (2 (1 - 3) vs. 3 (3 - 3) points between COVID-19 and recovered groups, $p=0.005$) and attention and calculation subtest score (5 (4 - 5) vs. 5 (5 - 5) points between COVID-19 and recovered groups, $p=0.007$).

We also determined concentrations of 45 cytokines in blood plasma of 40 individuals (9 in healthy group, 18 in COVID-19 and 13 in recovered) by using ProcartaPlex™ Human Cytokine/Chemokine/Growth Factor Convenience Panel 1 45-Plex. Statistically significant differences between tested groups were found for 3 cytokines (IP-10, HGF, SCF). Concentrations of IP-10 were statistically higher in blood plasma of COVID-19 patients compared to recovered subjects ($p<0.05$). Statistically significantly higher levels of HGF were detected in the plasma of COVID-19 patients compared to healthy ($p<0.05$) and recovered ($p<0.001$) subjects. Concentrations of SCF were statistically lower in COVID-19 patients compared to healthy individuals ($p<0.05$). Thus, our results show that COVID-19 significantly increases IP-10 and HGF levels and decreases SCF concentrations in the plasma of COVID-19 patients compared to healthy or/and recovered individuals.

As a summary, our study shows that there can be found statistically important differences regarding impact of COVID-19 in cognitive functions and molecular markers between healthy individuals, COVID-19 patients and recovered patients. However, further research is needed to determine links, causes and interdependence of these changes.

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A.51. ENERGY EXPENDITURE IN CRITICAL CARE PATIENTS ON CONTINUOUS RENAL REPLACEMENT THERAPY: CORRELATION BETWEEN THE PREDICTIVE EQUATIONSAND INDIRECT CALORIMETRY

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Introduction: Acute kidney injury patients on continuous renal replacement therapy (CRRT) are subjected to alterations in metabolism and variable energy expenditure (EE). Recent trials have validated indirect calorimetry as an appropriate tool for EE measurement for patients on CRRT. (1) Therefore, the aim of this research is to measure the EE and compare it to predicted EE in patients on CRRT.

Methods: This was a prospective observational study of critical care patients undergoing CRRT. The patients were enrolled in the study upon the initiation of CRRT. Demographic and descriptive variables were collected to describe the population. Predicted EE was calculated using the Harris-Benedict equation and by employing a 25kcal/kg/day value, representative of average EE in the critical care. The actual EE was measured using the indirect calorimetry sensor (E-sCOVX) integrated in the mechanical ventilation machine (GE Healthcare CARESCAPE). The measurements were compared in linear regression, statistical significance was set at p value 0.05.

Results: The study included 60 critical care patients, mean APACHE II score of 22.98±7.87, most of them female n=34 (59.7%), with therapeutic profile of the disease n=32 (53.3%). The mean Harris-Benedict predicted EE was 1630.43 ±313.4 kcal/d, the mean 25kcal/kg EE value was 2214.83 ±563.90 kcal/d and the mean with indirect calorimetry measured EE was 1560 ±517 kcal/d, providing a statistically significant difference $p<0.001$. However, a clinical difference was observed only between the 25kcal/kg EE and indirect calorimetry EE. A moderate linear correlation was present between the variables ($R=0.584$, $Beta=0.854$, $P<0.001$ for Harris-Benedict equation and $R=0.483$, $Beta=0.483$, $p=0.017$ for 25kcal/kg EE).

Conclusions: The EE in critical settings is variable and difficult to predict. Only moderate strength correlation between actual and predicted EE is present when employing predictive equations, with some advantage for the Harris-Benedict equation. Further research is needed to determine the cause of the high variability in EE in the critical care setting.

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A.52. VISUALISATION OF COLLAGEN IV IN HYALINE CARTILAGE WITH QUANTUM DOTS

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Cartilage is a unique connective tissue as it has no innervation and blood/lymphatic supply, consists just of one type of cells and has very limited regenerative capacity. Three zones of cartilage are visible under the microscope, depending on cells shape[1]. Collagen II is the most abundant protein in extracellular matrix of the cartilage, which provides biomechanical properties of the cartilage, however a vast majority of minor collagens also comprises pericellular and extracellular matrix of the cartilage [2,5]. Chondrocytes comprises just 10% of cartilage volume, but they are responsible for matrix biosynthesis. Collagen IV (Coll IV) and collagen VI (Coll VI) comprises pericellular matrix of chondrocytes, and expression of them depends on the stage of the degeneration of the cartilage, in other words, in more advanced stages of osteoarthritis expression of collagen IV diminishes, and expression of collagen VI - vice versa [3].

We have visualized collagen IV and collagen VI by immunohistochemistry and by using streptavidin-binded quantum dots (QD). Visually healthy cartilage was fixed in 10% formalin for 24 hours, sliced in a 4µm thickness, put on the slides, and stained with collagen VI (Fig.1) and collagen VI (Fig. 3) by using horseradish peroxidase (HRP) and quantum dots 655 (red) (Fig. 2) and 565 (green) (Fig.4). Collagen IV appears just near the chondrocytes, as it comprises basal membrane, and collagen VI is in the pericellular matrix, and binds basal membrane to the extracellular matrix. Moreover, collagen VI appears in extracellular matrix in the upper third of the healthy cartilage, while collagen IV does not [5,6].

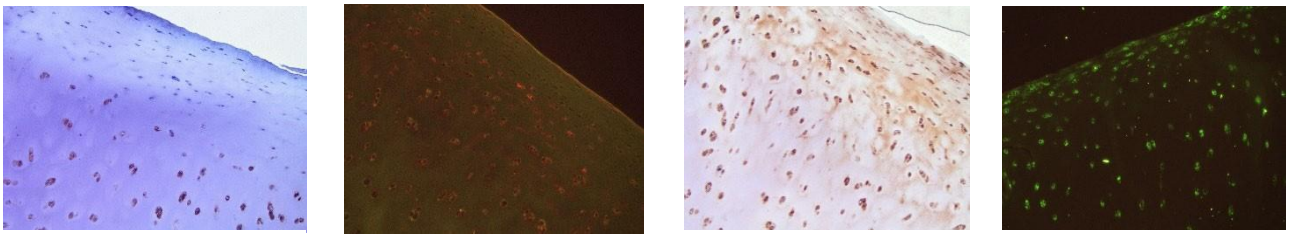

Figure 2. Coll IV+QD655

Different colors of quantum dots give us an opportunity for visualization more than one type of the biomolecule in the same slide, which may be beneficial in diagnosing early changes of the cartilage in osteoarthritis.

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A.53. EXTRACTION OPTIMIZATION OF PHENOLIC MARKERS OF AGRIMONIA EUPATORIA L. USING RESPONSE SURFACE METHODOLOGY

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Recently, with the change in people's lifestyle and perception of a healthy lifestyle, there is an increasing interest in plant-based products, food supplements and herbal medicines. Preparations made from plant raw materials have been used in various countries since time immemorial to improve human health, prevent and treat diseases [1]. The most common biologically active substances in the world are phenolic compounds, which are natural antioxidants that protect human body cells from oxidative stress. Due to the positive biological effects of phenolic compounds, medicinal plant raw materials that could be applied in medical practice are being sought. Discovering new health properties of plant-based foods is an increasingly important area of scientific endeavour.

Agrimonia eupatoria L. is a perennial plant belonging to the Rosaceae family used in traditional medicine for its beneficial effects. Agrimonia extracts are used to treat diarrhea, pneumonia, bleeding disorders, diseases of the liver and urinary system, and inflammation of the oral mucosa [2]. In order to extract as many active phytochemical compounds from plant raw materials as possible, it is very important to optimize the extraction conditions.

Surface response methodology was used to determine the optimal parameters of the extraction process (concentration of aqueous acetone solutions, the ration of the raw material and the solvent, and the extraction exposition time) for the construction of the extraction optimization model. Using the Design-Expert program, a surface response methodology design including 15 experiments was created to help determine the optimal concentration of extraction solvent, the proportion of raw material to solvent and time. The influence of three variables on the amounts of individuals phenolic markers from phytochemical profile was evaluated. According to the combinations indicated by the model the extracts were prepared and HPLC analysis was performed for the phytochemical profile evaluation. Quadratic model equation where constructed for the following responses: Quercitrin – R^2= 0,7081, Adjusted R^2=0,5914, Predicted R^2= 0,3294; Agrimoniin – R^2=0,7668, Adjusted R^2= 0,6735, Predicted R^2 = 0,5017; Catechin – R^2=0,7036, Adjusted R^2 = 0,5851, Predicted R^2 = 0,3066.

The result showed that for the optimal extraction of the Quercitrin, Agrimoniin and Catechin determined conditions where 57% concentration of acetone, the ration of the raw material and the solvent 1:10 and extraction time 30 minutes. Under these proposed conditions the predicted values corresponding experimental values confirming the validity of the experimental model.

After the selected conditions where applied the determined amounts of these compounds where: Quercitrin - 3,445 mg/g, Agrimoniin - 7,807 mg/g, Catechin - 7,455 mg/g. These optimal condition can be used for targeted extraction of Agrimonia eupatoria L. phenolic compounds.

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A.54. MEDICINAL PLANTS ESSENTIAL OILS EFFECT ON ORAL PATOGENS ASPERGILLUS FLAVUS, MICROCOCCUS LUTEUS, LACTOBACILLUS ACIDOPHILUS

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Microorganisms perform various functions in the human body. The oral cavity contains 100 million bacteria per milliliter of saliva. Various types of bacteria, protozoa, fungi, archaea, viruses live in the oral cavity, some of which cause infections related to the oral biofilm and promote the development of infectious diseases such as tooth decay, gingivitis, periodontitis. In addition to the composition and concentration of oral microorganisms, temperature, pH of saliva, oxygen concentration, availability and type of nutrients, immune system, anatomical features of the oral cavity, and dental hygiene are important factors.

The aim of this study was to evaluate the antimicrobial activity of essential oils from medicinal plant *Litsea cubeba* (Lour.) Pers. (the main organic phytocompounds in essential oil are citral, limonene, linalool, geraniol), Rosmarinus officinalis L. (the main organic phytocompounds in essential oil are limonene, linalool), Pinus sylvestris L. (the main organic phytocompounds in essential oil are limonene) against Aspergillus flavus, Micrococcus luteus, Lactobacillus acidophilus. Antimicrobial activity of essential oils was determined by zone of inhibition on the growth microorganisms using the agar well diffusion method. The sample solutions of essential oils were prepared in different concentrations from 1 to 100 percent by diluting with distilled water and Tween 20. Essential oils of *Litsea cubeba* (Lour.) Pers., Rosmarinus officinalis L. were the most effective and show potent inhibitory activity against microorganisms Aspergillus flavus, Micrococcus luteus, Lactobacillus acidophilus with minimum inhibitory concentration of 1 percent with 3-10mm zone of inhibition. Essential oils from Pinus sylvestris L. show about 1-2mm zone of inhibition against microorganisms in concentration 5 percent. Essential oils of *Litsea cubeba* (Lour.) Pers., Rosmarinus officinalis L. have the potential to be developed into agents that can be used as preventative or treatment therapies for oral diseases. The use of essential oils from *Litsea cubeba* (Lour.) Pers. and Rosmarinus officinalis L. could be a great alternative to antibiotics in the treatment of oral diseases since they show low cytotoxicity levels and do not induce resistance in bacterial pathogens. Research regarding the use of medicinal plants on the treatment of oral ailments continues to be an extreme interesting topic [1] mainly due to the extensive variety plants that potentially have antimicrobial properties.

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A.55. MODELING OF PROTEIN-NUCLEIC ACID INTERACTIONS USINGTEMPLATE-BASED AND FREE DOCKING METHODS

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Protein-nucleic acid interactions are essential in many cellular processes, such as transcription, DNA recombination, replication, repair, and modification. It is important to know the structures of the resulting complexes, as they form the basis of our understanding of these processes. Experimental methods such as X- ray crystallography can provide high-resolution structures, but they are expensive and time consuming. Thus, structural knowledge for a large fraction of known and putative complexes is still lacking, and there is a need for computational methods that can model protein-nucleic acid complexes providing insights into interaction mechanisms.

When structures of homologous protein-nucleic acid complexes are known, the interactions can be predicted using template-based modeling. To this end, we have developed the PPI3D web server [1]. It allows querying the non-redundant set of interactions from the Protein Data Bank, identifying the templates and modeling the structures of protein-nucleic acid complexes.

When closely related templates are not available, free docking can be employed. Molecular docking is a structural molecular biology tool, which aims to predict the predominant binding mode of a ligand with a protein of known three-dimensional structure. We present a newly developed FTDMP software system for docking, scoring and ranking diverse protein interactions, including protein-nucleic acid complexes. Rigid body docking of protein and diverse simulated conformations of DNA can be performed to account for DNA flexibility. The protein–DNA [2] docking benchmark, which contains 47 protein-DNA complexes with experimentally determined structures, is used to evaluate the docking results.

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A.56. DEVELOPMENT OF A CONTACT AREA-BASED MODEL FOR PROTEINLIGAND BINDING AFFINITY PREDICTION

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Understanding the nature of protein-ligand interactions is of great importance for molecular biology, biochemistry and medicinal chemistry. Computational modeling of the inter-molecular interactions provides insights into the mechanisms of the molecular recognition process and reveals the contribution of important factors, such as the hydrophobic effect and hydrogen bonding. Moreover, the ability to evaluate the proteinligand binding is crucial for modern drug design.

The predominant way to model interactions between proteins and small molecular weight ligands is molecular docking, which consists of ligand posing in the protein binding site (usually by conformational search) and ranking of generated poses by a scoring function. One of the widely used ways to score the generated poses is by predicting the protein-ligand binding affinity. For this purpose, empirical scoring functions that make predictions based on the structural features of the protein-ligand complex are frequently used. Such a scoring function is, in most instances, a simplified model consisting only of several terms. It does not account for all physical phenomena of ligand binding in order to minimize computational costs while trying to retain prediction accuracy. Although numerous different scoring functions have been developed, designing an efficient computational model to precisely predict the binding affinity for diverse protein-ligand complexes remains an unsolved challenge [1].

Here we present the development of a new minimalistic computational model for the prediction of protein-ligand interaction affinity. The model consists of several terms, each of which accounts for contacts of a specific type between the interacting protein and ligand atoms. These contacts and their attributes are computed using the Voronota software [2] that identifies the inter-atom contacts by means of Voronoi tessellation of biomolecular structures. In order to account for different interaction types, we classify the contacting atoms based on their atomic properties, such as hybridization, aromaticity, etc. [3]. The developed model is then parameterized using a supervised learning approach utilizing the experimentally determined structures and binding affinity data obtained from the PDBbind database [4].

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A.57. FORGED ALLIANCES: VIRAL HETEROGENEITY AMONG *SACCHAROMYCETALES* YEAST

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Since the emergence of Holobiont thinking it is challenging to view species thriving solitarily — there are many points of interaction that favor joint survival strategies. One such instance can be seen in so-called *killer yeasts*, where a system of double-stranded RNA (dsRNA) viruses ensures biocidic capabilities of the cell. Our aspiration was to locate these symbiotic groups among wild *Saccharomycetales* yeast and characterize their virome by homologically juxtaposing depicted sequences. The findings may aid in our understanding of interspecies communication and the mobility of viruses within distant biological entities.

Our team assessed 407 wild yeast strains from diverse natural niches in Lithuania. Incubation on selective medium and RNA gel electrophoresis indicated that more than ten percent (N = 47) of the chosen colonies carried a killer phenotype. Only members of different species were analyzed further. Following the purification of viral genomes from the selected strains, 20 unique dsRNAs were obtained. Each of them was sequenced using *Oxford Nanopore Technologies* and described via *CLANS* (*CLuster ANalysis of Sequences*) tool. The results imply that a larger variety of viruses — not exclusive to yeast — are present in various strains; e. g., *Mitoviridae sp.*, usually solely belonging to filamentous fungi, and other non-yeast related totiviruses. This signifies that dsRNA genomes are considerably more moderately dispersed among organisms and that their aspects of operation are yet to be divulged.

A.58. BIOINFORMATICS ANALYSIS OF UNCHARACTERIZED MICROBIALLIPOLYTIC AND LIPOLYTIC-LIKE ENZYMES

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Lipolytic enzymes are an important group of biocatalysts, widely used in many different industries, in such as precise synthesis of organic compounds, production of pharmaceutical materials, cosmetics and household chemicals, production of biofuel, etc. Despite their substrate specificity, lipolytic enzymes are all characterized by their ability to digest lipid substrates, similar protein structure, and catalytic mechanism [1]. Bacterial and especially archaeal lipolytic and lipolytic-like enzymes, such as cutinases, lipases, esterases, and polyethylene terephthalate hydrolases (PETases) are a promising group of enzymes with a high potential for use in various aforementioned branches of industry or bioremediation due to their substrate specificity, relative insensitivity to inhibitors or organic compounds, and wide temperature range [2]. However, microbial lipolytic and lipolytic-like enzymes still are a relatively low characterized group of enzymes, as there is not much data in literature and various databases.

In this study, we performed bioinformatic analysis on sequences collected from free-access databases that encode putative lipolytic or lipolytic-like enzymes. Sequences were selected according to a few requirements: 1) selected sequence has to encode a putative lipolytic or lipolytic-like enzyme, such as cutinase, PETase, lipase or esterase; 2) sequence source has to be of microbial origin – newly sequenced bacterial or archaeal genome, metagenome or environmental sample; 3) if a sequence source is a microbial genome, then this organism must have uncharacterized or low characterized status. After database analysis, four sequences encoding putative lipolytic enzymes were selected: hypothetical protein from *Candidatus Muproteobacteria*, *Salinigranum rubrum* cutinase, *Candidatus Poseidoniales archaeon* cutinase, and esterase/lipase family protein from uncultured marine euryarchaeote. Selected sequences were aligned to sequences of known and characterized lipolytic enzymes to identify conservative regions and predict catalytic amino acids. Lastly, amino acid sequences of putative lipolytic enzymes were subjected to structure prediction software to predict possible tertiary structures.

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A.59. PERSIMMON: A NOVEL DATA-DRIVEN METHOD FOR IDENTIFYING DIFFERENTIALLY METHYLATED REGIONS

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Proximal CpG sites are known to harbor similar methylation patterns and, when affected by a disease, are likely to change together forming so called differentially methylated regions (DMRs). DMR analysis canfacilitate the identification of disease affected genomic regions and the development of novel treatment strategies. Gold-standard approaches to detect DMRs first perform CpG-level statistical analysis and thenaggregate the statistical estimates into contiguous regions [1,2]. Such an approach has limited possibilitiesto recognize wider co-methylation patterns interspersed with noisy or erroneous measurements. We developed a novel approach for data-driven identification of DMRs, called *persimmon*. It leverages the correlation and pairwise distance of CpG loci to derive clusters of proximal CpGs with similar methylationpatterns. Linear mixed effects regression is then used to identify regions that are statistically significantlyaffected between different conditions. In contrast to other DMR detection methods, the *persimmon* algorithmexploits the correlation patterns inherent to the methylation data and assesses all CpGs within a region simultaneously while considering that each CpG has a different baseline methylation. That way, *persimmon* minimises the false positive rate of DMRs and boosts the power to detect methylation differences in individualCpG variations [3].

We applied *persimmon* to a public methylation EPIC microarray data consisting of 45 blood samples collected from infants diagnosed with Zika virus-associated microcephaly at birth (n = 18), unaffected infants known to be exposed to Zika virus in utero (n = 7), and unaffected unexposed infants (n = 20) [4]. *Persimmon* identified 12 DMRs (FDR q-value < 0.05 and absolute methylation difference > 5%) located in regulatory regions of known and novel Zika infection-associated genes and confirmed previously identified DMRs of host immunity and brain development [4]. Four DMRs were hyper-methylated (*DHX58*, *TRIM14*, *SLFN12L*, *EBF4*)and eight were hypo-methylated (*RABGAP1L*, *ISG15*, *NRP2*, *DUSP22*, *PM20D1*, *IFI44L*, *CNOT6LP1*, *IFIT1*) (Fig. 1). Of the affected genes, three (*EBF4*, *PM20D1*, *CNOT6LP1*) were associated with Zika virus infection for the first time. Functional enrichment analysis indicated enrichment of development, neuron death, brain pathogenesispathways as well as olfactory signaling pathways which can be pivotal when explaining the outcomes of Zikavirus infection. These findings provide novel insights into the role of methylation changes during fetal development while also demonstrating the advances and possible application of our newly developedmethod.

Ultimately, our novel algorithm—*persimmon*—enables sensitive detection of co-methylated regions and accommodates diverse experimental designs leading to an improved DMR detection.

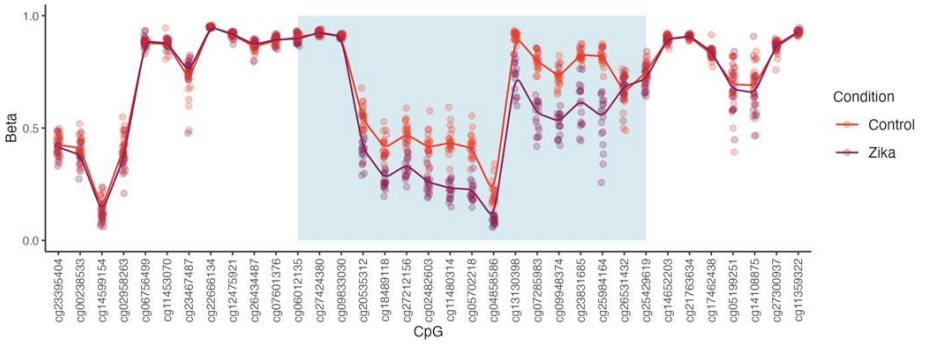

Figure 1. A differentially methylated region (DMR) of *RABGAP1L* gene, which is altered in infants with maternal ZIKA infection. The DMR is marked by the blue area and displays clear methylation differencesbetween the conditions.

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A.60. APPLICATION OF MONOCLONAL ANTIBODIES FOR THE DETECTION OF β-LACTAMASES USING TWO PHOTON EXCITATION MICROFLUOROMETRIC TECHNOLOGY (TPX)

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According to the World Health Organization (WHO) antimicrobial resistance (AMR) is one of the greatest threats to global health in this century. Currently 700 000 deaths are linked to AMR each year globally. It is estimated that 10 million lives a year may be lost due to AMR by 2050 (Figure 1.) [1]. Misuse of antimicrobials is the main driver in the development of new AMR mechanisms and difficulties in treating common infectious diseases. Therefore, development of reliable and rapid diagnostic tools is a priority in the context of AMR. In the healthcare system, about 60% of prescribed antibiotics consist of β-lactams [2]. Therefore, bacterial β-lactamases that degrade β-lactam antibiotics can be used as potential targets for diagnostics.

The aim of this study was to develop β-lactamase specific monoclonal antibodies (MAbs) to use them as molecular tools for the development of rapid diagnostic systems, such as fluorescent bead-based immunometric assays. Antibody based tests are rapid, simple to perform and highly promising for point-of-care diagnostics (PoC). Considering the WHO list of antibiotic resistant priority pathogens, we selected two β-lactamases – NDM-1 and CMY-34 – as targets for MAb development. Recombinant antigenshave been produced in the *Escherichia coli* and purified using affinity chromatography. Two collections of mouse MAbs against each target have been generated by hybridoma technology (25 MAbs in total). The most promising pairs of characterized MAbs have been selected and evaluated for their commercial and diagnostic potential by testing their ability to recognise natural β-lactamases in the pathogenic bacteria samples. Consequently, selected MAbs have been tested with ArcDia's (Finland) developed and commercialized novel bioaffinity platform based on its proprietary two-photon excitation microfluorometric technology (TPX). The technology is characterized in separation-free fluorescence detection allowing real-time monitoring of reaction kinetics and affordable PoC testing. Using recombinant antigens and clinical pathogenic bacteria isolates with characterized β-lactamase profile, the analytical and clinical sensitivity of MAbs have been determined in the TPX. All tested MAbs recognise natural NDM and CMY variants in the system. We believe that β-lactamase specific antibodies have high diagnostic potential in PoC diagnostics.

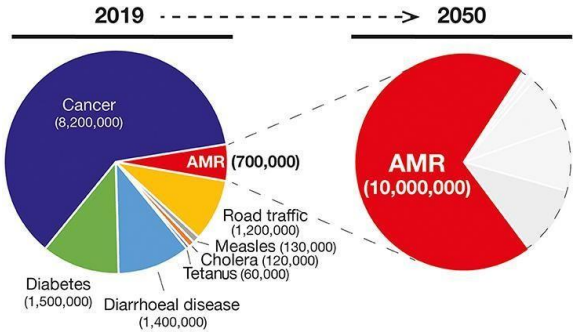

Figure 1. Number of deaths and the main causes of mortality in 2019 and 2050. AMR – antimicrobial resistance (Rosini et al., 2020).

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A.61. ACTIVATION OF NLRP3 INFLAMMASOME BY IMMUNE COMPLEXESOF VIRUS-LIKE PARTICLES IN PRIMARY MOUSE MICROGLIA

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The inflammasome is a vital component of the innate immunity. The best-described inflammasome is NLRP3, which contains three major components – nucleotide-binding and oligomerization domain-like receptor, adapter protein apoptosis-associated speck-like protein (ASC) and caspase-1 [1]. NLRP3 inflammasome activation results in release of inflammatory cytokines, like IL-1β, and inflammatory cell death – pyroptosis [2]. In the context of NLRP3 inflammasome activation, a non-receptor tyrosine kinase – spleen tyrosine kinase (SYK) has been shown to be involved. SYK plays a critical role in signal transduction pathways of immunoreceptors [3]. SYK participates in regulation of NLRP3 inflammasome activation. In previous research we showed that viral proteins triggered NLRP3 inflammasome activation depending on their structure [4]. Therefore, the aim of this study was to extend the latter research and determine whether immune complexes (IC) of oligomeric proteins could change the NLRP3 inflammasome activation in macrophages.

Primary microglia cell culture isolated from C57BL/6 newborn mice was selected as a cell culture model. Cells were treated with spherical virus-like particles (VLPs) of these human polyomaviruses (PyV): KIPyV, MCPyV, WUPyV [5] and their complexes with mouse immunoglobulins, called IC. Different subtypes of IgG class immunoglobulins were used: IgG1, IgG2a and IgG2b, to examine the differences between IC. NLRP3 inflammasome activation was studied by evaluating cell viability, IL-1β and TNF-α cytokine release and the formation of ASC specks. Specific inhibitor R406 was used to inhibit SYK kinase activity and define its role in activation of the inflammasome. Western blot analysis was performed to determine SYK kinase activity.

It was found that spherical PyV-derived VLPs and their IC induced cell death, IL-1β and TNF-α secretion and ASC speck formation in microglia indicating NLRP3 inflammasome activation. IC also activated SYK. In addition, immune complexes induced inflammatory response in microglia mediated a significantly higher cellular response compared to VLPs alone. To conclude, our results demonstrate that IC can enhance inflammasome activation.

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A.62. EFFECT OF DIFFERENT FORMS OF ATEZOLIZUMAB AND ITS INTERACTION WITH LECTIN ON PROSTATE CANCER CELLS DU-145

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Atezolizumab is an immune checkpoint inhibitor (ICI) that targets PD-L1 and is increasingly used in cancer treatment [1]. The expression of PD-L1 on tumor cells has been shown to correlate with patient's response to ICI's [2]. ICIs are protein-based anticancer drugs that do not induce tissue injury, upon direct contact with tissues, so alternative delivery methods that maintain their effectiveness are being sought. Additionally, posttranslational modifications of PD-L1, such as N-glycosylation, have emerged as important regulatory mechanisms that modulate immunosuppression in cancer patients [3]. Lectins, which have an intrinsic property of binding to specific sugar residues in glycoproteins and glycolipids [4], may possess immunomodulatory and antitumor properties [5] that could potentially enhance the effect of atezolizumab.

Therefore, this study aimed to assess the effect of immunomodulatory drug atezolizumab in different forms and to evaluate its interaction with the lectin *Bacillus subtilis* and its influence on the human prostate cancer cell line DU-145. In the atezolizumab binding experiments, cells were plated, allowed to adhere for 24 h, and then treated with atezolizumab in three different forms: native, nebulized, and heat-inactivated. Each form was tested in DU-145 cell binding assays at different concentrations and incubated at 37°C. For the lectin in cooperation with atezolizumab binding experiments, cells were processed as described previously at two different concentrations of lectin. All samples were analyzed by flow cytometry method.

The results showed that human prostate cancer cell line DU-145 exhibited characteristic spontaneous PD-L1 expression. The inhibition of anti-CD274 staining intensity of DU-145 cells depended on the concentration of anti PD-L1 drug atezolizumab. Comparing the CD274 blockade experiment with the different forms of atezolizumab (native and nebulized) resulted in very similar intensities as measured by flow cytometry. Nebulized atezolizumab retained cellular binding to PD-L1. *Bacillus subtilis* lectin did not bind to membrane PD-L1 in DU-145 cells and did not affect the ability of atezolizumab to block membrane PD-L1 when incubated together. However, when cells were exposed to lectin first and then subsequently to atezolizumab, PD-L1 was blocked less.

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A.63. COMPARATIVE ANALYSIS OF TWO MURINE BREAST CANCER MODELS

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Breast cancer remains the second leading cause of cancer deaths among women. Therefore, development and targeting of new breast cancer models remains a paramount task in tumour biology research. E0771 and 4T1 are murine breast cancer cell lines that are widely used in preclinical research to study the tumour microenvironment, particularly the role of immune cells in tumour progression and response to therapy. E0771 cells were derived from a spontaneous mammary tumour in a C57BL/6 mouse and represent the luminal subtype of breast cancer [1], while 4T1 cells were derived from a mammary tumour that developed in a BALB/c mouse and represent the triple negative subtype of breast cancer [2].

The objective of our study was to investigate and compare the characteristics of 4T1 and E0771 cell lines *in vitro* and *in vivo*.

For *in vitro* study, we cultured cells in supplemented RPMI medium and analyzed their cell surface markers using flow cytometry. The results showed that MHC I, PD-L1, CD11b markers are abundant in E0771 cell line (at 96%, 94%, 74% respectively). MHC I is lacking in 4T1 line while CD11b and PDL1 are present in 44% and 19% of 4T1 cells, respectively. CD44 marker can be found in most cells of both cell lines.

For *in vivo* study, we observed the growth rate of transplanted tumours in mice, and characterized their microenvironment dynamics formation using flow cytometry. Our findings show that qualitative and quantitative composition of E0771 tumor microenvironment remains the same during tumor development. In 4T1 tumours, difference in microenvironment dynamics was observed, with T cell infiltration decreasing over time. In addition, 4T1 tumours are more heavily infiltraded with T cells and tend to grow faster than E0771 tumors (Figure 1).

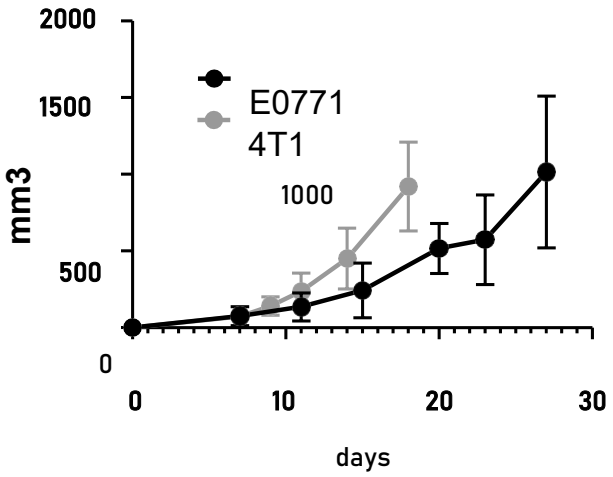

Figure 1. Growth curves of E0771 and 4T1 tumors.

By comparing and analysing these two murine breast cancer models, we can enhance our comprehension of the tumour microenvironment and identify clinical targets for the prevention and effective treatment of breast cancer. The examination of these breast cancer models is a valuable approach for investigating the molecular and cellular mechanisms that underlie breast cancer progression and metastasis, as well as evaluating the efficacy of novel therapeutic interventions.

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A.64. CAR-T CELL RECEPTOR FUNCTIONALITY ASSAY USING PROTEIN L AND CD19 CONJUGATES

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CAR-T cell therapy is a new, rapidly advancing field of immunotherapy. CAR-T cells can be infused into cancer patients for antitumor, antiviral or immunomodulatory effects. These cells are derived from genetically modified T cells ex vivo by expressing a CAR (chimeric antigen receptor) on the cell surface. CAR consists of 4 parts: antigen recognition domain, hinge region, transmembrane domain and intracellular signalling domain. From 2017 FDA approved four anti-CD19 CAR-T cell therapies: "Kymriah", "Yescarta", "Tecartus", "Breyanzi", which are used to treat B-cell malignancies and anti-BCMA therapies "Abecma" and "Carvykti" for the treatment of myeloma [3]. In addition to anti-CD19 and anti-BCMA therapies, dozens of antigen targets are being tested under preclinical and clinical settings for solid and blood tumours. To take CAR-T cell research from bench to bedside, first, genetically modified T cells have to be tested in vitro for CAR expression on the cell surface and functionality.

In 2022, B. Sharpless won a Nobel Prize for developing a more efficient way of conjugating molecules: he used water instead of organic solvents and catalyzed the reaction of azide and alkyne with copper [2]. The "click chemistry" reaction can take place in bioorthogonal conditions (in living cells), so the functioning of the body is not disturbed. Here, azide reacts only with compounds containing an alkyne group (BCN, DBCO).

GenieTAG™ technology was developed by Genie Biotech company [1] which allows the site-specific introduction of azide into the protein for subsequent "click chemistry"-based conjugation of various payloads for the vaccine, diagnostics, protein therapeutics and slow delivery applications. The linker is conjugated to proteins at a neutral pH of the solution (~7), the duration of the process is 1-3 hours, at 37 ℃.

In this project, we are developing a novel one-step modular detection system to determine the expression and functionality of CARs. For this purpose, we are conjugating protein L and CD19 to fluorescent dyes using GenieTAG™ site-specific conjugation technology. This one-step modular detection system will allow the identification and assessment of the functionality of CAR receptors on engineered T cells.

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A.65. DEVELOPMENT AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES AGAINST RECEPTOR BINDING DOMAIN OF SARS-COV-2 SPIKE PROTEIN

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Severe acute respiratory syndrome coronavirus 2 (SARS–CoV–2) caused COVID–19 pandemic in 2020 and became a major problem still threatening public health and global economy. Since the beginning of the SARS- CoV-2 outbreak, efforts have been taken to develop novel molecular tools to cope with the virus infection. Highaffinity and specificity monoclonal antibodies (MAbs) with broad neutralizing activity, which could block the viral infection by targeting structural proteins, have high therapeutic and diagnostic potential and are still relevant today. SARS-CoV-2 spike (S) protein is a key factor at the beginning of infection. Its receptor-binding domain (RBD), which spans 319–541 aa of the S protein, mediates viral entry into the host cells by interacting with cellular angiotensin-converting enzyme 2 receptor [1]. Therefore, anti-RBD MAbs tend to have high neutralizing potency; in fact, almost 90% of neutralizing antibodies are directed to the RBD [2]. Moreover, while new virus variants are emerging, inhibitory MAbs against conservative regions of RBD would be critical. These MAbs would be beneficial for diagnostics measuring the level of neutralizing antibodies against SARS–CoV–2.

Due to ongoing prevalence of COVID–19, this study aimed to generate anti–SARS–CoV–2 RBD MAbs and evaluate their application for molecular diagnostics. Recombinant RBD expressed in HEK293 cells was used for the immunization of mice. Evaluation of mice's humoral response proved that RBD was highly immunogenic. Thirty-seven hybridoma cell lines producing high-affinity IgG subtype MAbs against RBD were developed by hybridoma technology. MAbs were purified from the cells' growth media using protein A binding. The specificityof MAbs was analyzed by enzyme-linked immunosorbent assay and immunoblotting. Also, MAbs were tested with various SARS–CoV–2 variants' S proteins. The data indicated that part of anti–RBD MAbs' epitopes is conservative among different S variants. This study shows that developed MAbs are suitable for virus- neutralizing assays and could be used alone or in a mix in designing novel SARS–CoV–2 diagnostic systems.

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A.66. MAPPING OF RECOGNITION SITES OF MONOCLONAL ANTIBODIES DIRECTED TO CONSERVED SEQUENCES OF SARS-COV-2 SPIKE PROTEIN

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SARS-CoV-2 is responsible for the worldwide COVID-19 pandemic that has already cost more than 6 million lives. The virus spike (S) protein is essential in enabling the virus to enter host cells. The receptor binding domain (RBD) of the S protein binds human ACE2 receptor and triggers the fusion process, after which the virus infects the cell [1]. Many SARS-CoV-2 variants have emerged since the start of the pandemic. New virus variants have mutations within the S protein gene that helps the virus evade the host's immune system and avoid the defence of antibodies directed to the previous variants [2]. Therefore, antibodies against the conserved sequences of S protein are important for future research and diagnostics.

This study aimed to investigate the cross-reactivity of nine monoclonal antibodies (MAbs) with different S proteins of SARS-CoV-2 variants and to map recognition sites of the MAbs within the S protein. Six MAbs were generated against full-length recombinant SARS-CoV-2 S protein and three MAbs – against recombinant RBD. The interaction of the MAbs with six overlapping and seven truncated fragments of Wuhan- originated S protein were investigated by different enzyme-linked immunosorbent assay (ELISA) formats and by Western blot (WB). MAb reactivities with recombinant S proteins of SARS-CoV-2 B.1.1.7 (Alpha), B.1.351 (Beta), B.1.617.2 (Delta), B.1.617.1 (Kappa) and B.1.1.529 (Omicron) variants were analysed by indirect ELISA.

This study revealed six out of nine MAb recognition sites within the S protein. MAb 7E2 recognises the site between amino acid residues (a.a.) 268 and 293 of S protein. MAb 11A3 recognises a conformational epitope that is located within a.a. residues 620 and 695. MAbs 8E11 and 14G6 recognise epitopes between a.a. 719 and 743. MAb 5D7 epitope is between a.a. 764 and 775. MAb 3D7 epitope site was localised only with overlapping fragments between a.a. 790 and 1196. The epitopes of MAbs 11E11, 23B5 and 24A12 developed against RBD are within RBD sequence located between 319 and 541 a.a. of S protein. The investigation of MAb ability to recognise S proteins of different SARS-CoV-2 variants revealed that eight out of nine MAbs recognise conserved epitopes within S proteins tested. MAb 7E2 failed to recognise the S protein of the Omicron variant.

This study revealed six of nine MAb interaction sites distributed throughout the entire S protein. MAb 7E2 that does not recognise the Omicron S protein interacts with the N-terminal domain of the Wuhan-originated SARS-CoV-2 and S proteins of related viruses. MAbs 3G7 and 5D7 identified conserved recognition sites in the S2 domain responsible for membrane fusion. Broadly reactive MAbs 11A3, 8E11, and 14G6 recognised areas around S1/S2 cleavage site, which is essential for virus infection.

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A.67. INTERLEUKINS EXPRESSION IN PBMC AFTER CO-CULTIVATION WITH PANCREATIC CANCER CELL

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Pancreatic cancer has been known in the medical field for many years, from the end of the 19th century. Surgical methods of pancreatic cancer treatment were introduced, but they often did not prove effective due to the advanced stage of the disease even now days. Peripheral blood mononuclear cells (PBMC), which include various types of immune cells, are crucial for the body's immune response against cancer, that is a little-known area of research [1]. Understanding the interaction between PBMCs and pancreatic cancer cells is essential for developing effective cancer treatments that can harness the body's immune system to fight cancer invoke IL1b, IL6, IL10 that are known as procancerous cytokines, while IL4 – precancerous [2]. Ongoing research in this field can provide new insights into the mechanisms underlying the immune response to pancreatic cancer and offering better treatment methods than surgery.

The aim of our study is to understand how pancreatic cancer cells affect mRNA expression of cytokines in peripheral blood mononuclear cells (PBMC) in healthy humans.

This study was approved by the Kaunas Regional Biomedical Research Ethics Committee. Healthy humans' blood (n=7) was collected in a vacutainer tube with EDTA. A 50 ml tube was filled with 15 ml Ficoll and 20 ml of the blood sample diluted in PBS. After centrifugation, the Buffy layer was collected, and the laminar sterile cells were suspended in 1 ml RPMI + 10% FBS + 1% P/S medium. PBMC cells were counted using the Trypan Blue, and $36x10^6$ cells were Co-cultivated in well bottom +/-PDAC BxPC3 cells in the inserts, seeded three days prior to the experiment. After 20 h cells were collected.

0-day, non-co-cultivated (-C), co-cultivated (+C) PBMC cells ($3x10^6$) were stored in RNA later solution at -20C. Next, a commercial RNA isolation kit was used to extract total RNA. RNA quality and concentration were measured with Nano Drop spectrophotometer. The last step was to convert the cDNA. To perform real-time analysis, a reaction mix was prepared by combining three components (H2O, primers with TaqMan probes and master mix). Relative gene expression analysis was performed with delta delta CT method.

Our results revealed that procancerous IL1b, IL6, IL10 cytokines genes expression in PBMC's were statistically significant increased after co-cultivation with pancreatic cancer cells. While precancerous IL4 expression not changed.

This study concludes that immune cells, with the aid of three cytokines, can combat cancer in healthy patients. However, precancerous il4 does not exhibit any changes in PBMC. It is possible that there may be a mechanism at play that tricks the body, but a more extensive study and analysis would be required to make such a claim.

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A.68. DIFFERENCES IN MOLECULAR PROFILES OF TREE POLLEN, FRUIT,AND VEGETABLE ALLERGIES BETWEEN MEN AND WOMEN

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Background

Tree, fruit, and vegetable allergens can be encountered in everyday life. There are studies suggesting that there may be gender differences in the prevalence of allergies. Some studies have found that women may be more likely to experience certain types of allergic reactions, such as food allergies [1]. Another study shows that sensitivity to pollen has increased over the years, especially among men [2]. In this study, we aim to determine whether there is a difference in sensitization to tree pollen, fruit and vegetable allergens between men and women.

Methods

A retrospective analysis of data from patients examined at the Innovative Allergology Center, Vilnius, Lithuania was conducted. A total of 165 patients' data was collected and divided into two groups based on gender. 133 out of 165 patients were included in the statistical analysis: 73 women and 60 men. A detailed analysis of ALEX macroarray test results was performed.

Results

Data analysis revealed that in total 50 (37.59%) patients were sensitive to tree pollen, 32 (24.06 %) - to fruits, and 15 (11.28%) - to vegetables. Among the tested tree pollen, fruit, and vegetable allergens, Aln g 1, Bet v 1, Cor a 1.0103, Cor a 1.0401, Cor a_pollen, Fag s 1, Fra a 1+3, Mal d 1, and Api g 1 were the most frequently linked to sensitivity. The most common allergen was the birch pollen component Bet v 1, with a total of 46 (34.59%) patients being allergic to it. No statistically significant difference in sensitization rates was found between men and women for allergens Aln g 1, Cor a 1.0103, and Cor a 1.0401. However, male patients were found to be more likely to be sensitive to Bet v 1 allergen (27; 45%) than female patients (19; 26.03%) (p=0.035). A higher sensitivity was also observed in males' group for the Cor a_pollen and Fag s 1 allergens (15 (25%) and 22 (36.67%) of males, respectively), (p=0.0076 and p=0.0391). Furthermore, a higher prevalence of sensitivity was found among men for fruit allergens, 16 (26,67%) men and 7 (9,59%) women were sensitive to Fra a 1+3 allergen (p=0.018), and 13 (21.67%) men and 5 (6.85%) women were sensitive to Mal d 1 allergen (p=0.0257). Moreover, men were statistically more significantly allergic to celery allergen Api g 1: 10 (16.67%) men and only 3 (4.11%) women (p=0.033).

Conclusion

In this pilot study, it was found that there may be gender differences in the prevalence of certain types of hypersensitivity. The analysis suggests that male patients have a slightly higher prevalence of sensitivity to tree pollen, fruit, and vegetable allergens compared to female patients. However, a more extensive patient sample is required to evaluate differences more accurately.

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A.69. DEVELOPMENT OF MONOCLONAL ANTIBODIES AGAINST ANTIBIOTIC RESISTANCE PROTEINS OXA-48, OXA-134, SME-3,ADC-144 AND SHV-42

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Antimicrobial resistance (AMR) is the largest global health threat in the 21st century and it requires urgent measures. Major reasons behind the emergence of AMR – misusing and overusing different antibacterial agents in healthcare settings [1]. One of the steps in limiting the spread of AMR is accurate diagnostics of the disease and prescription of effective antibiotics. Currently applied diagnostic methods include phenotyping, biochemical, and genotyping methods which tend to be expensive and protractive. Immunodetection, on the other hand, is a rapid, specific, and sensitive method for identifying resistance factors. However, the lack of highly specific monoclonal antibodies (MAbs) limits the development of immunodiagnostic tests.

The aim of this study was to generate and characterize MAbs against five antibiotic resistance factors – β-lactamases OXA-48, OXA-134, SHV-42, ADC-144, and SME-3, which are widespread in pathogenic *Enterobacteriaceae* and confer resistance to carbapenems, penams and cephalosporins [2,3,4]. Using hybridoma technology three specific anti-OXA-48, three specific anti-OXA-134, two specific anti-SHV-42, two specific anti-ADC-144, and three specific anti-SME-3 MAbs secreting cell lines were created. Ten out of thirteen generated MAbs were shown to have high affinity towards respective antigens and twelve recognize their linear epitopes. One anti-OXA-134, one anti-SME-3, and one anti-ADC-144 monoclonal antibody pair were shown to have the potential to be applied in antibiotic resistance factors detection tools.

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Oral presentations

MAPPING VISCOSITIES OF LIPID BILAYERS IN LIVE CELLS AND MODEL MEMBRANES THROUGH FLIM

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Viscosity is the essential physical characteristic of cell membranes – it controls diffusion of lipids and macromolecules, affects the lipid raft formation, and influences the passive transport of solutes across the plasma membrane. Lipid membranes are inherently heterogeneous and are able to phase separate into liquid ordered (Lo) and liquid-disordered (Ld) domains. Viscous Lo phase is of particular biological importance – ordered microdomains of lipids and proteins, so called lipid rafts, play a key role in immune signaling [1,2], host-pathogen interactions [3,4], cardiovascular diseases [5], and cancer [6,7]. Thus, the ability to distinguish Lo and Ld phases and determine their precise viscosity values is of great interest and viscosity-sensitive probes offer a convenient solution for this task.

In this work, we present novel membrane-targeting viscosity probe – BODIPY-PM. Combining the use of BODIPY-PM with Fluorescence Lifetime Imaging Microscopy (FLIM), we demonstrate the ability of BODIPY-PM to recognize Lo and Ld phases in complex biological systems – large unilamellar vesicles (LUVs), tethered bilayer membranes (tBLMs) and live cancer cells (Fig. 1). Our method allows both imaging and dynamic monitoring of viscosity changes in real time in live cells, as well as model lipid systems.

In addition, we explore the effects of statins on lipid raft clustering in live cells. We demonstrate that simvastatin treatment induces aggregation of lipid rafts and segregates the plasma membrane into viscous and non-viscous domains. Importantly, non-viscous domains serve as an effective passive diffusion corridors for anti-cancer drugs, thereby increasing the susceptibility of cancer cells to anti-cancer drugs.

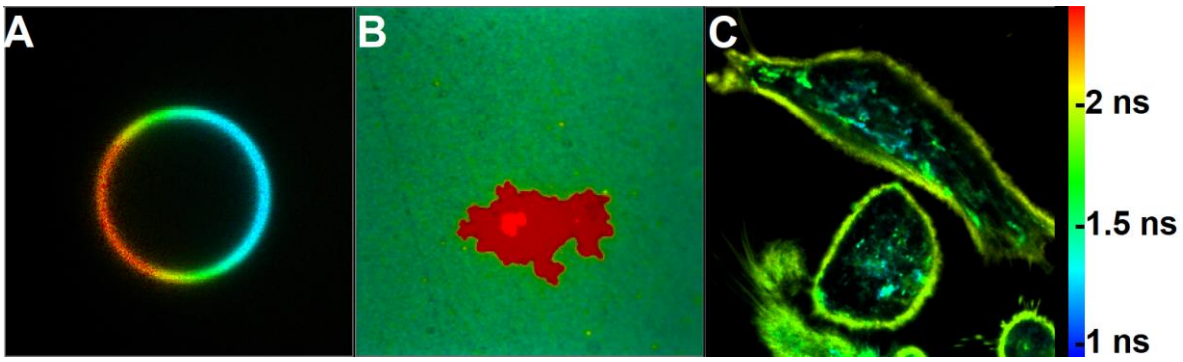

Figure 1. FLIM images of DOPC/DPPC/Chol LUV showcasing phase separation (A), DOPC/DPPC/Chol tBLM with Lo domain in the center (B), plasma membrane viscosity map of live lung cancer cells (C).

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GEOBACILLUS SPP. BACTERIA INDUCED BIOSYNTHESIS OF SILVER NANOPARTICLES

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The increasing resistance of bacteria to antibiotics and the strong side effects of antifungal agents are of great concern to scientists. Researchers are looking for new alternatives to combat these emerging problems for these reasons. Silver nanoparticles (AgNPs) can be applied in various fields due to their unique chemical and physical properties, including medicine. The most common methods used to obtain nanoparticles of various materials, including silver, are chemical and physical, but these methods are usually harmful to the environment. Meanwhile, biological methods of obtaining AgNPs, which use enzymes and extracts of microorganisms and plants, are cheaper and environmentally friendly alternatives [1]. One such bacteria that can be used for the biological synthesis of AgNPs is bacteria of the genus *Geobacillus*, which are thermophilic microorganisms [2]. Using thermophilic microorganisms to synthesize nanoparticles reduces the risk of microbial contamination during their synthesis. Although bacteria of various genera have been adapted for the synthesis of AgNPs, the exact mechanisms of the nanoparticles are not clear, so more detailed studies are needed.

In this study, AgNPs were successfully obtained by using the secretomes of four *Geobacillus* spp. stains, namely 18, 25, 95, and 612. Such synthesis is extracellular, and this has advantages such as easier purification of the resulting AgNPs. The AgNPs obtained by the bio-based method were characterized using UV-Vis spectroscopy, scanning electron microscopy (SEM), dynamic light scattering (DLS), and zeta potential measurements. The characteristic absorbance peaks at 410-425 nm were shown by UV-Vis spectroscopy. These peaks are indicative of AgNPs. With the SEM micrographs, it was found that the obtained AgNPs shape is spherical. However, DLS analysis revealed that the sizes of the AgNPs were widely distributed, with the majority less than 100 nm in diameter. Moreover, the Zeta potential values, ranging from -25.7 to -31.3 mV, depending on the *Geobacillus* spp. strain was also measured. In addition, research on the nitrate reductases of *Geocacillus* spp. bacteria was also carried out, allowing a better understanding of the importance of this enzyme in the synthesis of AgNPs. In conclusion, it can be stated that bacteria of the genus *Geobacillus* can be adapted for the synthesis of AgNPs.

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DEVELOPMENT AND CHARACTERIZATION OF MEMBRANOUS SUBSTRATE WITH SUITABLE ELASTIC MODULUS FOR THE EXVIVO EXPANSION OF HUMAN ARTICULAR CHONDROCYTES

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Osteoarthritis, the degenerative disorder in the articulating joints is one of the major healthcare challenges in today's world. Autologous chondrocyte implantation (ACI) in which autologous chondrocytes are implanted in the damaged cartilage is one of the promising therapeutic options. For ACI, the number of chondrocytes required for implantation in the cartilage defect is the defect-size dependent. Expansion of the patient derived chondrocytes under *in vitro* conditions is inevitable to produce the required chondrocyte number. Combining autologous chondrocytes with autologous/allogeneic mesenchymal stem cells (MSCs) has also been used to meet the required cell number for the transplantation procedure (Epanomeritakis et al., 2022). However, the *in vitro* expansion of the human articular chondrocytes or MSCs in conventional tissue culture plastic has been found to influence the chondrogenic functionality of these cells. The dedifferentiation, hypertrophy and osteogenic differentiation in chondrocytes and MSCs in response to stiff substrates are documented in the literature. Minimizing the dedifferentiation, hypertrophy and osteogenic differentiation in MSCs during their expansion is the key for producing the fully-functional cells for ACI procedure. Several studies in the literature have shown the influence of matrix stiffness in modulating the differentiation associated genes in chondrocytes and MSCs using hydrogel systems. However, a matrix system that can be scaled-up for the expansion of chondrocytes or MSCs has not been identified yet. Therefore, this study investigated the potential of using synthetic polymeric matrices with or without coating with hydrogel for the expansion of chondrocytes and MSCs. Towards this objective, electrospun polycaprolactone (PCL) nanofibrous matrices (NM) and NM coated with collagen based interpenetrating network hydrogel (NM-IPN) were developed and characterized. Further, effects of the NM and NM-IPN matrix stiffness in modulating the expression of chondrogenesis and hypertrophy associated genes in chondrocytes and MSCs were studied in comparison with tissue culture plastic (Li et al., 2003). The effect of the NM and NM-IPN matrices in modulating the proliferation of chondrocytes and MSCs were also evaluated. Our results revealed that the NM and NM-IPN had a low elastic modulus in comparison to the stiffness in tissue culture plastic. Both chondrocytes and MSCs showed significantly low proliferation in the NM and NM-IPN matrices in comparison to the tissue culture plastic system. However, human articular chondrocytes growing in the NM and NM-IPN matrices showed a stiffness dependent upregulation of chondrogenic and hypertrophy marker genes in comparison. In the case of MSCs, the expression of both the chondrogenic and hypertrophy markers found to be downregulated in the NM and NM-IPN matrices compared to the tissue culture plastic system. However, the downregulation of hypertrophy markers was several folds whereas chondrogenic gene downregulation was found to be minimal. In conclusion, the softer substrate was found to be unfavorable for proliferation of these cells, but it can be good for minimizing the dedifferentiation in the human articular chondrocytes.

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Biology and Ecology

B.1. EFFECTS ON HEMATOLOGICAL AND MORPHOLOGICALPARAME-TERS IN FISH AFTER CHRONIC EXPOSURE TOMICROPLASTICS

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In 2021, the total production of plastics climbed to 390.7 million tons, 90.2% of which were made from fossil fuels [1]. A synthetic aromatic polymer known as polystyrene (PS) is used in electrical, architectural, and packaging applications and it is one of most frequently used plastic polymers worldwide. PS has been discovered in a number of environmental and biological sources and frequently ends up as plastic debris in aquatic environments [2]. In terms of uptake, localization, and effects on various aquatic organisms, PS is one of the most researched polymers in the field of microplastics (MP) [3]. Hematological parameters, including hematocrit (HCT) and glucose, morphological parameters, such as weight and length, are useful markers for assessing the health of fish after exposure to a variety of environmental conditions, toxic chemicals, and bacterial infections [4]. The "coefficient of condition" is a measure of a fish's relative robustness or level of wellbeing (also known as condition factor (CF), or length-weight factor).

The goal of this study is to assess how chronic exposure to polystyrene (PS) microplastics affects hematological and morphological parameters of rainbow trout (*Oncorhynchus mykiss*). For hematological parameters blood samples were taken from a tail vein with a 1 ml insulin syringe and 25G needle. The microhematocrit centrifuge was used to determine HCT, and the automatic Glucose Analyzer was used to measure blood glucose (GLU). For morphological parameters total weight (TW), weight without organs (WWO) and weight of intestines (IW) were measured, as well as total length (TL), fork length (FL) and standard length (SL). After getting morphological parameters CF was calculated using formula:

$$CF = \frac{Total\ weight}{Total\ length^3} \cdot 100$$

We did not observe statistically significant changes in HCT or GLU when the PS-exposed group was compared to control (CTRL) fish. Changes in weight and length of fish were not statistically significant as well compared to control. While fish are particularly prone to ingesting microplastics because of their appealing colors, and resemblance to food in many experimental setups, organisms are frequently exposed to unrealistically high concentrations of microplastics. In this study we used environmentally realistic concentrations of artificially uncontaminated PS microplastic and exposure had no effect on morphometric data that characterizes fish condition. The usage of environmentally realistic concentrations is highlighted in many microplastic toxicological studies [5]. In conclusion, exposure to PS microplastics at environmentally realistic concentration did not alter the overall wellbeing and hematological parameters of rainbow trout.

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B.2. FIGHT FOR SURVIVAL: CASE STUDY OF THREATENED FUNGUS SARCOSOMA GLOBOSUM

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The soil saprotrophic fungi are strongly affected by natural and anthropogenic forest disturbances. The most important natural disturbance factors affecting Norway spruce (Picea abies) are fires, drought, wind storms and pathogens such as spruce bark beetle Ips typographus, fungus Heterobasidion annosum and the fungi of the genus Armillaria. Saprotrophic fungal communities respond differently to various forest management practices, e.g., clear-cutting, salvage logging or thinning. Increased soil temperature and exposure to sunlight, associated with reduced canopy projection cover, negatively affect the diversity of saprotrophic fungal species [1].

The saprotrophic macromycete Sarcosoma globosum (Pezizales, Ascomycota) produce large round gelatinous fruit-bodies on needle litter in spruce dominated forests mainly in early spring. It can live in only a restrictive range of habitats and is considered an indicator species of primeval and old-growth spruce forests.

S. globosum is very rare in North Europe and in Central-European mountains. Also known from Asian taiga zone and eastern parts of North America [2]. The decrease of its frequency occurred in the 20th century in many European countries. In Lithuania, S. globosum was not recorded more than four decades, and in the beginning of 21st century was evaluated as possibly extinct species. In contrast, an increase of sites is reported for the last decades in Finland, Russia and the Baltic states. The species has been included in the IUCN Red List of Threatened Species since the year 2015 (category Near Threatened) [3]. S. globosum has been protected in Lithuania since 1992 and in 2010 it was included in the list of strictly protected species in Lithuania.

The aim of our investigation was to assess the present conditions and situation of S. globosum habitats and to discuss the threats to fungal populations in Lithuania. We examined the diversity of habitats, various soil and tree stand characteristics, forest management activities and natural disturbances in all known fungus localities (43 sites in 28 localities) in Lithuania.

Our observations indicate that a large proportion of S. globosum sites are located in young Norway spruce stands: 25.6 % of the fungus sites were assigned to the age classes from 20 to 50 years. The youngest forest in which S. globosum has been recorded it was 27 year-old spruce plantation. Majority of S. globosum growth sites are situated in older forests: 16.3% – in forests from 51 to 70 years old, and 58.1% – in forests over 71 years old. A total of 3 habitat types (according EUNIS habitat classification) have been identified at the fungus sites: Boreo- nemoral bilberry western spruce taiga (51.1 %), Continental tall-herb western spruce taiga (25.6 %), and Native fir, spruce, larch, cedar plantations (23.3 %). P. abies was present in the tree layer of all sites. In 4 sites S. globosum was fruiting in spruce monocultures, which were planted on ploughed soil. The computed value of stand density index (SDI) varied from 231.5 to 1573.9, and the stand basal area (ba) values varied from 11.4 m2/ha to 468.8 m2/ha. The lowest SDI and ba values were estimated for the Paliūniškis forest, where salvage logging was carried out in 2022. The assessment of disturbances in S. globosum habitats showed that, in some cases, the fungus can survive forest management practices, such as salvage logging and thinning.

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B.3. *DIAPORTHE* SPP. GROWTH INHIBITION BY ENDOPHYTIC BACTERIA

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Fungi of the genus *Diaporthe* Nitsche (1870) have been identified as important plant pathogens causing large economic losses. These phytopathogens have a wide range of plant hosts and are found on plants and their living parts. Fungi of the genus *Diaporthe* clog the water vessels of the plants, damage the cells, and cause various symptoms as stem blight, dieback, canker. *Diaporthe vaccinii* is even included in the European Union's A2 list of quarantine pests.

The increasing resistance of fungi to fungicides has led to efforts to control these pathogens through biocontrol. The most common methods of controlling the spread of pathogenic fungi are the use of bacterial cultures or their secondary metabolites. Bacteria with an antagonistic effect on fungi can inhibit the growth of pathogens.

In order to control the spread of pathogens, it is essential to investigate the antagonistic relationship between bacterial isolates of the genera *Pseudomonas*, *Bacillus*, *Paenibacillus* and fungal isolates of the genus *Diaporthe.* In the present study, a double culture approach [1] was used to select the bacteria with the strongest inhibitory effect. Once the most antagonistically active bacteria were selected, their antimicrobial efficacy was tested by agar diffusion [2].

Using the double culture method, 76 antagonistic reactions between isolates of the genus *Diaporthe* and bacteria were selected out of 130 possible antagonistic reactions (Fig. 1). The inhibitory effect of the selected bacterial isolates was calculated. The inhibitory effect of the bacterial isolate AP6.1A ranged from 61 % to 87 % and the inhibitory effect of the isolate AP489B ranged from 53 % to 86 %. No inhibitory effect on the test fungi was detected for the metabolites of the secondary bacterial isolates using the agar diffusion method.

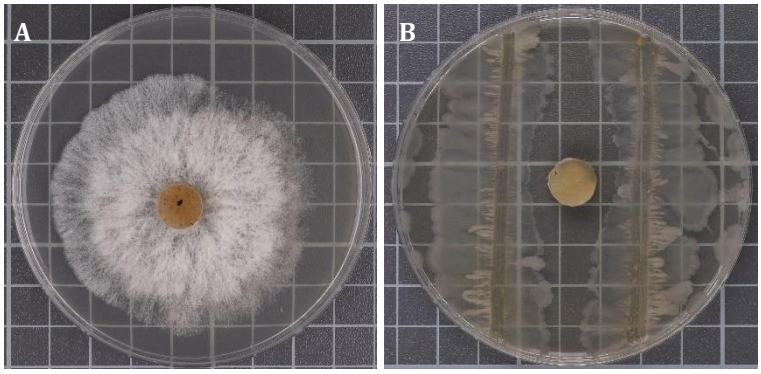

Figure 1. Antagonistic effect of bacterial isolate AP6.1A on the growth of fungal isolate FB316. A growth of the fungal isolate FB316 without the influence of bacterial isolate AP6.1A (control). B inhibitory effect of bacterial isolate AP6.1A on the growth of fungal isolate FB316 (fungal isolate in the middle and bacterial streaks on the left and right side).

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B.4. DIVERSITY OF ENDOPHYTIC FUNGI ON INTRODUCED PLANTS

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The increased international trade of plants and plant material has led to concerns about the potential damage of imported plants to native plant species. Thus, much attention is now being paid to the study of potentially invasive plants, but the risk of microorganisms arriving with these plants is often not assessed. One of the main reasons for biodiversity loss is invasive pathogens, and endangered plant species are especially vulnerable to these microbes. In addition, the spread could have significant economic consequences such as a decreased quality of the plant or plant product may limit it is exportability.

Endophytic fungi are known for their capability to improve host plant growth and provide protection against phytopathogens. Although, fungal endophytes colonizing healthy plant tissues may also switch to the pathogenic mode when the host is under stress. To date, few studies have been conducted in Lithuania to investigate which genera or species of invasive fungi may be potentially dangerous to native phytodiversity.

The aim of the study is to identify endophytic fungi on non-native plants.

Plant material for the study was collected from the Kairėnai Botanical Garden of Vilnius University. Samples were collected from total of 32 plants belonging to *Rhododendron* L, *Pinus* L, *Quercus* L, *Fagus* L, and *Pieris* D. Don genera. A total of 558 fungal isolates were obtained from needles, twigs, leaves, buds and soil samples.

The classification of the isolated fungi was carried out based on the main microscopic and macroscopic phenotypic characteristics, such as the color, shape and size of colonies, hyphae and spores. Molecular characterization is planned in the future. The cetyltrimethylammonium bromide (CTAB) method will be used to extract genomic DNA. For molecular identification of the isolates, the internal transcribed spacer (ITS) region will be used. ITS region amplification will be performed by polymerase chain reaction (PCR) using the ITS1/ITS4 primer pair [1]. In order to demonstrate the pathogenicity of the fungal isolates according to Koch's postulates, a pathogenicity test will be carried out.

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B.5. COMPARISON OF LEAFLET SHAPE VARIATION IN TWO SPECIES OF *AGRIMONIA* (*ROSACEAE*) USING GEOMETRIC MORPHOMETRICS

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Geometric morphometrics can be defined as quantitative study of shape distinguished by preserving the shape of the object in question by means of landmarks or an outline. Despite the opportunities offered bycurrent molecular methods, quantitative analysis of morphological features remains as a key component in classifying plants and artificial intelligence techniques are increasingly used for the task [1]. The main issue with analysing biological objects is that their shape and size are not consistent with how we interpret "perfect" symmetry. This is especially prominent in the plant kingdom where hybridization and changes intraits are highly prevalent. Also, different environmental conditions can account for allometry. The method of landmark-based geometric morphometrics is especially helpful when analysing compound leaves and can be complementary to other scientific research methods [2].

Agrimonia L. plants are perennial herbs mostly researched due to their pharmaceutical value. The genus is also important for researching evolutionary events as various cytotypes and different levels of polyploidy have been reported [3]. Three species of the genus *Agrimonia* are present in Lithuania: *A. eupatoria* L., *A. pilosa* Ledeb., and *A. procera* Wallr. Lithuania is along the westernmost border of the natural geographical range of *A. pilosa*. Previously, plasticity of *A. eupatoria* and *A. pilosa* had been researched and showed highly reduced active plasticity in marginal areas of species range. It is known that *A. eupatoria* can exhibit more active plasticity than *A. pilosa*. In addition, phenotypic plasticity can be influenced by light availability in the environment. The resulting changes in shoot and leaf morphology can be caused by either allometric dependence on the entire plant size (passive plasticity) or certain attributes associated with plant development (active plasticity) [4].

The aim of this study is to apply landmark-based geometric morphometric methods in order to analyseapical leaflets from the three middlemost compound leaves per individual plant in selected species: *A. eupatoria* and *A. pilosa*. Preliminary results of quantitative analysis indicate that the variability of morphological traits found in plants growing in a shaded environment is different than that found in plants inwell-lit areas. In addition, differences between fresh and herbarized material were detected. This study provides a foundation to further hypothesise possible explanations for developmental variation of leaves in both species of *Agrimonia*.

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B.6. INFLUENCE OF DIFFERENT SUBSTRATES ON OVIPOSITION OF *TENEBRIO MOLITOR*

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In a human's diet, insects could partially replace animal meat as they are very nutritious, reproduceand grow fast, mass rearing generates less pollutants compare to those produced in animal farms. Mealworms (*Tenebrio molitor* L.) are easy to cultivate, have a relatively large biomass increase, and are great sources of proteins. For insect breeders, it is essential to reduce the cost of production. Stimulation of egg-laying would be beneficial for reproduction increase. Assuming that females lay more eggs in a moresuitable substrate than in a less suitable one, we analyzed if chemosensory cues perceived by antennae could influence oviposition The following three tests were performed:

- Intact gravid females were allowed to lay their eggs in wheat flour;
- Females with removed antennae were allowed to lay eggs in wheat flour;
- Females with both antennae were allowed to lay eggs in quartz sand.

It was found that chemosensors on antennae do not influence egg laying activity - there was no statistically significant difference between the number of eggs laid by females either with intact or removed antennae (survival after surgery was not altered during 30-day period, while the duration of the test was 5days). However, females differentiate the substrate for oviposition: there was a statistically significant difference between the number of laid eggs in flour and sand. Further research is needed to reveal chemical cues involved in oviposition of *T. molitor* females.

B.7. CAN DAILY COLLECTION OF INVASIVE SLUGS IN SUMMER SAVE LITHUANIA FROM AN INVASION?

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Spanish slug (*Arion vulgaris* Moquin-Tandon, 1855) is a rapidly spreading invasive species in Europe, causing significant ecological and economic damage [1]. The main impact on biodiversity noted by scientists has been the decline in numbers and disappearance of local slugs in areas where *A. vulgaris* is abundant and invasive due to hybridization with local species [2]. In 2013, this slug was first recorded in Lithuania and in 2016 was added to the list of invasive species [3].

Good resistance to high and low temperatures, the ability to mate with other species and dietary flexibility helps the species to establish itself in new environments. To date, there are no standardized means of population control for A. *vulgaris*. No natural enemies have yet been shown to substantially reduce the populations of these slugs. The most popular control measure in Lithuania is hand-picking of the slugs and killing them with salt, although chemical control using ferric phosphate is available. Previous studies in VU laboratories [4] revealed that chemical control is not always effective enough, so the novelty of this work is that the newly approved molluscicide (containing ferric phosphate) in Lithuania was tested. Due to the fact that the majority of research is in a laboratory setting, lack of information about the possible impact of different weather or climate conditions makes it difficult for regular citizens to help in the fight against the invasive species, considering chemical use may be more effective.

This research was carried out in the Municipality of Palanga (Lithuania) during 2022 July-August. Two plots of land close to each other and similar geographic conditions, with recent A. *vulgaris* invasions affecting them were chosen – one for daily hand-picking, one - for molluscicide use.

We have found that collection by hand doesn't have a noticeable effect on the structure of the slug population whereas the molluscicide reliably reduced the average slug weight. Molluscicide use was faster, required less work and was more effective at reducing the numbers and possible killing of slugs (Fig. 1).

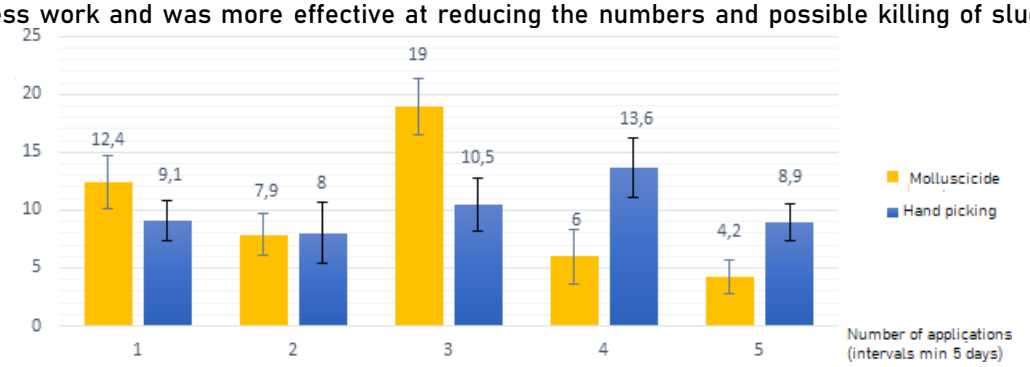

Figure 1. Abundance of invasive slugs (ind./m^2± SD) before and after application of molluscicide.

This research also showed that although rain and high temperatures had an effect on the average number of collected slugs, there were undeniable differences between hand-picking and chemical use effectiveness.

In conclusion, daily collection of slugs in summer can't save Lithuania from the invasion. However, application timing of molluscicide should be taken into consideration too: people usually use it in the middle of summer when they see large breeding slugs, so research will continue this year to evaluate the effectiveness of the product according to the professional recommendations, applying the molluscicide in early spring.

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B.8. EFFECTS OF QUANTUM DOTS ON BROWN TROUT

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Quantum dots (QDs) are a type of engineered nanoscale particles composed of a small number of atoms, possessing exceptional optical, electrical, and catalytic properties, which make them popular in fields such as biological imaging, electronics, solar power, and electrochemical sensing [1]. However, the increasing use and release of QDs into the environment has raised concerns about their impact on the environment and human health. Improper disposal or breakdown of QDs can result in the release of heavy metals into the environment, which can accumulate in fish tissues and lead to negative effects on fish populations. In addition, the use of QDs in various applications can increase their presence in the environment, highlighting the need for research to understand their environmental impact and develop effective strategies to mitigate potential harm. QDs can accumulate in the food chain and potentially harm aquatic life, such as fish, mollusks, and algae [2]. Metallothionein (MTs) are among the few biomarkers of oxidative stress and can be used to assess the effects of QDs on fish and other aquatic organisms [3]. MTs are small, cysteine-rich proteins that regulate metal levels, including cell growth and division, and serve as biomarkers of exposure to heavy metals and other toxicants [4]. MTs have a strong affinity for heavy metals and bind to foreign heavy metals to protect against toxicity [5]. When attached to necessary heavy metals such as copper and zinc, MTs control cell growth and division and defend against oxidative stress [6]. They also regulate the balance of zinc and copper within cells, which is important for cell division and differentiation, and act as antioxidants against free radicals and oxidative stress caused by mutagens, radiation, and antineoplastic drugs [7]. This ability to bind heavy metals makes MTs a reliable indicator of exposure to heavy metals and other toxicants and a means of assessing potential harm from exposure.

The aim of this study is to determine the level of MTs in the liver of brown trout after exposure to QDs. The MTs level was measured through a colorimetric method, using Ellman's reagent. The obtained results showed no significant change in MTs level as a result of exposure to QDs compared to the control group, but showed an increase in MTs level during exposure time. Studying the effects of QDs on aquatic organisms and their environment is important to ensure their safe and sustainable use and to inform risk assessments, environmental policies, and regulatory measures. Additionally, understanding the impact of QDs on organisms and the environment could improve the safety and sustainability of industries that rely on them and protect human health and the environment.

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B.9. IMPACT OF UV LIGHT ON TOXICITY AND DEGRADATION OF ACTIVE SUBSTANCE THIAMETOXAM IN NEONICOTINOID PESTICIDE ACTARA

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Pesticide overuse is a modern-day agriculture problem, which may cause significant damage to natural ecosystems, and it poses a threat to human health. One of the most widely used classes of insecticides are neonicotinoids [1]. These pesticides are neurotoxins which are used for leaf and tree trunk treatment, coating plant seeds or for spraying onto soil directly in order to control pests [2]. One of the biggest concerns of pesticide use is their effect on non-target organisms. High water solubility of neonicotinoids also contributes to its harmful impact, since pesticide which was directly sprayed onto soil, can be dissolved in runoff water and transferred to surface water bodies and can negatively affect the diversity of aquatic organisms. It is known that neonicotinoid thiamethoxam can undergo photodegradation [3]. However, there is a lack of data on the rate of this process speed and the toxicity of its byproducts.

The objective of this research was to study the impact of UV light on the toxicity of thiamethoxam based neonicotinoid pesticide Actara WG 25 to model biotest organism *Vibrio fischeri* and on levels of thiamethoxam degradation. Commercially available pesticide Actara WG 25 was exposed to UV light for different periods of time (10, 30 and 60 min). The toxic effects of untreated and UV treated pesticide samples were determined using bacteria *Vibrio fischeri* bioluminescence inhibition test. The comparison of medium effective concentrations (EC_{50}) of untreated (EC_{50} = 45.52 mg/L) Actara WG 25 and treated with UV light for 10 min showed that after 10 min exposure time, toxicity of pesticide was reduced more than twice (EC_{50} = 100.23 mg/L). When UV exposure time was increased up to 30, and up to 60 min, the effect to bioluminescence did not change significantly (EC_{50} = 109.21 mg/L; EC_{50} = 114.50 mg/L). Using fluorescent detection method, the impact of UV treatment to the degradation of Actara's WG 25 active ingredient thiamethoxam was evaluated. After conducting comparative analysis of UV treated and untreated Actara WG 25 suspension fluorescence spectrum under the excitation of 230 nm, it was determined that in all cases the highest peak was detected at 312 nm. When time of UV treatment was increased, the intensity of fluorescence at this wavelength was correspondingly reduced. Based on the obtained results it was concluded that UV treatment reduces toxic effect and the concentration of the active ingredient thiamethoxam in pesticide Actara WG 25.

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B.10. PREVALENCE OF *SARCOCYSTIS* PARASITES IN ENVIRONMENTAL SAMPLES FROM LITHUANIA

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Sarcocystis are intracellular protozoan food-borne parasites that can pose a threat to animal and human health [1]. Due to significant economic, medical, and veterinary impacts, sarcocystosis is an important public health problem worldwide [2]. However, there is a lack of information on *Sarcocystis* spp. infection in livestock farms. To date, the prevalence of *Sarcocystis* infection was mainly studied by analyzing animal carcass. Therefore, the aim of this study was to determine the prevalence of *Sarcocystis* parasites infecting livestock from environmental samples in Lithuania using a molecular-based method.

Samples of water, hay, and soil were collected from 10 livestock farms in Lithuania during the summerof 2022. Water samples were concentrated directly using the filtration method [3], while hay samples were washed in a 1 L glass beaker before filtration. Concentrated water and hay samples were used for gDNA isolation, meanwhile, soil samples were used for direct gDNA extraction. *Sarcocystis* parasites were identified using nested PCR targeting *cox1* gene. Primer pairs suitable for the identification of *S. cruzi*, *S. bovifelis*, *S. arieticanis*, *S. tenella*, *S. capracanis*, *S. bertrami*, and *S. miescheriana* were selected [4]. Based on molecular methods, *Sarcocystis* spp. DNAs were detected in all investigated farms.

Comparing the prevalence of *Sarcocystis* species in different types of environmental samples, the highest occurrence rate was found in the hay (43%) and water (40%) samples. The lowest number of *Sarcocystis* parasites were detected in samples from soil (31%), which may have been due to the relatively small amountof the tested soil sample. *S. cruzi* (70%) infecting cattle has been identified as the most common species inhay samples. A high infection rate was also determined for *S. tenella* (50%) pathogenic to sheep and *S. bertrami* (50%) infecting horses. The distribution of species in water samples was quite similar, e.g., high detection rate (60%) was estimated for *S. cruzi* and *S. arieticanis*, meanwhile, moderate detection frequency (40%) was established for *S. tenella*, *S. bertrami*, *S. miescheriana*. The highest contamination in the soil samples was determined by *S. bertami* (60%) and *S. arieticanis* (50%) species parasites, while identificationrates of other species exceeded 10–30%. Comparing the prevalence in individual farms, *Sarcocystis* spp. were detected in all 3 types of environmental samples (water, hay, soil) in 6 out of 10 farms. Meantime, in two farms *Sarcocystis* were identified only in water and hay samples, and in one farm only in hay and soil samples. Usually, five (40%) or six (30%) different species were identified in individual farm, rarely three (20%) or seven (10%) species. To conclude, the most common *Sarcocystis* species in livestock farms in Lithuania were *S. cruzi* (53%), *S. arieticanis* (50%), and *S. bertrami* (50%).

This study is the first to analyze the prevalence of *Sarcocystis* spp. in different environmental sources (water, hay, soil). The prevalence of the analyzed *Sarcocystis* species did not vary significantly between different types of examined environmental samples. It can be concluded that domestic animals have an equal chance of becoming infected with *Sarcocystis* parasites regardless of environmental source.

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B.11. THE EFFECT OF POLYSTYRENE DIET ON THE FITNESS OF *TENEBRIO MOLITOR* AND *ZOPHOBAS MORIO* LARVAE

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Polystyrene is a valuable material, widely used for insulation or packaging. Driven by the many applications, global demand for the polystyrene is high: in the 2020 it had reached nearly 18.6 million metric tons. By 2026 the demand is expected to reach 20.8 million metric tons [1]. In most cases polystyrene packaging is used only once, later it becomes a pollutant. The biggest problem with polystyrene is that it does not degrade easily and stays in the environment from decades to centuries, or even longer, if it is not exposed to the sunlight [2] or mechanical abrasion. Growing public awareness and amounts of nondegradable waste leads to the search of novel ways of disposal. One of such ways could employ insects, as some of them have been observed to possess plastic eating behaviour. Among those – *Tenebrio molitor (L.)* [3] and *Zophobas morio (F.)* [4]. The aim of this study is to determine whether polystyrene consumption affects *Tenebrio molitor* and *Zophobas morio* survival and fitness. The experiment consisted of two treatment groups and two control groups. First treatment group had been fed oatmeal supplemented with dry yeast and polystyrene. Second treatment group was fed with polystyrene only. Yeast supplemented oatmeal diet was fed to the first control and the second control was not given any food. Experiment was conducted for twenty days. Dead larvae were counted, and the weight of alive ones was measured every two to four days. The data has shown that the mortality rate of the larvae of both species does not depend on the chosen diet. The findings show that polystyrene consumption is not lethal for either of insects (*Tenebrio molitor* larvae survival rate was 100%, *Zophobas morio* larvae survival rate was 98.33%) and it does not have an effect on their fitness. *Zophobas morio* is more effective at consuming polystyrene than *Tenebrio molitor*. When larvae were fed with polystyrene only, *Zophobas morio* larvae consumed almost twice as much polystyrene as *T. molitor*.

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B.12. EVALUATION OF THREE FUNGI SPECIES WOOD DEGRADATION ABILITES IN VITRO

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Xylotrophic fungi occupy an important niche in ecosystems by degrading various wood polyphenolic molecules, polysaccharides and other compounds. For these processes, the fungi commonly use extracellular enzymes, which, depending on the type of wood and the relative degradation rate of the polymers, lead to either white or brown rot. Understanding the processes by which these phenomena occur can not only help to appreciate the delicate energy transactions of ecosystems, but also help in maintaining the quality of man-made wooden structures and help the similar enzyme employing paper, beverage, pharmacology, and other industries.

The aim of this work is to quantitively evaluate the wood biodegrading capabilities of three fungi species – *Flammulina velutipes, Fomitopsis pinicola* and *Trametes versicolor*. This is accomplished by assessing the difference of dry mass between fresh, and fungal enzyme affected standardized wood blocks, *in vitro*. The dry mass value is established by drying the wood blocks at 65 °C temperature, followed by individually weighing them 3-4 times until the mass value stabilizes.

Pure fungal cultures were obtained from naturally occurring xylotrophic fungi sporophores, according to P. Stamets (1983) methodology [1]. 13-day old pure culture mycelium in MEA medium was used.

Standardized wood material was prepared according to the methodology of Zvyagintsev et al. [2]. Wood was acquired from 11 native tree genera – *Acer, Betula, Fraxinus, Larix, Picea, Pinus, Populus Quercus, Salix, Tilia,* and *Ulmus*. The wood of tree genus *Entandrophragma* was acquired commercially, as it is non-native to Europe, but widely used locally for furnishing and decoration.

The experiment was conducted in MEA medium by following a particular design: in the middle of a Petri dish lay a 10 mm diameter disc of mycelium and around it symmetrically lay 4 sterile standardized wood blocks. 3 Petri dishes, representing 3 replications, were used for each tree genera wood – a total of 12 specific wood blocks for each of the 3 fungi species tested. In total, 456 wood blocks were included in the study.

Once 40 days had passed after full wood block colonization by the mycelium, they were taken out, carefully cleaned, dried, and weighed according to the previously described method. The mass difference was assessed. This routine will be repeated, accordingly, 80 and 120 days after wood colonization by the fungi.

After 40 days, *F. velutipes* had induced the most mass loss in wood of *Quercus* and *Acer* (9,75 %), and the least in *Picea* wood (2.05 %). *F. pinicola* had the biggest effect on the wood of *Quercus* (7.6 %) and *T. versicolor* induced the greatest mass loss in *Acer* wood (11.62 %).

This work will be supplemented by a qualitative evaluation of the three fungi species' oxidase-type enzyme activity, according to the reagent-based methodology of Gramss et al. (1998) [3].

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B.13. ANALYSIS OF THE DEPOSIT SYSTEM IN LITHUANIA AND ASSESSMENT OF DEVELOPMENT OPPORTUNITIES

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Continuous human consumption leads to the generation of waste, which is mostly landfilled [1]. People's consumption behaviour is a key parameter of environmental pollution [2]. In Europe, 10% of waste is reused [3]. The landfill system is one of the solutions to develop the circular economy and improve the sustainability of the system, as it focuses on the reduction, reuse and recycling of its elements [4]. The problematic question is how would the public react to an extended deposit system in Lithuania?

The aim of this Master's thesis is to analyse the deposit system in Lithuania and to assess the possibilities for its expansion. The objectives of the thesis are: to show the relation of the deposit system to the circular economy; to identify the deposit systems in the European Union countries and the concept of the deposit system in Lithuania; to analyse the amounts of recyclables collected in Lithuania after the introduction of the deposit system; to find out the opinion of the population on the deposit system and to compare the differences in opinion between different groups of the population; to assess the opinion of experts on the deposit system.

Scientific literature was analysed and used to design the questionnaire and interview questions. Data analysis was used to find out the number of collections of plastic, glass and metal packaging following the introduction of the deposit system. A questionnaire survey was carried out in 2022 and 202 questionnaires were collected. In the same year, interviews with four experts were conducted. MS Excel and PAST were used to process the data obtained. The results of the interviews are divided into categories with sub-categories based on the experts' statements. A SWOT analysis was drawn up from the data obtained.

The aim of the deposit system is to prevent waste from being dumped in landfills but to reuse it. The disposal system is about reducing pollution, saving energy and raw material resources and economic benefits due to the cost of producing the product. In Lithuania, a deposit system is used for used beverage packaging, where consumers pay a deposit fee for the packaging and reclaim their fee when they return it to certain collection points. There is no common deposit rate and labeling system in the EU countries, nor is there a common capacity for collection. The amount of recyclables collected in Lithuania is increasing year on year, reducing the negative impact on the environment. The majority of respondents and experts are positive about the deposit system and the potential for its development, as it is efficient, the amount of waste collected is recycled, and the culture of waste is improving. More education is needed to achieve acceptance by consumers, producers, traders and importers. Women, respondents who return the deposit themselves and residents with knowledge of waste management are more positive about the deposit system and its development.

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B.14. IDENTIFICATION OF ROOT ENDOPHYTE FUNGI IN FESTUCA GIGANTEA

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Endophytic fungi are associated with almost all plants in natural ecosystems. Fungi are common inperennial grasses and may be specific to a particular host plant. Different endophytic fungi can be detected in the host plant, and the actual number may vary up to dozens. A number of studies have been conducted to identify and analyze the influence of endophytes on the host plant, but there is no data on endophytic fungi in the root system of *Festuca gigantea* (Poaceae). *F. gigantea* is a perennial grass common in woodlands, preferring shady places. The aim of this study was to identify and determine the frequency of endophytic fungi in the roots of *F. gigantea* by examining plant samples from Vingis park in Vilnius. We calculated the frequency of endophytic fungi in cytological root specimens by staining them with Trypan Blue (0.025%). Endophyte fungi were found to colonize 40 percent of *F. gigantea* roots. Endophytic fungi were isolated from two hundred 1–2 cm long root segments on PDA medium by incubation at 27℃ in the dark. For the isolation of the genomic DNA of endophytic fungi, 10–day–old fungal colonies were used. The sequence data of rDNA ITS, *ACT*, *TEF*, *SSU*, and *RPB2* were obtained and aligned to fungi reference DNA data by BLAST. The morphological structure assessment of the mycelium of endophytic fungi was carriedout by describing the color and other characteristics. The microscopic structure was analyzed from the mycelium specimens mounted in glycerol : lactic acid (1:4) under the phase-contrast microscope. Our results show that *F. gigantea* roots are colonized by five species of endophytic fungi: *Alternaria alternata*, *Cadophora* sp., *Coprinellus disseminatus*, *Microdochium bolleyi* and *Sistotrema brinkmanii*. This is the firstreport describing endophytic fungi in the roots of *F. gigantea*.

B.15. CONDITION FACTOR ANALYSIS OF *SALMO SALAR* IN LITHUANIA DURING SPAWNING MIGRATION

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Atlantic salmon (*Salmo salar* L.) is one of the world's best-known and most economically important fish species that has attracted scientific interest due to their overall decreasing stocks and spawners migration alterations. Scientists conducted studies to identify the factors that caused Atlantic salmon stocks to exceed sustainability and collapse in eastern North America and western Europe. Climate change, predation, parasites, decreasing food availability at sea, and escaped farmed salmons have been identified as the major threats to the wild Atlantic salmon population in recent years [1], [2], [3]. Salmonids population conservation and restoration programs are being implemented in Lithuania and across the world. Unfortunately, there is a lack of knowledge on the health status of Atlantic salmon throughout their spawning migration. The study was carried out to examine the changes in the Atlantic salmon condition factor over a four-year period (2019-2022) and to investigate possible differences between male and female during their spawning migration. The results revealed significantly lower levels of Atlantic salmon condition factor in 2021 when compared to the previous investigation year and the following year (both p=0.001). The condition factor of males and females of Atlantic salmon was similar, corresponding to equivalent life conditions of individuals during the respective research year. However, the obtained data revealed that the condition factor of Atlantic salmon females was significantly lower in 2021 compared to the entire study period, which may be attributed to the small sample size and high standard deviation of the mean. More detailed information regarding the health condition of Atlantic salmon throughout the spawning migration period is required to make recommendations for future research targeted at conserving declining salmon populations.

Acknowledgments: this research was funded by the Research Council of Lithuania, Project No. S-MIP-21-10, MULTIS.

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B.16. DI(2-ETHYLHEXYL)PHTHALATE AND DIBUTYLPHTHALATEGENOTOXIC EFFECT ON RAT ERYTHROCYTES

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Phthalates are substances that have mutagenic and endocrine disrupting properties and have a tendency to accumulate in the environment and human body [1]. They reach only the amount in micrograms but, when released into the water, are likely to pose a significant risk to the health of people and ecosystems. The aim of this project was to evaluate the effect of two types of phthalates (which consistently exceed themaximum allowable concentration in Lithuanian waters) in rat bone marrow cells using an *in vivo* micronucleus assay. The rats were supplied from Department of Biological Models (Vilnius University) and were kept here as well. The study has been reviewed and approved by the Ethical Committee on Animals Experiments of State Food and Veterinary Service (2022-08/09 No G2-221). To find out the genotoxic effect, an analysis of micronuclei in polychromatic erythrocytes of rats was carried out.

Female rats of *Wistar* straine, 5-8 weeks old, were divided into control and 5 experimental groups, which get standard food and additionally received a piece of ecological biscuit with different doses ofphthalate dissolved in olive oil: 1) DEHP 200 µg/kg; 2) DEHP 1000 µg/kg; 3) DBP 100 µg/kg; 4) DBP 500 µg/kg; 5) mixture of phthalates (DEHP 200 µg/kg, DBP 100 µg/kg). Control animals received only a piece of the same biscuit with oil and without phthalates. At the end of the study, after 2 months, the rats were killed in a CO_2 chamber. Later, during the autopsy, femurs were dissected and bone marrow slides were prepared for theobservation of micronuclei in polychromatic erythrocytes [2]. Micronucleus analysis was performed by calculating 2000 PCE (polychromatic erythrocyte) and the ratio of PCE and NCE (normochromatic erythrocyte) (Fig.1) and data were analysed using one-way ANOVA. Statistical analysis was performed usingGraphPad Prism 9.0.0.

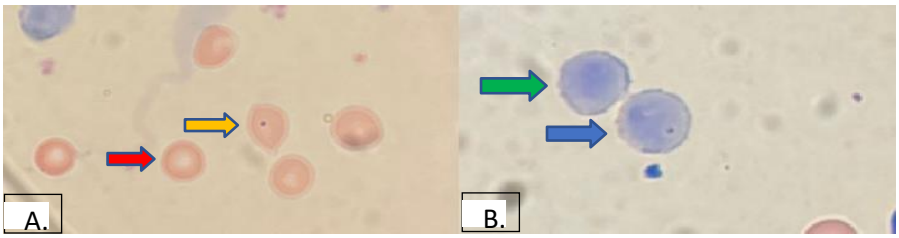

Figure 1. Giemsa-stained rat bone marrow cells: (A.) Normochromatic erythrocyte (NCE) shown by red arrow and NCE with micronucleus shown by yellow arrow; (B.) Polychromatic erythrocyte (PCE) shown by green arrow, PCE with micronucleus shown by blue arrow. Imagination: × 100 oil immersion.

It was found that even small doses of phthalates during daily continuous consumption have a negative genotoxic effect on rats. All results were statistically significant ($p<0.05$). A direct correlation was found with increasing phthalate dose, the number of micronuclei in PCE also increased. Significantly more micronuclei were found in bone marrow preparations of rats exposed to DBP than to DEHP.

The results of the study reveal that it is necessary to regulate the amount of phthalates in Lithuanian wellfields more effectively and to regulate wastewater treatment more strictly.

Acknowledgments: We would like to thank dr. Virginija Bukelskienė for help in organising the research, medical students Justina Alčauskaitė and Evita Sėrikovaitė for help in weighing the rats and preparing the biscuits for the study experiment. This project was financed by the Science Promotion Fund for Scientific projects of Vilnius University, agreement No MSF-JM-18/2022.

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B.17. COMPARISON OF THE EFFECTIVENESS OF MOLLUSCICIDES FOR INVASIVE SLUGS

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Arion vulgaris (Moquin-Tandon, 1855) (syn. *Arion lusitanicus*, Mabille, 1868) belongs to the Arionidae family, and is considered one of the hundred most invasive species in Europe [1]. In Lithuania, it has been included in the Lithuanian list of invasive organisms since 2016. According to the European Parliament and Council Regulation (EU) No. 1143/2014 "On the prevention and management of the introduction and spread of invasive alien species" Lithuania must implement effective management measures for this species as well as for other species. When transitioning to a green economic course, metaldehyde molluscicides were banned in Lithuania, so in this study a comparison of the effectiveness of molluscicides with different amounts of ferric phosphate was performed.

The research was carried out in the Zoology scientific laboratory of Vilnius University (from July 10, 2022 until August 13, 2022) with slugs of different body mass (Fig. 1), collected in in the village of Bukiškis. Slugs were maintained in a Fitotron thermo–climate chamber (Wess Technik, USA) at 15°C, with a 12 h light/12 h dark photoperiod and 80% relative humidity [2]. Each slug was provided with a portion of food that comprised 5 g of lettuce ("Iceberg"), 2 g carrot ("Nanto"), one dry cat food pellet ("Purrrfect", Czech Republic),and two oatmeal grains ("Skanėja", Lithuania) per container. Each treatment contained 20 slugs of the same size group and five pellets of one molluscicide for each slug. We studied three body mass groups and three molluscicides approved in Lithuania, here named as M1, M2, M3 (Fig. 1).

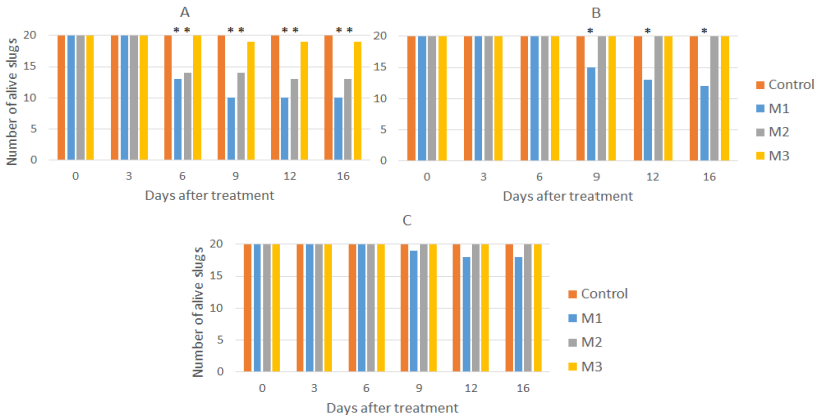

Figure 1. Number of alive slugs (body mass: A – 1,18±0,48 g; B – 2,97±0,64 g; C – 7,72±1,94 g) after treatmentof different molluscicides with different concentrations of active ingredient ferric phosphate: M1 (24.2 g/kg), M2(29.7 g/kg), M3 (9.9 g/kg). Asterisks indicate significant differences by Kruskal Wallis test, R (version 3.5.3).

Our results showed that the molluscicides reduced the consumption of food: the control slugs ate significantly more lettuce, carrot, oatmeal and cat food, comparing to the slugs in the containers with the molluscicides. By the way consumption of food differed in different groups: it depended on body mass and the amount of pellets eaten. The most effective molluscicide was M1 which contains 24.2 g/kg ferric phosphate and the most effective impact was on the smallest slugs.

Acknowledgments: Part of the study was supported by grants from the BASF SE (Germany) and the Vilnius University (Lithuania) R&D Service Agreement No (1.57) 15600-INS-43

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B.18. THE USE OF *UNIO TUMIDUS* FOR DETECTION OF WATER POLLUTION IN THE CURONIAN LAGOON

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In January 2020, a cardboard plant owned by Grigeo Klaipėda, a company operating in the city's seaport, was caught releasing untreated waste water into the Curonian Lagoon for years every night. That company has been polluting waterbody on the edge of the UNESCO's world heritage site, the Curonian Spit, since 2012.

Unio tumidus Retzius, 1788 was used for measuring impact of local pollution, as they are able to filter large quantities of water, are sedentary and could live more than 12 years [2]. Their interaction with pollutants was established in sediments and water column. The aim of this research was to determine genotoxic impact of total water pollution upon *U. tumidus* mussels inhabiting different areas of the Curonian Lagoon. The level of DNA damage was determined in the epithelial gill cells using the micronucleus test [1]. Samples of surface bottom sediments and water column were taken, mussels were collected upstream in relation to Klaipėda city from four distinct locations in the Curonian Lagoon in 2022. Water and sediment parameters, such as phthalates, phosphates, nitrates, ammonium, water hardness, ph, salinity, concentration of organic carbon and heavy metals (Cu, Zn, Ni, Pb, Cr, Cd, and Hg) were determined.

It was revealed that alive *U. tumidus* were only in Ventės ragas and Dreverna where fresh water circulated. It should be noted that the salinity level was increased in those sites where molluscs were not found (Kairiai and Smeltė). We have found that *U. tumidus* individual biomarkers of genotoxicity did not show consistent trend and the cumulative criterion of damages did not reveal significant differences of pollution impact in Ventės ragas and Dreverna (Fig. 1).

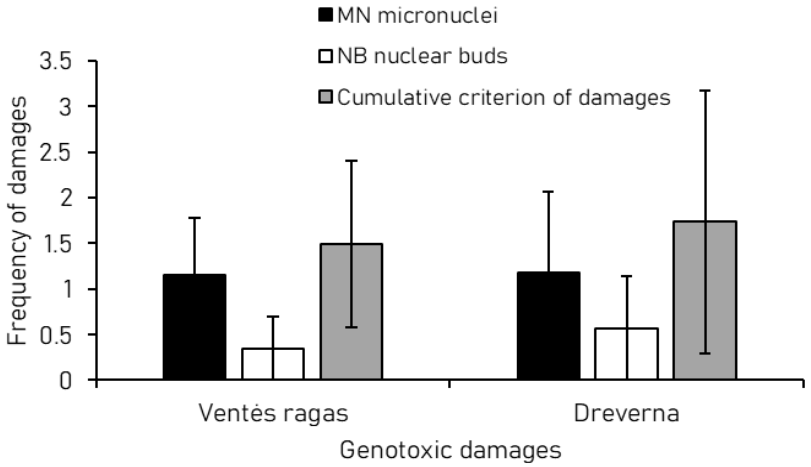

Figure 1. The frequency of genotoxic damages of gill epithelial cells of *U. tumidus.*

It was determined that the permissible norms of water quality only in Ventės ragas were exceeded by dibutyl phthalate (DBP = 0,2 µg/L). There were also measured the highest concentrations of Di(2-ethylhexyl)phthalate (DEHP = 0,1 µg/L) and Lead (Pb = 1 µg/L).

In conclusion, local impact by Grigeo Klaipėda pollution remains unclear because no molluscs were found close to the pollution source. Thus other methods need to be researched.

Acknowledgments: We are thankful to Dalia Juškevičienė from Curonian Spit National Park for accommodation and provided data; Dr. Milda Stankevičiūtė and Dr. Roberta Valskienė for methodological consultations; Normantas Volodko and Jonas Skuja for help in collecting molluscs. This project has received funding from the Research Council of Lithuania (LMTLT), agreement No P-ST-22-232.

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B.19. CHALLENGES INVESTIGATING TRYPANOSOMATIDS INBLOODSUCKING DIPTERA

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Trypanosomatids are a big group of Protozoa, which are subdivided into two non-taxonomic groups: dixenous trypanosomatids develop in two different hosts (vertebrate and invertebrate), while monoxenous develop only in invertebrate hosts. Parasites belonging to genus *Trypanosoma* infect various vertebrate hosts, including reptiles, birds and mammals. Avian *Trypanosoma* are less studied trypanosomatids, although almost one fifth of all known *Trypanosoma* are found in birds [1]. Vectors of avian trypanosomes are various bloodsucking dipterous insects – biting midges, mosquitoes and blackflies. These insects are being used as a hosts not only by *Trypanosoma* parasites, but by monoxenous trypanosomatids as well. Mixed infections of dixenous and monoxenous parasites in insects are very common in the wild. Nowadays molecular methods are highly used to detect parasites in their hosts, however, mixed infections make it difficult to detect target species. Research from previous years [2] showed that the widely used primers amplify the 18S rRNA gene fragment of all trypanosomatids including both dixenous and monoxenous. In case of mixed infection these primers often underestimate *Trypanosoma* parasites. Our results show that wild dipterous insects can be infected with monoxenous trypanosomatids up to 13 % and laboratory reared insects can be infected even more if the infection enters the insect colony. This is an obstacle to obtaining trusty results in vector research if using just PCR-based methods.

The aim of this study was to develop and test primers which would detect only parasites from the genus *Trypanosoma* which would help to detect trypanosomes in insects infected with mixed trypanosomatid infections. For this purpose, we tested samples from experiments carried out in 2018 and 2022. Both wild-caught and laboratory reared mosquitoes (Culicidae, n = 120) and biting midges (Culicoides, Ceratopogonidae, n = 41) were experimentally infected with avian *Trypanosoma* parasites. Microscopy of insect gut preparations, traditionally used nested PCR and sequencing were carried out to test the samples. All samples in which mixed infections or infections with monoxenous trypanosomatids were found were tested with newly developed primers TRYP F and TRYP R. Overall 15 samples out of 17 were positive for *Trypanosoma* parasites using newly developed primers TRYP R and TRYP F. The use of both traditional and newly developed primers in parallel should be used for detecting mixed monoxenous trypanosomatid and *Trypanosoma* infections. Our newly developed PCR primers proved to be effective distinguishing *Trypanosoma* parasites in both wild-caught and laboratory reared dipterous insects. Although it might be a useful tool in trypanosomatid research, further research is needed.

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B.20. CAN AVIAN MALARIA DISRUPT MICROBIOME OF THE HOST?

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Avian malaria parasites are a group of protozoans, which have a complex life cycle and are transmitted by mosquitoes. Avian *Plasmodium* can infect a wide range of bird species worldwide and have an impact on various aspects of their host's biology while causing severe illness or even death [1]. The mechanisms of how avian malaria parasites affect host health are not well known; however, recent studies have shown that parasites could affect host immunity through microbiome modulation [2]. In this study, we investigated whether experimental infection with avian malaria parasite, *Plasmodium homocircumflexum*, causes changes in the gut microbiome of birds at different stages of infection.

We collected faecal samples at different time points throughout the experiment and used 16S rRNA amplicon sequencing to characterize the microbial communities in infected and control birds. Our findings show that avian malaria infection did not have a significant impact on bacterial diversity of infected birds. However, taxonomic and functional profiling of microbiome in infected and control birds revealed an emergence of infection-specific taxa and pathways at later stages of infection. Differential abundance and co-occurrence network analysis showed the changes in the microbiome assembly of infected birds, affecting its structure and robustness.

Presented results shows that avian malaria infection can alter the gut microbiome of birds, causing deviation from normal development, which potentially could impact host health.

The study was funded by the FGIAP (No. ANR-10-LABX-62-IBEID) and the Research Council of Lithuania (No S-MIP-22-52).

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B.21. SALMO TRUTTA JUVENAILES BODY CONDITION COMPARISON BETWEEN WILD AND STOCKED SPESIMENS

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Supplemental fish stocking is one of the most popular and effective way to restore and support local fish populations worldwide. The main aim of fish restocking plans can be achieved only if the stocked fish can grow qualitatively under natural conditions [1]. Artificial stocking method is widely used for brown trout (*Salmo trutta*, hereafter *S. trutta*) populations enhancement in small Lithuania lowlands rivers by the Fisheries Service under the Ministry of Agriculture of the Republic of Lithuania, but there is lack of knowledge about wild and artificially stocked (*S. trutta*) fry specimens' body condition – do they differ in the first year of life?

Naturally spawned/ hatched and artificially incubated/ reared fry specimens of the wild *S. trutta* populations were studied for four year period (2019-2022) in natural Plaštaka river, a fifth order tributary (Nemunas, Nėris, Šventoji, Siesartis and Plaštaka rivers) of the Nemunas river. Because of the possible negative genetic effects, the annual S. trutta stock augmentation in Lithuania is conducted by using juveniles produced from wild stock spawners, obtained from the fourth river Siesartis every year. To separate artificially stocked *S. trutta* juveniles from wild ones, marking procedure by immersion of 100 mg L^{-1} Alizarin red S dye for 3 hours was applied. After a post-marking 2-day acclimatization period, marked specimens were released directly in the same 100 m long section located 435-535 m up of the researched stream mouth.

S. trutta fry catchment survey was performed by running electrofishing survey. To evaluate the growth of stocked and natural *S. trutta* we measured total length (TL, cm) and weight (W, g) of every fish specimen (g) and then used the data to calculate Condition factor (K), which is broadly used for standardized assessment of fish condition [2].

The results show that despite statistically significant difference between some of the measured TL or W through the years, there was no statistically significant difference in calculated K among wild and stocked

S. trutta juveniles' specimens at the end of each study season. Obtained results in difference may be related to similar research which proved that stocked *S. trutta* juveniles grow bigger due to hatchery diet and environment, although such individuals are at high risk of potential energy deficits caused by lower ability of feeding on wild prey, reduced swimming abilities or stamina as well as higher metabolic rates [3]. Results of this study further suggest that stocked *S. trutta* adaptation and further growth may vary across different latitudes and ecosystems. Therefore, in order to reach development of sustainable management strategies of fisheries in the future, a deeper understanding of the interactions of stocked and wild S. trutta juveniles in the natural environment are of great fundamental and applied importance.

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B.22. IN VITRO EVALUATION OF LOW-DENSITY POLYETHYLENE MICRO(NANO)PLASTICS EFFECT ON HUMAN AIRWAY EPITHELIUM

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Micro(nano)plastics (MnPs) are defined as plastic particles with a diameter of <5 mm for microplastics, and <1 µm for nanoplastics. MnP pollution has become a major concern worldwide, with the potential to cause adverse health effects. Airborne MnPs have been found in various environmental matrices, including the air we breathe. Recent studies have shown that micro(nano)plastics can penetrate deep into the human lungsreaching the alveolar regions [1]. The limited data available on the effects of airborne MnP on pulmonarytoxicity highlights the urgent need for more research in this field. Low-density polyethylene (LDPE) is a commonly used plastic material that can degrade into micro(nano)plastic particles. It is among the mostcommon polymers detected in atmospheric dust [2]. However, current in vitro research on micro(nano)plastics' cellular effects is mostly focused on polystyrene beads. Therefore, in this study, weaimed to investigate the biological impact of less explored LDPE MnPs of various shapes and sizes on human airway epithelium.

LDPE MnP was produced using photocatalysis for the degradation of LDPE granules (laboratory standard), producing MnPs of different sizes and shapes similar to native atmospheric plastic particles. Normal human bronchial epithelial cells BEAS-2B were exposed to different concentrations of LDPE MnP for 24 and 48 hours. After exposure, intracellular particle distribution, cell viability, morphology, and metabolic response were evaluated using various assays. Our results showed that exposure to LDPE micro(nano)plastic particles resulted in a significant decrease in cell viability and proliferation at higher concentrations. Furthermore, exposure to LDPE MnP altered cell morphology causing epithelial cell elongation and significant change in cell shape index. Atomic force microscopy was employed to observe the intracellular distribution of MnP. Moreover, the altered energy metabolism induced by LDPE MnP was confirmed by a Seahorse Real-Time Cell Metabolic analysis.

In conclusion, these findings suggest that exposure to LDPE micro(nano)plastic particles may haveadverse effects on human airway epithelium by interacting with the cellular membrane, inducing phenotype change, reducing viability, and changing the regular cell metabolism. Further studies are needed to determinethe long-term effects of exposure to LDPE MnP on human health and the environment. This study highlights the importance of reducing plastic waste and implementing sustainable practices to protect public healthand the environment.

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B.23. RESEARCH ON DUST POLLUTION BY HEAVY METALS IN SCHOOLS OF VILNIUS CITY

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The importance of an indoor microenvironment in schools for children's health is widely recognized.Many reviews and original researches have determined a relationship between indoor school exposure and respiratory morbidities in school children [1]. Children are significantly affected by the impact of the environment due to biological immaturity, and ongoing prenatal and postnatal lung development, hence, health professionals should increase their role in managing the exposure of children [2]. The large surfaceareas of indoor settled dust can adsorb large amounts of heavy metals that originated from various indoorsources as well as from outdoor sources. Most of the heavy metals with toxic vulnerability to the environment originated from anthropogenic activities as well as a natural constituent of soil minerals and can tenaciousto indoor settled dust [3].

This study aims to determine concentrations of cobalt (Co), chromium (Cr), lead (Pb), zinc (Zn), arsenic (As), copper (Cu) their contamination characteristics and health risks, in the samples of dust collected from 24 different schools in the Vilnius region and were investigated. X-ray fluorescence spectrometry was used to analyze the collected dust samples and determine concentrations of heavy metals. The geo-accumulation index (Igeo) and pollution load index (PLI) were used to determine the contamination level and Health risk assessment (HRA) index.

The purpose of this research was to investigate and determine the contamination of dust by heavy metals accumulated in schools located in different micro-districts of the city of Vilnius. Furthermore to distinguish zones in the city of Vilnius according to different levels of pollution determined by solid particles, to determine the concentration of heavy metals and compare with the results of other scientific studies.

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B.24. A NEW INTERMEDIATE HOST FOR *MICROSOMACANTHUS PARVULA* (KOWALEWSKI, 1904) (CESTODA: HYMENOLEPIDIDAE)

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The Cestoda (tapeworms) is a large group of endoparasites, the majority of which are found in the intestine of vertebrates. These worms have a complex life cycle involving one or two intermediate hosts. Theirlarval forms (cysticercoids) parasite in mollusks and leeches. *Microsomacanthus parvula* is quite widespread inEurope, from its western to the eastern borders [1]. Despite their broad geographic distribution, relatively little is known about interactions between this cestode and its second intermediate hosts *Erpobdella octoculata*, and final host waterfowl (duck).

The aim of this study was to investigate the helminth parasites infection in leeches in Lithuania. In the period of 2020-2022 between May-November, leeches were sampled from different slow-flowing and standing water bodies (lakes, ponds, streams and rivers). About 800 individuals of 9 freshwater species (*Alboglossiphonia heteroclite*, *Glossiphonia complanata*, *Helobdella stagnalis*, *Hemiclepsis marginata*, *Theromyzon tessulatum*, *Placobdella costata*, *Erpobdella octoculata*, *Erpobdella nigricolis* and *Haemopis sanguisuga*) were collected, and dissected in order to determinate their parasites. Each leech was compressed slightly between 2 glass slides and examined for the presence of parasites larve stage under a binocular microscope. Befor necropsy the leeches were anesthetized with 10 % ethyl alcohol. The cysticercoids found were further isolated from the leeches, examined using light microscopy and preserved in

70 % ethyl alcohol. The preliminary identification of the parasite larves is based on morphological characteristics. The molecular studies are planned in the near future. Among investigated leeches, cysticercoides of *M. parvula* were detected in the digestive system not only of *E. octoculata* but of *H. stagnalis* too. Of the 345 *E. octoculata* investigated, parasites were detected of 3 (prevalence 0,8 %) and of 298 *H. stagnalis* – 1 (prevalence 0,3 %).

It is the first record of *M. parvula* from *H. stagnalis* as an intermediate host.

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B.25. DIFFERENTIAL BLOOD CELL RATIONS IN RAINBOW TROUT (ONCORHYNCHUS MYKISS) AFTER MICROPLASTIC EXPOSURE

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Global plastics production increased by 4 % in 2021, reaching 390.7 million metric tons [1]. Microplastic (pieces of plastic less than 5 mm, MPs) is widely used in cosmetics, air blasting technology, as drugs vectors in medicine, but the main source is environmental weathering and forces breaking down larger plastic pieces [2]. MPs are present at all aquatic habitats, posing a major danger to health of aquatic biota. For this reason, MPs regarded as one of the main environmental issues of a global concern due to their toxicity effects.

Hematology is a promising area in fish physiological research. The blood indices are considered to be the vital physiological markers for examining the stress responses in fish caused by disease, toxicants, and other environmental stressors [3]. Differential blood count in fish is used as a non-destructive biomarker of exposure to pollutants [4].

The aim of the present study was to determine the effect of a long-term exposure to different microplastics polymers on differential blood count in rainbow trout (Oncorhynchus mykiss). During experiment the effect of MPs particles were investigated using most demanded plastic polymers in the European Union and most commonly founded polymers in marine habitats. After long-term microplastic exposure, the relative proportion of each type of leukocytes (lymphocytes, neutrophils (non-segmented, segmented and poly-segmented), eosinophils, basophils and monocytes) and thrombocytes was obtained by microscopic examination of 500 leukocytes per slide. Giemsa-stained fish blood smears were analyzed under the light microscope Olympus BX51 at 1000× magnification. Differential leukocyte count (DLC) and thrombocyte count (TC) were expressed as percentages (%).

Obtained results showed that there were no significant differences in DLC and TC in O. mykiss exposed to different microplastic polymers and those of the control group, except for TC which significantly increased in polypropylene-treated group.

Blood analysis is an effective method to evaluate the physiological status of fish. Blood analysis in the current study reveals alterations in hematopoietic regulation in MP-exposed fish. Yet, more thorough research on fish hematological parameters is required to characterize the harmful impacts of microplastic.

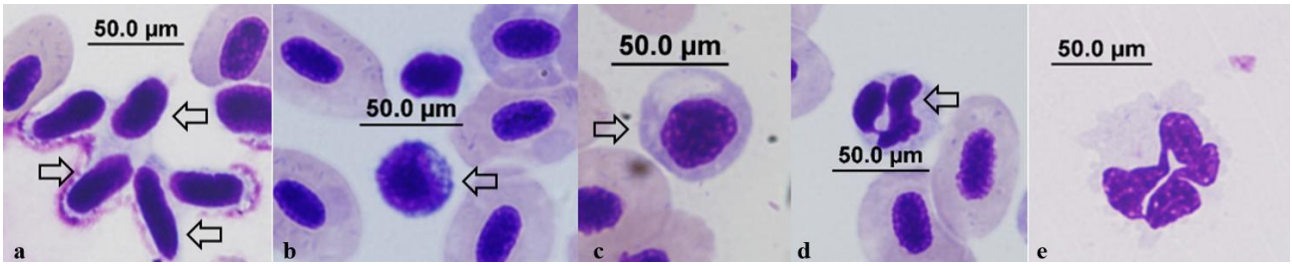

Figure 1. Different types of peripheral blood cells in O. mykiss: thrombocytes (a), lymphocytes (b), non-segmented neutrophil (c), segmented neutrophil (d), poly-segmented neutrophil (e).

Acknowledgement
This research was funded by the Research Council of Lithuania, Project No. S-MIP-22- 51, ARFA.

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B.26. TOXICITY STUDY OF GRAPHENE OXIDE AND METAL MIXTURES FOR MICROALGAE (Scenedesmus quadricauda) AND CRUSTACEANS (Daphnia magna)

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Graphene oxide (GO) due to unique structure and exceptional properties increasingly used in the various fields of industry and inevitably released into environment, where it can interact with different pollutants [1].

The aim of the presented study was to examine the toxicity of GO using microalgae (*S. quadricauda*) and crustaceans (*D. magna*) as the test-organisms and to evaluate the ability of GO to change the toxicity of tested metals on these organisms.

The metal mixture (MIX) (Ni 0.034, Zn 0.1, Cr 0.01, Cu 0.01 mg/L) was prepared according to maximum-permissible-concentrations (MPC) accepted for the inland waters. The mixtures (MIX1, MIX20, MIX40, MIX80) were prepared by increasing the MPC of every single metal by 20, 40 and 80 times. The concentrations of GO (1, 20, 40 and 80 mg/L) were chosen according to the previous study on the GO ability to adsorb metals. The toxicity of mixtures containing different concentrations of MIX and GO (MIX+GO) was investigated, also. The experiments were conducted according to the OECD (2011) and ISO 6341:2012 recommendations [2, 3].

Our data indicates that the tested concentrations of GO, MIX, and MIX+GO in the most cases affect the amount of algae cells and structure of their coenobia. The obtained data showed that single cell coenobia dominated in the culture of *S. quadricauda* exposed 96 h to all GO and MIX concentrations. Meanwhile, a higher amount of multicellular coenobia was observed in algae culture after exposure 96 h to MIX+GO concentrations. It was found that GO at all concentrations after 24 and 48 h exposure did not cause significant mortality of *D. magna* juveniles. Meanwhile, all tested concentrations of MIX caused significant mortality of *D. magna*, but MIX+GO caused significantly lower mortality. The results obtained showed that GO did not cause changes in heart rate of *D. magna* juveniles, both with increasing concentration and duration of exposure. However, MIX1 induced bradycardia of *D. magna* juveniles after 24 and 48 h. Meanwhile, after 24 h of impact of the lowest concentration MIX+GO, the heart rate of *D. magna* juveniles did not significantly differ from the control. So, the effect of MIX+GO on the heart rate of *D. magna* juveniles showed the ability of GO to reduce the toxic effects of metals on crustaceans.

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B.27. CLASSIFICATION OF UNDERWATER IMAGERY IN BALTIC SEA REEFS USING TEXTURAL EXTRACTION ALGORITHMS

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Underwater imagery is widely used in marine biology field for seabed habitat identification, classification and mapping. Underwater imagery is a favorable method for environmental scientists as it is relatively cost-effective, offers shorter field survey time and in turn allows more rapid collection of large amounts of data. Due to technological advances and better availability of underwater cameras, data collected underwater increased significantly over the last decade. However, because of laborious and time-consumingimagery analysis procedure there is an increasing need for automatic imagery classification techniques. Mostprevalent approaches for classifying objects is based on colour, shape and texture. Colour and shape are quite helpful for distinguishing objects in traditional classification tasks, however they are not as reliable underwater. As a result of different water properties, colours can change radically underwater. Furthermore, object shape is irrelevant when attempting to classify abstract shapes such as algae or sessile colonial organism coverage. Yet, different object textures may be more useful in object classification underwater.

In our work, multiple textural features extraction algorithms were investigated for geological and biological object classification. For textural features extraction we used underwater video material collected in SE Baltic Sea reefs located in Lithuanian marine area. We selected images that clearly represented biological or geological textures of boulders, sand, red algae *Furcellaria lumbricalis* and blue mussel *Mytilus edulis trossulus* (Fig. 1). Images were then divided into smaller (100x100 px) patches and 104 patches wereused for each class in the experiment.

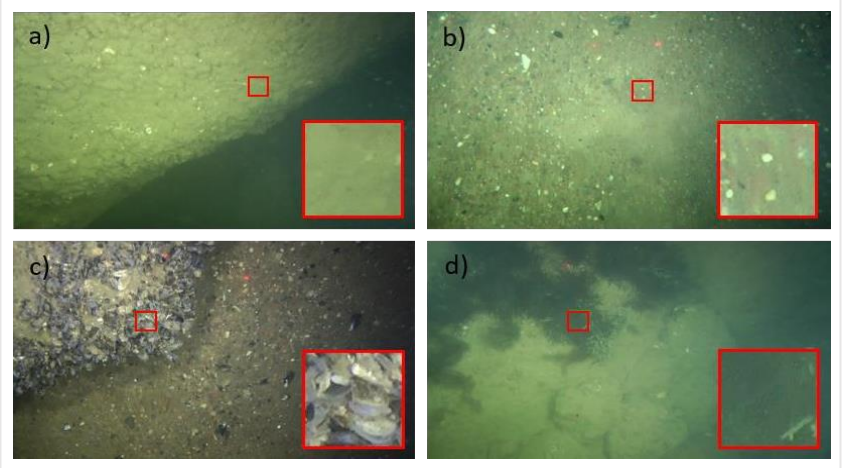

Figure 1. Texture examples of four classes with 100x100 px size patches a) Boulder b) Sand c) blue mussel *M. edulis trossulus* d) red algae *F. lumbricalis*

From our 7 tested algorithms Median Robust Extended Local Binary Pattern [1] using 24 most important features showed the best performance, with random forest model accuracy of 89.2 %. Highest precision (91 % and 92 %) was achieved when classifying geological features (boulders and sand), while red algae *F. lumbricalis* and blue mussel *M. edulis trossulus* showed 87 and 86 % precision respectively.

Textural analysis proved to be a promising method for classification of underwater photos where object shape and colour are irrelevant factors. In the future, we intend to test and adjust more textural features extraction algorithms for improved model classification results, as well as automate the patch extraction and classification process to the point where a user might obtain a classification result straight from an image.

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B.28. IMPACT OF GRAPHENE OXIDE AND METALS MIXTURES ON SALMONIDS-SPECIFIC BACTERIA CULTURE

Renata Butrimienė[1], Vita Raudonienė[1], Vesta Skrodenytė-Arbačiauskienė[1], Nataliia Matviienko[1], Danguolė Montvydienė[1], Živilė Jurgelėnė[1], Sergej Šemčuk[2,3], Nijolė Kazlauskienė[1]

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Graphene nanomaterials, such as graphene oxide (GO) due to unique structure and properties have been widely used in various fields. However, the rapid increase in GO production and application may lead to their inevitable release into aquatic and terrestrial environments, which could result in potential health and ecosystem risks [1]. There are only some studies on the ability of GO to adsorb heavy metals from aqueous solutions and natural media [2].

The aim of this study was to evaluate the effects of GO, metals (MIX), and GO+MIX on test bacterial cultures specific to salmonid fish.

The metal ion mixture (MIX) (Ni(II) 0.034 mg/L, Zn(II) 0.1 mg/L, Cr(III) 0.01 mg/L and Cu(II) 0.01 mg/L) was prepared according to the maximum-permissible-concentrations (MPC) accepted for the inland waters in the European Union. The mixtures MIX20, MIX40 and MIX80 were prepared by increasing the value of MPC of every single metal ion by 20, 40 and 80 times, respectively. The GO concentrations (1, 20, 40, and 80 mg/L) were chosen in accordance with the previous study [3] concerning the ability of GO to adsorb heavy metals. The colony forming unit (CFU) counting method was chosen.

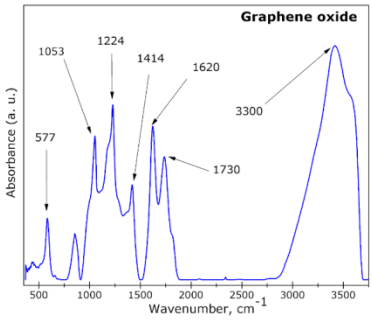

Figure 1. The FTIR spectrum of syntheed graphene oxide [3].

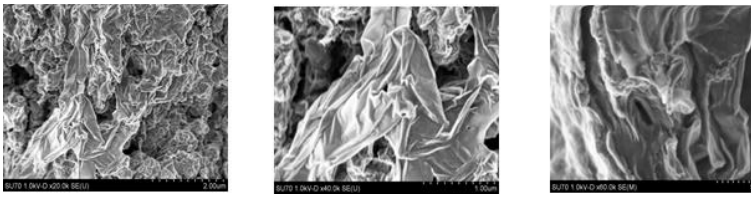

Figure 2. The SEM images of synthesized graphene oxide [3].

After six hours of exposure, higher concentrations of GO were more toxic to *Escherichia coli* in comparison with *Aeromonas salmonicida* and *Pseudomonas sp*. The higher MIX concentrations (20- and 40-fold increases in metals) inhibited the growth of all tested bacteria. Meanwhile, GO+MIX was observed to reduce the toxicity of metals to *Escherichia coli* and *Pseudomonas* sp. The study revealed that GO are promising and advanced adsorbent of metals.

This research was supported by the research infrastructure Open Access Centre for Nature Research at the initiative of the Open R&D Lithuania network. This work was done according to the aims of the Research Council of Lithuania Project No. S-MIP-20-22.

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B.29. WILD CUCUMBER (*ECHINOCYSTIS LOBATA* (MICHX.) TORR. ET A. GRAY.) PHENOLOGICAL TRENDS AT PRIVATE AREA IN KAUNAS CITY

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Biological invasions are one of the greatest threats to biodiversity of natural ecosystems. The majority of alien plant species were intensively spreading at the end of 19th century and at the beginning of the 20th century. Most invasive species in the European temperate zone came from warmer climate regions of the world. In recent decades a number of alien species are expanding their invasive range of distribution to more northern areas, most often such phenomenon is related to climate change. Wild cucumber (*Echinocystis lobata*) is recognized as an invasive species in Lithuania and other European countries of temperate climate and is considered to be one of the most dangerous in terms of its intensity of the spread in the continental part [1, 2]. Wild cucumber (*Echinocystis lobata*), *Cucurbitaceae* representative, is vine, climbing with the help of branched tendrils, forming big capsules with spines [3, 4]. This annual herb is cultivated in the gardens due to numerous fragrant whitish flowers, attention attracting fruits, hanging on the fences [5]. *E. lobata*spread, as introduced to Europe species, is related to ornamental features. Romania is proposed as the first country of *E. lobata* settlement in Europe, where it has arrived from North America. *E. lobata* has escaped from the sites of anthropogenic origin to overmoistured natural habitats of temperate climate zone. Within last decades it is recognized as an intensively spreading invasive species along riverbanks of Lithuania. Till now this annual herb is grown in private gardens for its abundant fragrant whitish flowers and hanging fruits that attract attention. Once planted, it can germinate again the following year and grow unattended for many years. The aim of this study was to compare the phenology of wild cucumbers growing in the same location over four years (2017-2020). Morphological and gravimetric parameters have highlighted the huge potential of the aboveground part for development of this species. The gravimetric and morphometric parameters per individual were much higher than what could be expected from the literature. The maximum morphological parameters observed were as follows: stem length close to 300 m, aboveground mass 1600 g and number of seeds 1000. The results show that among the useful strategic features of the species might be establishment of the huge aboveground part, which helps producing large quantities of seeds and scattering them away from the point where germination of the invader took part.

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B.30. DETERMINANT OF CRISPR NUCLEASE GUIDE RNA FUNCTION

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CRISPR (clustered regularly interspaced palindromic repeats) systems are sought-after genome editing tools for therapeutics and diagnostics. The use of CRISPR systems for gene editing is not without difficulties: the specificity of Cas12a nuclease, a Cas9 alternative, presents a limit in its use for gene editing due to its inability to successfully edit some genes. The primary cause of this problem is believed to be gRNA underperformance, caused specifically by gRNA secondary structures[1].

The gRNA plays a critical role in CRISPR systems. Experimental analysis has shown that the use of CRISPR for genome editing can result in off-targeting, causing a high degree non-specific editing at undesired genomic [2]. Strategies used for gRNA evaluation have several drawbacks as well, such as a need for substantial protein engineering, incompatibility with viral packaging restrictions, and an increased number of system components. Published findings on the impact of the nucleotide composition and secondary structure of the gRNA contradict one another [3]. Although, guides with slight modifications like changing the nucleotide composition and extension of the secondary structures can efficiently improve Cas12a nuclease efficiency for gene editing [4]. Several bioinformatic tools help users design guide RNA with high targeting capability [5]. Yet, the effectiveness of gRNAs in guiding Cas12a for genome editing varies substantially [6]. Therefore, the field needs methods for designing highly effective gRNAs that can improve target identification and reduce nonspecific targeting.

For this research, 12,000 gRNAs, spanning different nucleotide compositions, targets and secondary structures will be analyzed to show how these features influence transcription, ribonucleoprotein complex formation, and target cleavage. We will apply high-throughput next-generation sequencing to characterize gRNAs at each stage. A microfluidic droplet platform will also be used to perform in vitro assays during the study.

This study will provide a strategy for increasing the specificity of Cas12a gene editing. This will be beneficial in determining how gRNAs influence CRISPR Cas12a DNA cleavage efficiency. There is a need to increase the performance of Cas 12a endonucleases so that they are resilient and are ready for biotech applications. This study's logical design of gRNAs is a viable way to meet this demand. Using well- characterized oncogene target locations, we will show that rationally designed RNA secondary structures can boost the efficacy and specificity of gene editing tools like Cas 12a.

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B.31. PROFILING THE SPECIFICITY OF THE TNPB – TRANSPOSON ASSOCIATED NUCLEASE

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Genetic engineering provides the scientific industry with many benefits. Up to date, science has established a perfect approach to such problems as treating inherited diseases, eliminating pests and increasing harvest in agriculture, providing more food supplies to maintain the growing human population, and even executing rapid molecular diagnostics [1]. On the other hand, gene editing comes with several issues of which successful delivery of an editor to a target cell is one of the most common burdens. Nowadays, CRISPR (*clustered regularly interspaced short palindromic repeats) associated proteins (Cas)* are proven to outperform other editors by being faster, cheaper, more accurate, and more efficient [2]. They also depend on a single targeting molecule – gRNA (guide RNA) for target sequence recognition and gRNA can be easily reprogrammed to target the desired locus in the genome. The main drawback of Cas proteins is their size. Packaging SpCas9 (*Streptococcus pyogenes* Cas 9) and a gRNA together (~4.2 kb) into an AAV (Adeno-associated virus) vector is challenging due to AAV packaging capacity (~4.7 kb). Due to this problem, scientists are looking for new nucleases that would be smaller and therefore easier to deliver. Here we focus on TnpB – a transposon-associated nuclease that is two times smaller than Cas9. TnpB is believed to be a progenitor for Cas nucleases and exhibits similar biochemical patterns. It has been already claimed that TnpB is capable of editing genomes by introducing indel (insertion and deletion) mutations to human genes [3].

TnpB is a newly discovered gene editing tool and some of its features are unknown. This motivates **the main goal of our project:** determine TnpB's target specificity profile with multiplexed, next-generation biochemistry. This comes to the main method termed NucleaSeq [4]. NucleaSeq is a platform that combines DNA digestion by a nuclease with deep sequencing. NucleaSeq reveals the cleavage kinetics of a nuclease and benchmarks its fidelity by quantifying the rates and sites of cleavage across DNA targets, including thousands of off-targets that do not fully match the nuclease's gRNA. One of the main **expected results** from this project is a broader understanding of how TnpB's kinetics compares to that of well-studied Cas nucleases: whether it creates more or fewer off-targets, and exhibits a lower or higher cleavage rate. This project will help to identify the suitability of TnpB for gene editing and which environmental conditions should be held for the best results. If TnpB turns out to be exhibiting high fidelity and desirable cleavage rates it could become one of the mainly used gene editing tools with a size of miniature nuclease.

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B.32. THE ROLE OF DNA TOPOLOGY IN CRISPR-CAS9 NUCLEASE SPECIFICITY

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Bacteria are susceptible to infection by bacteriophages (phages); they use their hosts for replicating their genetic material and for assembling new phages, leading to the demise of the host. To prevent their destruction, bacteria have various mechanisms dedicated to the identification and removal of any foreign genetic material. One such mechanism is clustered regularly interspaced short palindromic repeats (CRISPR) – CRISPR-associated protein 9 (Cas9) system. It can recognize a specific sequence with the help of the complementary to a target single guide RNA (sgRNA), which directs the nuclease to the cleavage site [1].

Due to such specific guidance of the nuclease, the CRISPR-Cas9 system is a desirable tool for eukaryotic gene editing approaches. Nevertheless, such an application requires more data on how Cas9 works in a eukaryotic environment, which has several topological differences compared to prokaryotic DNA.For example, prokaryotes prefer adenine over cytosine methylation, which helps with self and non-self differentiation, while eukaryotes methylate only cytosines to help control gene expression [2]. Supercoilingalso differs as eukaryotic DNA not only is more complexly compacted but it can also allow having positive and negative supercoils together, which is highly restricted in prokaryotes [3]. Knowing this, the differenceswe have decided to test within the scope of the study were target methylation patterns (on adenine/cytosine) and supercoiling (positive/negative) as well as their effects on how Cas9 interrogates its targets.

To test methylation effects, we designed five targets with different patterns of methylation on adenine (6mA) and cytosine nucleotides (5mC). Within these five targets we were able to methylate every single nucleotide within the target and assess its effects on Cas9 cleavage. After exposing these targets for Cas9 digestion over time, we did not observe cleavage rate differences between methylated and non-methylatedon- and off-targets.

For the second part of the study, we will test supercoiling effects for on- and off-target cleavage witha library of supercoiled or relaxed plasmid DNA targets. These targets will include matched and mismatched (consisting of insertions, deletions and mispairs) sequences compared to the sgRNA. The results from this study will further contribute to a better understanding of how Cas9 reads eukaryotic DNA, which should helpto better minimize the off-target promiscuity of Cas9.

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B.33. INVESTIGATION OF NOTCH SIGNALING PATHWAY IN ENDOMETRIAL CANCER CELLS KLE

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Endometrial cancer is the sixth most common type of malignancy in women worldwide [1]. The increasing incidence is thought to be due to improvements in quality of life: longer life expectancy, the obesity epidemic, malnutrition and hormonal changes [2]. According to the American National Cancer Institute, the increasing number of new cases of endometrial cancer and the constant mortality rate show that there are still no effective treatment strategies to control the disease [3]. Current treatment strategies, including chemotherapy, surgery, extend life expectancy of women but not always cure completely, leaving a further risk of recurrent cancer. As a result, it is important to look for biological therapeutic targets that fundamentally eliminate oncogenesis factors.

The molecular mechanisms underlying endometrial carcinoma are not yet fully understood. It is well established that the development of endometrial cancer is linked to alterations in signaling pathways in endometrial cancer. One of these is the NOTCH signaling pathway, which regulates cell differentiation, proliferation and homeostasis. In mammals, NOTCH signaling is activated through 5 ligands. Ligand binding to the NOTCH receptor leads to the sequential progression of proteolytic cleavages and intracellular nuclear translocation of NOTCH receptor domains (NICDs), which regulate NOTCH-dependent dependent transcription. Disrupted NOTCH pathway signaling can promote tumorigenesis by increasing cancer stem cell activity, or conversely, act as a tumor suppressor.

To investigate the importance of this pathway in more detail, it is relevant to study the transcriptional changes of the components of NOTCH signaling pathway. As an *in vitro* model, we used endometrial carcinoma cell line KLE. Our study will describe the optimization of growth conditions of KLE cells, determination of the appropriate concentration and incubation time of the chemotherapeutic drugs cisplatin, paclitaxel and gamma secretase inhibitor nirogacestat which are important for further experiments. Also, we will evaluate the effect of these chemotherapeutics compounds on mRNA and protein levels of the components of NOTCH signaling pathway

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B.34. ADAPTATION IN A TYPE II-C CRISPR-CAS SYSTEM

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CRISPR-Cas are widespread bacterial and archaeal adaptive immune systems, which protect cells against mobile genetic elements, such as viruses or foreign plasmid DNA. The mechanism of CRISPR-Cas systems comprises three stages, called adaptation, CRISPR RNA maturation and interference, while the CRISPR loci contain inverted sequences (CRISPR repeats), interspaced by short fragments acquired from foreign DNA (spacers). A key component in the adaptation step is the heterohexameric integrase complex composed of a Cas2 dimer sandwiched by two Cas1 dimers. The role of this complex is to capture fragments of foreign DNA (prespacers) and integrate them into the CRISPR locus, leading to formation of new spacers that confer immunity against the corresponding viral DNA. So far, CRISPR-Cas adaptation is best described in systems of subtypes I-E and II-A [1], yet the mechanism of spacer acquisition in subtype II-C systems remains unclear.

In order to investigate adaptation in a subtype II-C CRISPR-Cas system, we purified the Cas1-Cas2 integrase complex and demonstrated that this complex preferentially integrates pre-spacers consisting of 22 base pair duplex DNA with 4 nucleotide 3'-overhangs. Additionally, we determined the CryoEM structure of the Cas1-Cas2 integrase complex (Fig. 1), which revealed molecular interactions between the integrase and pre-spacer DNA as well as explained the preferred 22 base pair pre-spacer duplex length. Finally, *in vitro* spacer integration assays showed that new spacers are integrated near the leader-proximal CRISPR repeat, however, unlike in most CRISPR-Cas systems where the leader is positioned upstream and is responsible for initiating the transcription of CRISPR RNA, our subtype II-C system has a leader sequence downstream of the CRISPR array. Therefore, CRISPR RNA transcription is initiated at the individual CRISPR repeats, which seems to be a characteristic feature of subtype II-C systems [2, 3].

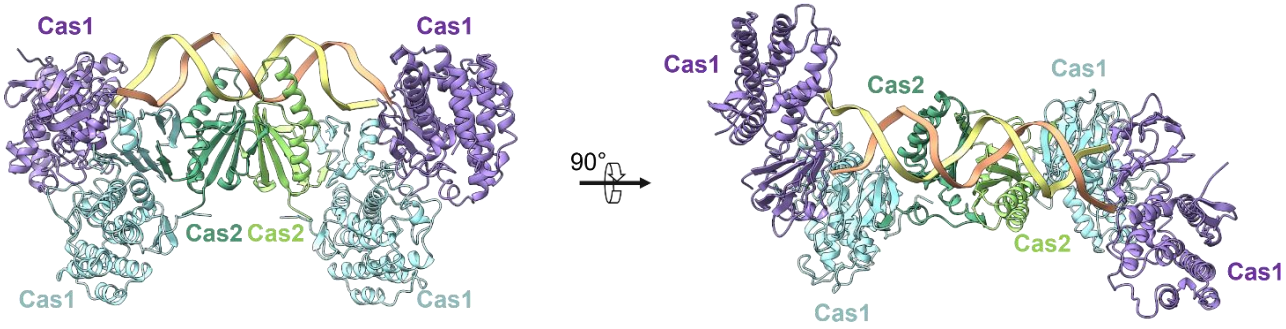

Figure 1. The structure of subtype II-C Cas1-Cas2 integrase complex.

Our results suggest that the adaptation in a subtype II-C CRISPR-Cas system might be generally similar to other type II systems. However, as it lacks accessory proteins like Csn2 and Cas4 found in subtypes II-A and II-B respectively, the overall adaptation mechanism may differ and further investigation is needed to elucidate it.

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B.35. THE EFFECT OF dGAE FRAGMENT CONCENTRATION ON ITS SELF-AGGREGATION AND ASSOCIATION WITH TAU PROTEIN

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Protein aggregation into amyloid fibrils is associated with several widespread neurodegenerative disorders. Alzheimer's disease is one of the most prominent cases, with an ever-increasing number of afflicted patients. The disorder is characterized by memory loss and is considered to be caused by amyloid-β extracellular plaques and neurofibrillary tangles made from hyperphosphorylated Tau. The main physiological function of the Tau protein is to maintain microtubule dynamics and promote polymerization. *In vitro* Tau protein filament formation is induced by the presence of polyanions such as heparin. A truncatedform of Tau protein (dGAE), which can assemble into filaments without polyanions, forms a proteolyticallystable core region in full-length Tau paired helical filament (PHF) [1]. The dGAE fragment could serve as amodel system for the search for inhibitors of Tau protein aggregation [2].

Considering the rise of Alzheimer's disease incidences worldwide, understanding the process of amyloid aggregation is a crucial step in developing drugs. Research of dementia is heavily focused on the process of how pathogenic Tau can spread throughout the brain, as it may provide a key therapeutic targetfor slowing the development of tauopathies. There are a lot of disputed questions concerning the physiological and pathological consequences of PHF–core Tau self–assembly, therefore we aim to elucidate the influence of dGAE fragment on Tau protein aggregation.

Tau 2N4R gene was inserted into pET Champion His-SUMO vector by TA cloning method to create the ULP1–cleavable N–terminally His–SUMO–tagged Tau. The recombinant His–SUMO–tagged Tau (2N4R isoform) protein was purified by immobilized metal affinity chromatography and consequently cleaved by the ULP1 protease to remove His–SUMO–tag. A truncated form of Tau protein (297–391) (dGAE) was purified by cation exchange chromatography. Both proteins were purified further by size-exclusion chromatography. We usedpurified Tau 2N4R protein and dGAE fragment for monitoring the amyloid aggregation process. All performedaggregation kinetics were followed using a thioflavin-T fluorescence assay. Atomic force microscopy was performed to analyze the morphology of the formed aggregates. We used FTIR spectroscopy to observe thedifferences in secondary structures of the dGAE fragment and dGAE+Tau protein aggregates.

Looking at the results, it is visible that the aggregation halftime and lag time values decrease when dGAE fragment concentration increases. These results confirm the data which is presented in the literature that at higher concentrations dGAE fragment aggregates faster [2]. The apparent rate constant varies within error at different concentrations. FTIR spectrum of dGAE fragment shows formed β-sheets which is typical for amyloid fibrils. However, dGAE peptide does not stimulate Tau aggregation as FTIR spectra show two components of dGAE fibrils and native Tau protein.

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B.36. DELETION OF THE *LACTICASEIBACILLUS CASEI* SMALL RNA SLCB2236– USING CRISPR–CAS9^{D10A}

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Lacticaseibacillus casei is a type of lactic acid bacteria commonly found in fermented foods. *L. casei* can help to regulate the gut microenvironment and relieve inflammation, which can improve digestionand strengthen the immune system. These bacteria are capable adapt to a wide range of unfavorable conditions, making them particularly attractive in studies on the fermentation of pharmaceuticals, biofuels,and traditional foods [1].

In order to study *L. casei* and their industrial applications, it is important to find out which genes are important for their adaptation and how they are regulated. In bacteria, changes in gene expression are usually regulated by small non-coding RNA molecules (sRNAs), which interact with mRNA or directly bind to the protein. Not much information on these sRNAs is available in the aforementioned *L. casei* bacteria, therefore a conserved sLCB2236-sRNA, among the *L. casei* group bacteria, was chosen for this study, which is likely to increase lysozyme susceptibility and confer resistance to nisin, penicillin G and hydrochloric acid.

The first step to understanding the significance of the selected sLCB2236-sRNA in *L.casei*, is to remove this gene from the genome, compare this mutant with the wild type and look for differences.
Precise gene removal from the *L. casei* genome can be performed using the PLCNICK system, which is based on CRISPR–Cas9^{D10A} nickase–mediated chromosomal single–strand breaks and allelic exchange dependent on homologous recombination [1].

On this basis, a PLCNICK plasmid with suitable homologous arms was prepared and inserted into *L.casei* bacteria by electroporation. The deletion was identified by PCR followed by restriction analysis. After the verifications, the plasmid from the mutant was removed by plating on non-selective media. Additionally, the deletion was confirmed by sequencing and RT-PCR method to ensure that no extraneous mutations have occurred. In addition, the obtained mutant was complemented with a nisin inducible plasmid containing the reconstituted target sRNA and the complementation was confirmed by RT-PCR.

In conclusion, the aim of the present study was to develop an *L.casei* ΔsLCB2236-deletant using CRISPR–Cas9^{D10A}, which can be further exploited in future studies to elucidate the influence of sLCB2236–sRNA on the adaptation of bacteria to different environmental conditions.

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B.37. FORMATION OF CALPROTECTIN (S100A8/S100A9) INHIBITS AGGREGATION OF S100A9 INTO AMYLOID COMPLEXES

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S100A8 and S100A9 are members of the S100 calcium-binding protein family. They are established as biomarkers of inflammation with various extracellular and intracellular functions [1]. Both of the proteins can be heavily upregulated in Alzheimer's disease (AD) patients' brain tissues [2]. S100A9 forms neurotoxic amyloid fibrils [3], which may contribute to the pathology of AD [4]. Differently from S100A9, S100A8 assembles into non-fibrillar aggregates in the brain tissues of transgenic APP or ageing wild-type mice [5]. Moreover, S100A8 and S100A9 are able to form heterodimeric complex (calprotectin (CP)). Calcified amyloidogenic fibrils of calprotectin can be found in the ageing prostate, which additionally emphasizes the connection between inflammation and amyloid deposits formation [6].

Assembly into amyloids is related to the changes in the protein's secondary structure. During the aggregation process, a transformation from the native state into cross β-sheet structures occurs [7]. However, existing evidence implies that S100A9 has a distinct aggregation pathway as S100A9 fibrils retain a significant amount of a-helical motives alongside β-sheets [3]. Thus, S100A9 amyloid fibrils structural propensities remain unclear. Moreover, there are no detailed studies of S100A8 and CP aggregation *in vitro*. Therefore, in this study, we were investigating S100A8 and S100A9 fibril's secondary structures and morphologies, as well as their aggregation rate dependence on protein concentration. Additionally, we examined CP formation influence on S100A8 and S100A9 aggregation.

In order to evaluate S100A8 and S100A9 aggregation propensities, Thioflavin T Fluorescence Assays were conducted under 37 °C. Aggregation kinetics results indicated that both proteins have a short lag-phase period and half-time values of the amyloid formation kinetics elevate notably in a concentration-dependent manner. However, distinct characteristics of S100A8 and S100A9 aggregation curves were observed, hence, formed aggregates were examined using Atomic Force Microscopy (AFM). AFM imaging indicated S100A8 aggregates as spherical oligomers, differently from S100A9 short worm-like fibrils. Lastly, we observed that CP formation inhibits S100A9 assembly into fibrils *in vitro*. In summary, our conducted research provides important and previously unknown insights into S100A8 and S100A9 aggregation, which is tightly connected to various pathologies.

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B.38. IDENTIFICATION OF RNA-RNA INTERACTION PARTNERS USING MODIFIED MAPS METHOD

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Lactic acid bacteria – Gram-positive, non-pathogenic, mesophilic bacteria that are widely used in food industry for their ability to ferment various sugars, such as lactose, to produce lactic acid. These bacteria must rapidly adapt to changing conditions to survive. The presence of small RNA molecules plays a significant role in adaptation. They are post-transcriptional regulators, typically less than 300 nt in length and act as non-coding molecules. The categorization of sRNAs is based on the mechanism of interaction with their target RNA molecules. Following that, sRNAs can be divided into two major groups: those that mediate target RNA regulation through base-pairing interactions, and those that modulate target RNA stability or translation through ribonucleoprotein (RNP) complex formation with proteins. However, such mechanisms of interaction between small RNAs and their targets require more analysis. For that reason, it is important to identify the RNA-RNA interaction partners.

In this work we used a modified MAPS method for the identification of RNA-RNA interaction partners. It involves the tagging of sRNA molecules with MS2 sequences and their purification from lysed cells using a dimeric MCP bound with Glutathione S-transferase (GST) affinity tag. We employed this method to isolate MS2-s1042 RNA molecules from the L. lactisΔs1042 mutant, based on our prior results of the purification of these molecules from the wild-type Lactococcus lactis strain. We then evaluated the efficiency of MS2-s1042 RNA extraction from the lysed mutant cells compared to the untagged controls and wild-type, using reverse transcription (RT) and real-time quantitative PCR (RT-qPCR) analysis. A search for potential RNA-RNA interaction partners among all coding sequences in L. lactis was performed, using the IntaRNA bioinformatics tool and a previous RNA sequencing data. In addition, a total of 13 hits were selected: 5 were chosen using a bioinformatics tool that screened genome-wide genes based on the highest interaction probabilities, while other 8 were from previous RNA sequencing work that detected changes in the level of gene expression upon overproduction of s1042 RNA. The coextraction of these 13 genes was tested by the RT-qPCR method, with primer pairs amplifying the targets. The expression of one gene, selected with the IntaRNA bioinformatics tool, was obtained, and showed an enrichment of approximately 71 times, while no changes in expressions were determined for the other genes selected based on RNA sequencing data. The analysis of the enriched gene indicated that it is a hypothetical aquaporin encoding the GlpF (glycerol uptake facilitator) protein. Thus, modified MAPS method can be applied for identifying RNA-RNA interaction partners.

B.39. PROTEINS INHIBITING CRISPR-CAS DEFENCE

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CRISPR-Cas are bacterial and archaeal defense systems that provide resistance against phages and other invasive genomic elements. Short DNA fragments of extracellular origin are inserted within repeated sequences of the CRISPR locus. The CRISPR transcript is matured into small crRNA molecules, which, together with Cas proteins, assemble an effector complex destined to detect and destroy foreign nucleic acids [1]. The bacteriophages evolved small anti-CRISPR (Acr) proteins, which block CRISPR-Cas protection [2]. To date, over 100 Acr families have been discovered, but only a fraction of them have been studied in detail [3]. Recently, Acr proteins have been utilized to regulate CRISPR-Cas editing tools, demonstrating their immense practical potential.

Our research object is the Acr proteins inhibiting the type I-F CRISPR-Cas system from Aggregatibacter actinomycetemcomitans. The system encodes a ribonucleoprotein complex, termed Cascade, which binds foreign DNA and then recruits the Cas2/3 nuclease to destroy the invader [4]. We show Acr proteins blocking the CRISPR-Cas system in bacterial cells. We employ biochemical assays for the analysis of their molecular mechanisms. Our findings provide insight into the inhibitory mechanism of these previously uncharacterised proteins.

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B.40. STRUCTURAL AND FUNCTIONAL STUDIES OF THOERIS ANTIPHAGE DEFENSE SYSTEM

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Prokaryotic antiviral (antiphage) defense system research is a rapidly developing field. In recent years, known arsenal of antiphage systems has expanded to over a hundred of confirmed unique instances [1]. Detailed investigation of these systems reveals unique molecular pathways and deepens our knowledge about microorganism ecology. However, only a small number of antiphage systems are characterized in depth.

Thoeris is a bacterial antiviral defense system composed of two genes *thsA* and *thsB*. ThsB protein contains Toll/interleukin-1 receptor (TIR) domain which is a canonical component of animal and plant immune systems. ThsA is composed of a sirtuin-like (SIR2) and SLOG domains [2]. Representative structures of ThsA and ThsB proteins and their functional studies were published recently [3, 4]. ThsB protein recognizes phage infection and produces a signaling molecule cyclic ADP ribose isomer 1"-3'gcADPR [5]. 1"-3' gcADPR is then bound by the ThsA SLOG domain and activates NAD^+ hydrolysis by the SIR2 domain resulting in NAD^+ depletion and host cell death [4]. However, it was not clear how binding of the sigaling molecule turns on NADase activity of ThsA. In this work we reconstituted Thoeris enzymatic activities *in vitro* and using structural and biochemical methods showed the ThsA activation mechanism.

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B.41. EXPLORING THE NOVEL CRISPR-CAS12 EFFECTOR COMPLEXES

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Genome engineering is one of the fastest developing technologies of the 21st century. Using the latest sequencing technological achievements, the genome sequences can be obtained almost instantly, butto easily change the DNA, encoded in cells, universal, precise and easily programmable DNA manipulation tools are needed. The breakthrough in genome engineering was achieved with the discovery and adoption of CRISPR-Cas systems for programmable DNA cleavage [1, 2].

CRISPR–Cas (Clustered Regularly Interspaced Short Palindromic Repeats – CRISPR associated protein) systems, which are found in prokaryotes, provide resistance against foreign mobile genetic elements and have a wide range of genome editing and biotechnological applications. Class 2 CRISPR-Cas systems are most commonly used as molecular tools and includes types II, V and VI, characterized by Cas9, Cas12 and Cas13 respectively [3]. Cas9 (type II) and Cas12a (type V) nucleases from CRISPR-Cas systems have revolutionized the field of genome engineering. Due to the use of DNA target recognition by an RNA molecule, these nucleases have allowed genome engineering to be very efficient and easily reprogrammable. In *in vivo* therapy, the delivery of these nucleases to cells is limited by the capacity of adeno-associated viruses (AAVs) and the packaging of large Cas12a (850–1400 aa) or Cas9 (1000–1600 aa) nuclease-coding genes into these carriers is complicated [4]. As a result, recent efforts have focused on expanding the number of known CRISPR–Cas systems to identify novel nucleases. Smaller CRISPR–Cas nucleases in particular are being very attractive to treat genetic disorders *in vivo* using AAV although the functional diversity of these compact nucleases within each subtype remains poorly explored.

In this study, we describe a set of Cas12 protein candidates, ranging 500-700 aa in size, which function still remained unknown. Using biochemical methods, we provide evidence that these proteins areable to form effector complexes with RNA molecules and describe key elements, needed for DNA target recognition *in vitro.* Further investigation of chosen Cas12 proteins revealed their ability to provide DNA interference in cells.

Altogether, this study expands the CRISPR-Cas toolbox and provides a thorough characterization of DNA target elements, needed for successful RNA-guided interference. Our results indicate that the natural diversity of Cas proteins provides a source of potential novel gene editing tools with unique characteristics.

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B.42. MECHANISM OF CRISPR-CAS3 HELICASE USING MAGNETICTWEEZERS

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CRISPR-Cas provides RNA-guided adaptive immunity against invading genetic elements. CRISPR systems consist of multiple Cas proteins, which are responsible for CRISPR-dependent cell immunity mechanisms. The effector complex in CRISPR I-E consists of Cascade and Cas3. Cascade is responsible for foreign DNA targeting. Meanwhile, Cas3, which possesses helicase and nuclease activities, is a key protein of the system, necessary for crRNA-guided interference against virus proliferation.

Although single-component Class 2 CRISPR systems, such as type II Cas9 are widely used for genome-editing, the research on multi-component Class 1 proteins, including Cas3, of the same system has been less developed. Components of the I-E CRISPR system have already been used as a genome-editing tool to generate big deletions. However, the detailed mechanism by which Cas3 achieves its function is not well understood. The aim of this study is to elucidate the mechanism of Cas3 DNA unwinding and shredding. We are using single- molecule force microscopy, namely, magnetic tweezers, to probe the mechanical aspects of Cas3 unwinding activity. Greater knowledge of the Cas3 mechanism of action would improve the application of Cas3 as a tool for genome editing.

B.43. INHIBITION OF RECOMBINANT SARS-COV-2 MAIN PROTEASE WITH NATURALLY DERIVED FLAVONOIDS

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In 2020, the severe acute respiratory syndrome coronavirus 2 rapidly spread worldwide and was declared a pandemic. This strain of coronavirus causes COVID-19, which has been seen to be highly contagious and dangerous, especially to the elderly and immunocompromised. Researchers started to search for methods to mitigate this threat, such as creating antiviral drugs against SARS-CoV-2.

The viral genome of SARS-CoV-2 encodes two polyproteins, which, when cleaved in 11 conserved sites, cause active viral replication and assembly of viral particles in the host cells. The main or 3- chrymotrypsin like protease (Mpro or 3CLpro) is a non-structural protein (nsp5), which is responsible for the cleavage of viral polypropein and therefore is widely explored as a potential drug target[1]. Synthetic compounds and natural substances are investigated as possible antiviral drug candidates using various scientific approaches[2]. Potential inhibitors of Mpro could be flavonoids that include many secondary metabolites found in fruits, vegetables, and several plants. Flavonoids that are extensively studied for theiranti-inflammatory and antiviral properties could also serve as a future therapeutic utility for drugging COVID-19[3].

Two recombinant Mpro plasmids were designed and expressed in *E. coli* and the proteins were purified by affinity chromatography for the study. The catalytic efficiency of recombinant Mpro proteins wasdetermined using fluorescence resonance energy transfer-based enzymatic assay and later compared with commercially available wild-type Mpro. More than 250 flavonoids were screened as potential recombinant Mpro inhibitors using fluorescent thermal shift assay (FTSA). The compounds showing a stabilizing or destabilizing effect on the protein thermal stability were investigated using in vitro FRET-based enzyme inhibition assay. Several promising flavonoids were analyzed as inhibitory compounds and their *Kd* values were determined using dose-response analysis. The inhibition of the recombinant Mpro and the inhibitory effects of selected flavonoids on commercial wild-type Mpro were investigated and the determined Kd values were compared. The study not only suggests that naturally derived compounds could be considered as potential Mpro inhibitors but also compares the effect of amino acid sequence difference on inhibition ofMpro.

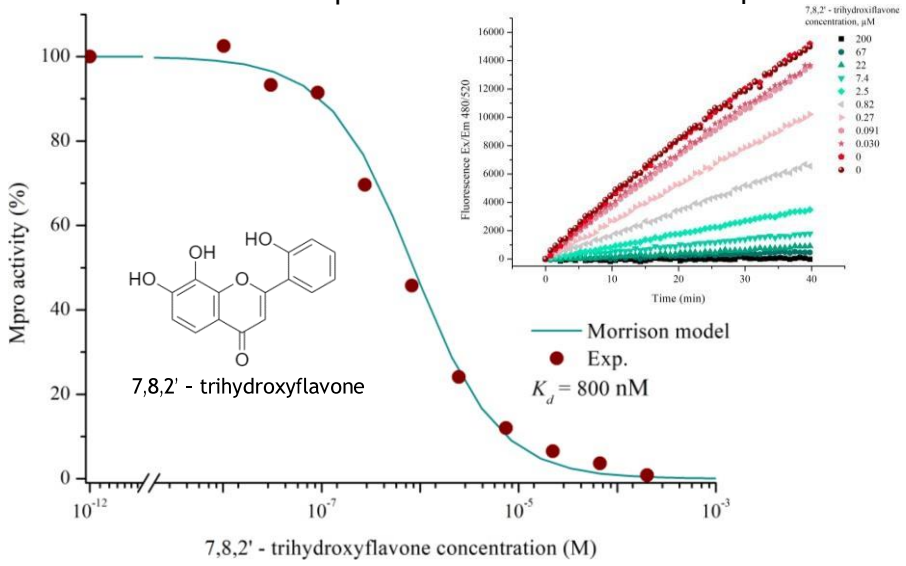

Figure 1. Dose-response curve of 3CLpro inhibition with 7,8,2' - trihydroxyflavone.

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B.44. SYNTHESIS OF NOVEL 1-(2-HYDROXY-5-METHYLPHENYL)-5- OXOPYRROLIDINE-3-CARBOXYLIC ACID DERIVATIVES

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Pyrrolidinone derivatives exhibit various pharmacological properties: antibacterial, antioxidant, antifungal, anti-cancer, antiviral, antihypertensive and anticonvulsant [1, 2]. This group of compounds are the active ingredient in many drugs, for example metadoxine, piracetam, raltegravir and rolipram [3]. Moreover, some benzimidazole derivatives hold even better anti-cancer and antiviral properties. Thus, compounds containing a benzimidazole condensed ring system are often pharmacophores of bioactive molecules. Examples of this include mebendazole and albendazole. The latter indirectly promotes the processes of apoptosis of cancer cells, stops the cell cycle and the formation of microtubules [4]. Therefore, the aim of this study was not only to synthesize new pyrrolidinone derivatives but also to obtain novel benzimidazole derivatives containing pyrrolidinone cyclic structure as potential new candidate drugs. The desired compounds were prepared by a multi-step synthesis process as shown in figure 1.

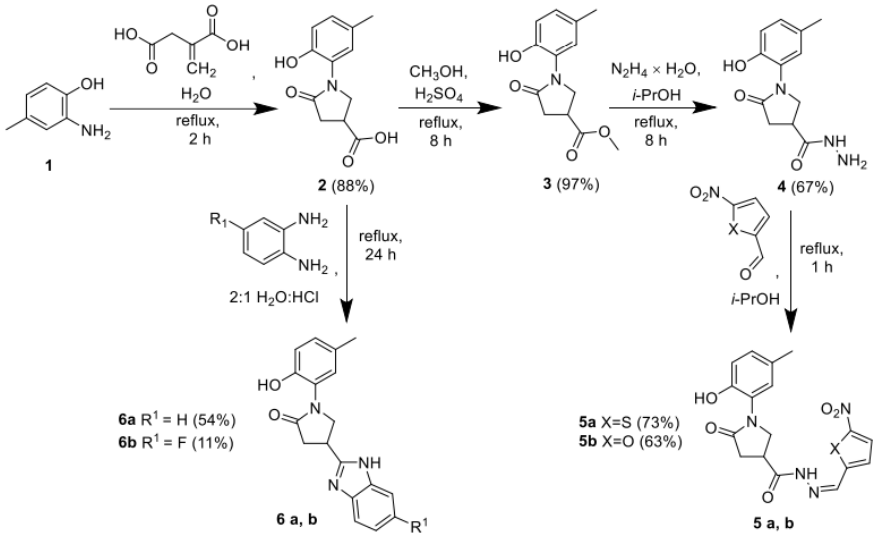

Figure 1. Synthesis scheme of novel target pyrrolidinone and benzimidazole derivatives

The 1-(2-hydroxy-5-methylphenyl)-5-oxopyrrolidine-3-carboxylic acid 2 was obtained from the reaction of the 2-amino-4-methylphenol 1 and itaconic acid in refluxing water solution. The carboxyl group of the compound 2 was esterified using methanol and a catalytic amount of sulfuric acid. Purified methyl ester 3 was further transformed to carbohydrazide by reaction with hydrazine monohydrate in an isopropanol solution. The hydrazide functional group of previously obtained compound 4 participates in condensation reactions. Therefore, interactions of compound 4 with aromatic carbaldehydes were investigated. The following target compounds 5a and 5b were synthesized during the reaction in isopropanol solution using accordingly 5-nitro-2-thiophenecarboxaldehyde and 5-nitro-2-furaldehyde. Benzimidazole derivatives 6a and 6b were obtained by the Phillips method from compound 2. Further studies on the biological activity of all these compounds are planned. The structure of the compounds has been proven by 1H NMR, 13C NMR, FTIR spectroscopy and elemental analysis.

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B.45. SYNTHESIS OF PYRAZOLE-CHALCONE DERIVATIVES

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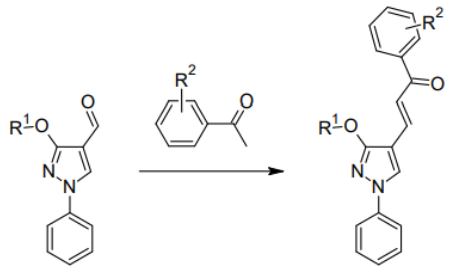
Chalcones (or 1,3-diaryl-2-propen-1-ones) constitute an important group of natural products and synthetic compounds. They are known as biogenetic precursors of flavonoids and isoflavonoids, which are abundant in all plant parts – flowers, leaves, fruits, roots and stems. Synthetically obtained chalcones are generally the condensation products of aromatic aldehydes with acetophenones in the presence of a catalyst. Chalcones are known to exhibit a wide range of therapeutic activities, such as anticancer, antioxidant, antibacterial, antiviral, anti-inflammatory and antimalarial [1]. Nitrogen heterocycles with a chalcone fragment have recently been reported to be active against lung adenocarcinoma and breast cancer cells [2], as well as against some gram-positive and gram-negative bacteria [3].

Pyrazole-ring containing compounds, which are usually obtained by synthesis, also have a wide range of biological activities: antiviral, antibacterial, antifungal, anti-tubercular, anti-inflammatory, anti-diabetic effects, and are included in many biologically active substances. Synthesis and investigation of pyrazole chalcones and other hybrid molecules is an important task in organic synthesis. These derivatives may have enhanced biological activity or the potential to develop new biologically active compounds. Thus, the synthesis of hybrid pyrazole-chalcones derivatives is important and relevant for the development of new materials that can have good biological activity.

Herein we report the synthesis and structure elucidation of 3-alkoxy-1*H*-pyrazole moiety containing chalcones.

The synthesis of various (*E*)-3-(3-alkoxy-1-phenyl-1*H*-pyrazol-4-yl)-1-phenylprop-2-en-1-ones was carried out, as depicted in Scheme 1. Easily accessible 1-phenyl-1*H*-pyrazol-3-ol was converted to 3- methoxy-, 3-propoxy- or 3-(2-methoxyethoxy)-1-phenyl-1*H*-pyrazoles and corresponding 4-carbaldehydes *via* O-alkylation and Vilsmeier-Haack formylation procedures. The obtained compounds were then subjected to a Claisen-Schmidt condensation reaction with variously 4-substituted acetophenones in the presence of ethanolic sodium hydroxide. The heating reaction mixture at 55 °C for 30 min afforded chalcones as final products in fair to excellent yields (58-97 %)



Scheme 1

The structure of the synthesized pyrazole-chalcones was proved by NMR and IR spectroscopy, MS spectrometry, HRMS analysis methods [4].

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B.46. CRYSTALLIZATION AND X-RAY STRUCTURAL ANALYSIS OF THSB PROTEINS FROM BACTERIAL ANTIVIRAL SYSTEM THOERIS

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Recently, bacterial antiviral systems have been shown to cluster side-by-side in bacterial genomes, forming 'defence islands', which lead to an explosion of new discoveries of prokaryotic antiviral systems [1, 2]. One of these new systems is named after the ancient Egyptian deity of protection and fertility, *Thoeris*. This system consists of two genes, *thsA* and *thsB*. The ThsB proteins share homology with the Toll- interleukin receptor (TIR) domains, and in prokaryotic genomes the homologous *thsB* genes are often located side-by-side in a single operon. Two types of ThsA are found: the first (TH1) consists of SIR2 and SLOG domains, the second (TH2) of transmembrane and Macro domains [2].

During bacteriophage infection, ThsB proteins of TH1 Thoeris system have been shown to produce the signalling molecule 1'-3'gcADPR from NAD^+ [3-5]. These molecules are bound by the SLOG domain of the ThsA protein, which activates the NADase activity of the SIR2 domain when bound. Thus, when the phage infects the bacterium, the Thoeris system consumes all of the cell's NAD^+, leading to cell death (figure 1). To date, it is not yet known how ThsB recognises bacteriophage infection and how these proteins are activated to synthesise the signalling molecule [3].

Based on protein sequence ThsBs are classified into 7 phylogenetic clusters. To gain more insight into the function of the ThsB proteins, we decided to determine X-ray structures of ThsBs belonging to 3 different clusters – c2, c3 & c5. For this type of analysis, protein crystals are required, which are obtained by performing crystallization experiments with the purified recombinant protein. Crystal structures of the selected ThsB proteins would provide the basis for further studies on the recognition of bacteriophage infection and the regulation of the enzymatic activity of the ThsB proteins.

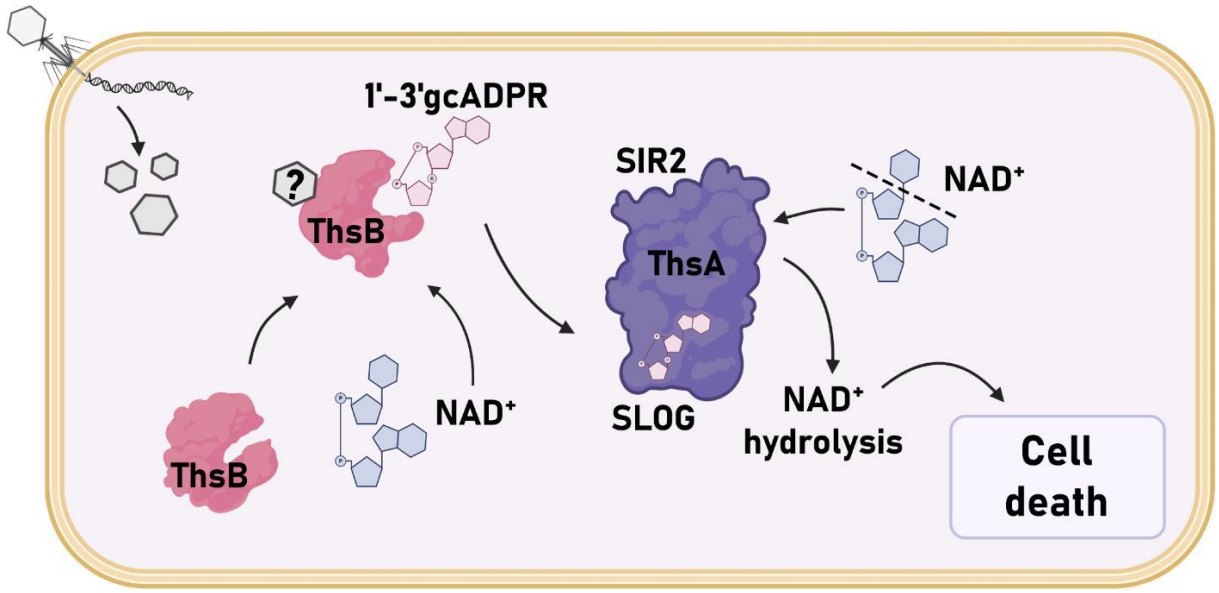

Figure 1. Proposed mechanism of action of the TH1 Thoeris antiviral system.

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B.47. IDENTIFICATION OF AMINO ACIDS CONTRIBUTING THE SPECIFITY OF METAGENOMIC CYTIDINE DEAMINASE CDA_F14

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Cytidine deaminases (CDA) belong to hydrolase class of enzymes (EC 3.5.4.5) that catalyse deamination of cytidine or deoxycytidine to uridine or deoxyuridine, respectively. CDAs are divided into two groups: homo-dimeric CDAs, found in Gram-negative bacteria such as *E.coli* or in plants, and homo- tetrameric CDAs, found in Gram-positive bacteria and in most eukaryotes. In both types each subunit coordinates zinc ion, which is required for deamination reaction.

Unpredictably, our research revealed, that some CDAs also catalyse the conversion of various N^4-acyl-, N^4-, S^4-, O^4-alkyl- and N^4-, S^4-, O^4-arylpyrimidine nucleosides directly into uridine and corresponding leaving groups [1].

In this study, to elucidate a structure-function relationship, it was aimed to identify important amino acids for catalysis and substrate binding by a site-directed mutagenesis. Changing of catalytic cysteines to histidines showed the impact of zinc ion coordination on the enzymatic activity of CDA_F14. While investigating CDA_F14 structure, it was revealed that an β3α3 loop (79-88 a.a.) determines the space for binding of N^4–substituted nucleosides. Mutagenesis of several amino acids in this region showed effects on specificity and k_{cat}/K_M values of CDA_F14 enzyme. Mutations of C-terminal site, like deletion at 127-130 and 127-132 positions or random changing at 127-130 position, as well as changing of Phe126 into alanine or tryptophan also created the enzymes with altered substrate binding and k_{cat}/K_M values.

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B.48. SEQUENCE-SPECIFIC RECOGNITION OF GUIDE AND TARGET STRANDS BY *ARCHAEOGLOBUS FULGIDUS* ARGONAUTE PROTEIN

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Argonaute proteins (Agos) are found in all domains of life. Eukaryotic Argonautes (eAgos) are the catalytic core of RNA interference machinery, whereas prokaryotic Argonautes (pAgos) provide innate immunity to exogenic nucleic acids, such as plasmid or bacteriophage DNA. All pAgos share a similar mechanism of action, they use DNA or RNA guides to recognize complementary DNA or RNA targets. Upon target recognition, pAgo either cleaves the target strand or employs effector proteins, which leads to degradation of invading nucleic acids or, in some cases, cell death [3,4]. Knowledge of preferred guides and targets reveals valuable information about how pAgos obtain their guides and recognize invading nucleic acids.

The object of this study is an archaeal Argonaute protein AfAgo from *Archaeoglobus fulgidus*. Several groups published crystal structures of AfAgo with various nucleic acid substrates [2]. Therefore, it served as a structural Argonaute model in RNA interference studies. Although AfAgo was widely-studied, recently new features of AfAgo were described, such as ability to form homodimers and create DNA loops [1]. Latest crystal structure of AfAgo obtained in our group revealed several base specific contacts with three terminal base pairs of guide-target duplex. In addition to that, we showed that *in vivo* AfAgo binds RNA guides (gRNA) enriched in 5'-AUU sequence. Studies show that similarly to eAgos, pAgos may exhibit specificity to the guide strand 5'-terminal nucleotide and respective complementary target strand nucleotide. However, this is the first example of more extensive specificity towards guide and target sequence [3].

In this study we compare AfAgo affinity towards RNA guides with different 5'-end sequence using electrophoretic mobility shift assay (EMSA). Results provide experimental evidence that AfAgo specifically recognizes RNA guides with 5'-AUU sequence. We also demonstrate that AfAgo:gRNA binary complex preferentially binds complementary DNA targets. These results challenge previous knowledge about AfAgo, since it was thought that AfAgo uses ssDNA guides and recognizes DNA targets, possibly due to the use of nonoptimal 5'-sequence guides in experiments [2].

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B.49. CRYO-EM STRUCTURES OF *STREPTOCOCCUS THERMOPHILUS* CAS9 BOUND TO TARGET AND NON-TARGET DNA

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Cas9 is a programmable RNA-guided DNA endonuclease [1; 2]. It recognises a short DNA sequence, known as a protospacer-adjacent motif (PAM), and cleaves DNA at a fixed position in accordance to PAM when there is full complementarity between DNA and RNA strands. This feature has made the Cas9 protein a highly attractive and popular molecular tool for genome editing. Nevertheless, there are still gaps in full understanding of the Cas9 biological role in prokaryotic cells. Cas9 belongs to II-type CRISPR-Cas systems and is essential for all three stages of the CRISPR-Cas mechanism (adaptation, RNA biogenesis and interference). During adaptation, viral DNA is selected, cut and integrated as a spacer into the CRISPR region in a bacterial chromosome. The precise mechanism of adaptation in II-A subtype CRISPR-Cas systems is yet to be determined. It has been shown that during adaptation Cas9 aids in viral DNA selection by recognising the PAM sequence [3]. In some way Cas9 has to convey the information of PAM recognition to macromolecular Cas1-Cas2-Csn2 adaptation complexes, which then finalise the adaptation stage [4]. When viral DNA is inserted into the CRISPR region, it is transcribed by RNA polymerase and processed by Cas9 and RNase III into CRISPR RNA (crRNA), which is hybridized with trans-activating crRNA (tracrRNA) forming a functional guide RNA. In the final interference stage, Cas9 uses guide RNA to find the target DNA molecules and induce DNA double-strand breaks. In this way, CRISPR-Cas system confers hereditary and adaptive immunity against viruses.

Here, we report the structure of *Streptococcus thermophilus* Cas9 in complex with guide RNA and target DNA (interference mode). We also present the structure of *Streptococcus thermophilus* Cas9-RNA bound to non-target DNA, with the PAM-adjacent target DNA strand non-complementary to the guide RNA. We show that Cas9-RNA adopts different conformations, which might be exploited for the distinct roles of Cas9 in adaptation and interference.

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B.50. *IN VIVO* STUDIES OF HEPN-MNT TOXIN-ANTITOXIN SYSTEM

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Bacterial and archaeal viruses are the most diverse entities on Earth [1]. Bacterial anti-phage defense systems are found in microbial genomes, where they form defense islands. Based on their genomic co-localization with known systems, this genetic feature allowed for the recent discovery of numerous defense systems, but the full arsenal of anti-phage mechanisms in bacteria is highly understudied [2].

Toxin-antitoxin (TA) systems are genetic elements that target key cellular processes such as replication, transcription, or translation. Here we look into the HEPN-MNT toxin-antitoxin system encoded in the vicinity of a subtype I-D CRISPR-Cas system in the cyanobacterium *Aphanizomenon flos-aquae*. This TA system codes toxin that can interfere with key cellular processes and antitoxin that acts as an antidote by neutralizing toxin. In this TA system, HEPN (higher eukaryotes and prokaryotes nucleotide-binding protein) acts as a toxic RNAse and cleaves 4 nt from the 3' end of tRNA molecules, and MNT (minimal nucleotidyltransferase) that inhibits the HEPN RNAse. Usually, in a type II toxin-antitoxin system, an antitoxin forms a complex with a stable toxin and keeps its activity low. In normally growing cells, the toxin is inhibited by the continuously synthesized antitoxin, but under certain conditions, a decrease in antitoxin concentration may lead to toxin activation. What makes the *Aphanizomenon flos-aquae* HEPN-MNT system different is the additional toxin inactivation mechanism via covalent modification performed by the MNT antitoxin. It is proposed that HEPN could be activated at low ATP concentrations, that could interfere with the MNT- catalyzed AMPylation reaction. The MNT antitoxin activity requirement for ATP suggests that the TA system could also act as an ATP sensor [3].

Here we test whether our selected ATPase expression reduces ATP concentration. We then express ATPase in *E. Coli* cells to reduce the ATP concentration and to test if it could activate the HEPN protein.

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B.51. CRISPR-CAS PROTOSPACER INTERACTION WITH ADAPTATION COMPLEX

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CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and their associated genes (*cas*) provide an adaptive immunity against exogenous nucleic acids in bacteria and archaea [1]. During the adaptation a recognition of a short motif known as a PAM (protospacer-adjacent motif) initiates the binding of adaptation complex to a target DNA. Adaptation complex cleaves out a segment of the target DNA and generates a protospacer. After duplication of the repeat, protospacer is inserted into the leader end of the CRISPR array and becomes a spacer [2]. Therefore, the main purpose of this research was to investigate how the sequence of a protospacer affects the adaptation process.

In this study the adaptation complex was co-expressed in *E. coli* BL21(DE3) cells and purified using a two-step affinity chromatography. Afterwards, *in vitro* integration assay was carried out. In total 7 different protospacers were analysed. All sequences of protospacers contained an identical segment for the integration into CRISPR array. However, they differed from one another at their 5' and/or 3' ends.

Here we purified a functional adaptation complex and showed that the sequence of a protospacer has an impact for the interaction with adaptation complex. Protospacer that contained sticky ends and double- stranded PAM had the best integration efficiency, which indicates a stronger interaction with adaptation complex.

Taking everything into account, these results provide insight into the mechanism of adaptation complex and protospacer interaction affecting the process of adaptation.

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B.52. MECHANICAL PROPERTIES OF CASCADE-CAS3 USING SINGLE-MOLECULE MAGNETIC TWEEZERS

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CRISPR is an antiviral defence system in bacteria and archaea. These systems require nucleoprotein complexes to detect and neutralise viral DNA. Cascade-Cas3 is a functional complex found in type I - E CRISPR system and works as a DNA shredder. Here we are using a single-molecule approach, namely magnetic tweezers (MT), to elucidate the mechanism of action of Cascade-Cas3.

Invading DNA is detected by a multisubunit DNA targeting ribonucleoprotein complex – Cascade. As a result, an R-loop is formed which enables accessory helicase-nuclease protein Cas3 to bind and cut the foreign DNA (Sinkunas et al, 2013). However, the full mechanism of how Cas3 unwinds and destroys DNA is yet to be known. That is the key question that we are trying to address here.

Knowledge about the mechanism of this complex would present us with a genome-editing tool which would be suitable for performing large deletions. Since Cas3 acts as a motor protein and requires ATP togenerate mechanical work (Sinkunas et al. 2011), understanding the mechanical properties is crucial to elucidating the mechanism. Thus, the aim of this research is to characterise the mechanical properties ofCascade-Cas3.

Magnetic tweezers allow manipulation of single DNA molecules attached to paramagnetic beads. We prepared a double stranded DNA substrate for MT baring Cascade target site. To observe our samples, we are using a WOSM which was constructed in our laboratory.

Applying this technology enables us to observe protein-DNA interactions in real time and perturb it,making it a suitable method to examine the mechanical properties of helicase and nuclease activities of Cascade-Cas3. Since knowledge about the movement of Cas3 is scarce, we are hoping that results of thisproject will enable us to learn more about it and elucidate the mechanism of this functional complex. Additionally, described method is applicable to study other helicases making it even more appealing.

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B.53. NOVEL *BACILLUS* ANTIVIRAL DEFENSE SYSTEM

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The arms race between prokaryotes and bacteriophages gave rise to numerous antiviral defense strategies in bacteria and archaea. In prokaryotic genomes constituents of defense systems tend to be encoded in close proximity to each other in regions termed "defense islands". Without genes of well-known defense systems, defense islands also contain operons of unknown function [1]. By investigating uncharacterized genes in vicinity of established defense genes, multiple novel antiviral systems werediscovered. One of recently described systems that was first identified in bacteria of *Bacillus* genus is namedSeptu [2].

Bacterial defense system Septu provides resistance to viruses by means of two proteins – putative ATPase PtuA and HNH nuclease PtuB [2]. Although variants of Septu is isolated from representatives of *Bacillus*, Septu system of *B. thuringiensis* provides protection from both *B. subtilis* and *E. coli* phages [3]. Inthis study, we purified proteins encoded by PtuAB operon and examined *in vivo* activity against *E. coli* phages of *B. thuringiensis* Septu defense system.

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B.54. OXIDATION OF ALKENYL-SUBSTITUTED PYRIDINES USING PMLABCDEF MONOOXYGENASE

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One of the most commonly used reactions in the industry - oxygenation reactions - is carried out with the help of organic chemistry methods. However, these methods often require peroxides or metal catalysts. Therefore, more environmentally friendly methods are needed, and as a result, more attention is shifting tothe enzymes that catalyse such reactions - various oxygenases. Non-heme diiron monooxygenase PmlABCDEF possesses a broad substrate specificity and can oxidize different chemical groups, including C=C double bonds and ring heteroatoms [1].

Oxiranes, also known as epoxides, are important intermediates in organic chemistry and they bear the capacity of wide-ranging ring-opening reactions, which usually occur with predictable regioselectivity and stereospecificity [2]. *N*–oxides have increased reactivity compared to regular *N*–heteroaromatic compounds. They can be applied in the agriculture, and pharmacy industries [3]. However, it is a challenging task to selectively oxidize chemical groups of different reactivity (e. g. *N*-oxidation versus epoxidation) in a singlemolecule, therefore diverse synthesis strategies are employed with multiple reaction steps [4].

In this work, we investigated the selectivity of PmlABCDEF monooxygenase with substrates bearing two possible reaction sites – terminal C=C double bond and nitrogen atom in the pyridine ring. Hence, alkenyl-substituted pyridine compounds having different lengths of carbon chains were synthesized from 3-pyridinol and appropriate alkenyl bromides. Produced compounds were used in the bioconversion reactions with *Pseudomonas putida* KT2440 producing recombinant PmlABCDEF monooxygenase. Reaction products were identified as *N*–oxides and epoxides. Chemical oxidation of alkenyl–substituted pyridine derivatives using reagents such as *m*CPBA (meta-chloroperoxybenzoic acid) yields the *N*-oxide or dioxide. Oxidation using biocatalytic methods with PmlABCDEF monooxygenase will allow us to obtain pure epoxide.

The reaction products were extracted and purified using column chromatography on a silica gel. The obtained compounds were analysed with nuclear magnetic resonance (NMR), thin-layer chromatography (TLC), and high performance liquid chromatography – mass spectrometry (HPLC–MS).

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B.55. SINGLE-CELL RNA SEQUENCING OF CLEAR CELL RENAL CELL CARCINOMA TUMOR MICROENVIRONMENT REVEALS NOVEL TUMOR ENDOTHELIUM SUBPOPULATION

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Clear cell renal carcinoma (ccRCC) is the most prevalent renal cancer, accounting for over 75% of cases. The asymptotic nature of the disease often leads to diagnosis in late III or IV stages, where 5-year survival probability drops to 59% and 20%, respectively. First line of treatment is partial or complete removal of kidney as well as immune-checkpoint blockade (ICB) therapy for metastatic disease treatment. Nevertheless, approximately 30% of cases metastasize despite surgical intervention, while the fraction of patients benefiting from ICB treatment remains low [1].

Considering high vascularization, immune system infiltration and heterogeneous tumor microenvironment of ccRCC, establishing reliable biomarkers for disease progression, prognosis or treatment remains a challenge. Understanding the mechanisms and interactions by which infiltrating immune cells contribute to disease progression or treatment response can provide valuable insights in developing new treatment strategies.

Here, using droplet-based single-cell RNA sequencing we profiled 50,236 cells from paired tumor (n=8) and healthy adjacent (n=9) kidney tissues. Our analysis revealed high heterogeneity and inter-patient variability of the tumor microenvironment. In healthy-adjacent samples we identified all major epithelial and endothelial populations of healthy kidney, including an epithelial progenitor-like population previously described as a potential ccRCC cell of origin. Tumor samples were marked by high infiltration of immune cells, including several populations of tumor-associated macrophages and T cells. Moreover, tumor samples displayed abundant stromal and endothelial cell compartments, as well as tumor cells. Our analysis uncovered a previously uncharacterized tumor vasculature subpopulation associated with epithelial-mesenchymal transition. Additionally, cell-cell communication analysis revealed multiple modes of immunosuppressive interactions in the tumor microenvironment, including interactions between tumor vasculature and stromal cells with immune cells. Furthermore, expression of the genes involved in these interactions associated with worse survival in the TCGA cohort.

Overall, our work reveals the unappreciated interplay between tumor vasculature and stromal cell populations in shaping the tumor microenvironment, and uncovers a novel tumor vasculature subpopulation associated with invasive phenotype.

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B.56. AN ULTRA-HIGH THROUGHPUT PLATFORM FOR THE EVOLUTION OF TYPE V CAS NUCLEASES

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CRISPR-Cas provides adaptive immunity in bacteria and archaea against invasive mobile genetic elements. The Class II enzyme Cas12 (type V) does not only cut double-stranded DNA for genome editing, but also displays non-specific ssDNase trans-activity triggered by cis-target cleavage[1]; this unique feature is being actively exploited for the detection of nucleic acids and pathogens in diagnostics[2] and environmental monitoring[3]. This is achieved by triggering the cleavage of a fluorogenic single-stranded molecular beacon upon recognition of the target DNA in the tested sample (Fig. 1). Given the inherently slow activity of this enzyme, its enhancement is highly desirable to improve its efficiency as genome editing tool[4] and to increase the sensitivity in diagnostics[5]. Droplet microfluidics allows for the generation of an emulsion, composed of monodisperse microdroplets, each working as a separate independent reactor, representing a powerful technology for screening of large mutant libraries, enabling time- and cost-effective directed evolution outcomes compared to traditional processes using microtiter plates[6](Fig. 2). For this reason, the aim of this project is to evolve an improved Cas12 variant, developing an appropriate droplet microfluidics pipeline.

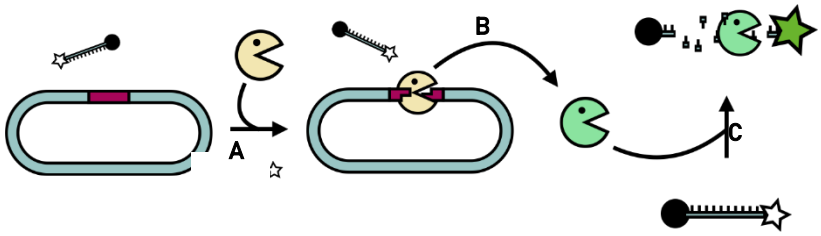

Figure 1; Generation of the fluorescent signal upon target cleavage. The enzyme (in yellow) binds to the target sequence (scarlet) and cleaves it (reaction **A**): this triggers the trans-nuclease state (reaction **B**), that allows the enzyme to cleave non-specifically the ssDNA molecular beacon (reaction **C**). Upon cleavage of the beacon, the quencher (black circle) and the fluorophore are separated, allowing the fluorophore to emit a fluorescent signal (green star.

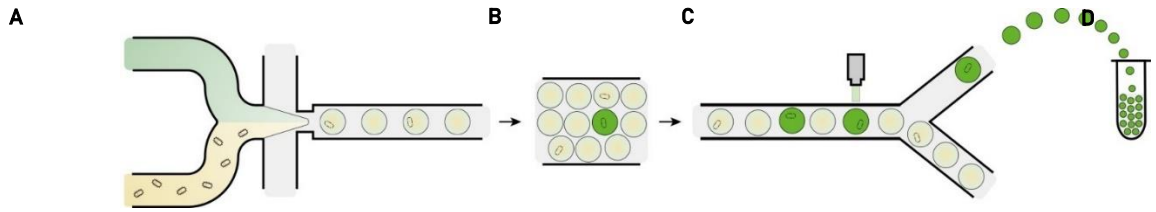

Figure 2; droplet microfluidic wokflow. **A**: droplet generation by co-flow of the mutant library (yellow flow) with lysis reagents, gRNA, target DNA and reporter (green flow). **B**: droplet incubation and signal generation. **C**: droplet sorting. **D**: droplets collection and downstream applications

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B.57. STABILITY OF *S. CEREVISIAE* L-BC VIRUS-LIKE PARTICLES PURIFIED FROM HETEROLOGOUS HOST

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L-A and L-BC, members of the virus family *Totiviridae*, are among the most common viruses that infect *Saccharomyces cerevisiae*. They are distinguished by a double-stranded RNA genome packaged into a capsid made of a single major capsid protein Gag and Gag-Pol fusion protein, which includes RNA-dependent RNA polymerase. Current evidence indicates that Gag modification by N-acetylase complex is crucial for viral assembly of L-A. In contrast, L-BC Gag can self-assemble into a viral particle without N-acetylation [1]. Consistently with this observation, successful efforts to produce L-BC virus-like particles (VLPs) in *Escherichia coli* have been made.

VLPs are icosahedral or rod-shaped nanoparticles that self-assemble *in vitro* from viral capsid proteins. The main difference between VLPs and native virions is the absence of genetic material which makes them unable to replicate in host cell. VLPs have immunogenicity comparable to that of natural viruses and, therefore, can be applied as vaccines. Moreover, due to their empty internal cavity and enhanced permeability, VLPs have been utilized for the delivery of drugs, genes, peptides, proteins and other biological material. While VLPs can be generated artificially in a broad range of host cells such as yeast, insect, plant and mammalian cell lines, bacterial expression systems (especially *E. coli*) are frequently used for this purpose due to low cost of culturing, rapid cell growth and efficient protein expression [2].

The primary goal of this study was to examine the stability of L-BC VLPs synthesized in *E. coli*. In order to produce L-BC VLPs, recombinant protein synthesis was induced in *E. coli* and particles were purified by ultracentrifugation of bacterial lysate through sucrose cushion and cesium chloride. Particles formed by the recombinant L-BC Gag protein were visualized by transmission electron microscopy (TEM) and their size matched the particle size earlier determined by dynamic light scattering (DLS) method (40.4±1.57 nm), as well as the size of native L-BC virus (38.5 nm) [3]. Prior to considering these VLPs suitable for nanodelivery, their stability in different conditions was tested. After monitoring the particle size for 8 weeks by DLS method, it was found that these VLPs are stable in both Tris-HCl- and phosphate-based buffers, different NaCl concentrations, pH values and in the presence and absence of magnesium ions. Stability of the particles was affected only by temperature: particle aggregation was observed after eight weeks at room temperature and after one week at 37 °C. Thus, it can be stated that the purified VLPs are considerably stable. Nevertheless, additional research on cytotoxicity must be done before utilizing them as nanocarriers.

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B.58. DE NOVO METHYLTRANSFERASE-SPECIFIC DNA TRANSALKYLATION IN VITRO

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A family of enzymes known as DNA methyltransferases catalyze the transfer of a methyl group from the cofactor S-adenosyl-L-methionine (AdoMet) to a cytosine on a CpG site in mammalian cell genome. The enzymes are classified into two groups based on their catalytic functionality: 1) Dnmt1, a DNA methyltransferase that targets a hemimethylated CpG sequence in the DNA chain and restores cytosine methylation levels after DNA replication; 2) Dnmt3 (Dnmt3a, Dnmt3b) de novo methyltransferases, which target unmethylated CpG sequences in the DNA strand. As a result, genome methylation is maintained by the combined action of all three AdoMet-dependent DNA methyltransferases. However, the catalytic interplay of each methyltransferase during global DNA methylation maintenance in cells remain poorly understood.

There are several methods for assessing the global methylation profile of genomic DNA. However, the main disadvantage of most technologies is that it cannot determine the individual contribution of each DNA methyltransferases. TOP-seq is a recently developed technique that involves tagging CpG regions with a genetically modified DNA methyltransferase and a synthetic AdoMet cofactor analog, followed by sequencing of the tagged CpG region[1]. Furthermore, the adaptation of TOP-seq in mouse live cells containing an engineered DNA methyltransferase Dnmt1 paved the way for the discovery of epigenetic mechanisms involved in DNA methylation maintenance[2]. Therefore, a similar genetic manipulation strategy of de novo methyltransferases could significantly broaden the understanding of the individual contribution of each Dnmts to the global DNA methylation profile in cells.

The goal of this study was to investigate the catalytic activity of Dnmt3a and Dnmt3b variants *in vitro* using AdoMet cofactor and its synthetic analogue. To begin, vectors containing mouse wild-type Dnmt3a and Dnmt3b as well as preferred mutant coding sequences were constructed and electroporated into Pichia pastoris yeast strain cells. Next, the biomass of G418 antibiotic-selected yeast cell clones was grown, protein expression was induced with methanol, and Dnmt variants were purified by affinity chromatography using a HiTrapTM IMAC HP column from the prepared soluble fraction of cell lysates. Finally, DNA modification activity of purified Dnmt3a and Dnmt3b proteins was assessed with liquid chromatography-mass spectrometry (HPLC-MS) using AdoMet cofactor or synthetic analogue Ado-6-azidohex-2-ynyl (Ado-6-azide), and poly(dI-dC)-poly(dI-dC) or poly(dG-dC)-poly(dG-dC) as DNA substrates.

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B.59. BIOCHEMICAL CHARACTERIZATION OF TRANSPOSON-ENCODED TNPB

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Intercellular DNA transfer is one of the driving forces of evolution in prokaryotes. Mobile genetic elements (MGE), such as insertion sequences (IS), transposons, plasmids, or phages, are responsible for high genomicplasticity in bacteria, allowing rapid adaptation to changing environmental conditions [1]. The IS200/IS605 family insertion sequences are one of the most widely distributed ancient groups of mobile genetic elements among prokaryotes. These sequences are flanked by LE and RE terminal imperfect palindromic motifs and may encode TnpA, TnpA and TnpB, or single TnpB. While the TnpA transposase is responsible for IS transposition, the function of TnpB remains unknown [2]. The comparative analysis of bacterial and archaealgenomes has recently suggested that TnpB might be an evolutional predecessor of CRISPR-Cas9 and Cas12 nucleases [3]. Therefore, the characterization of TnpB could provide new insights into the transposition mechanism of IS200/IS605 family MGEs, and allow us to acquire more knowledge about the evolution of Cas9 and Cas12 nucleases, which are widely adopted in genome editing.

The aim of this study was the characterization of *Deinococcus radiodurans* ISDra2 transposon-associated TnpB protein. First, we detected substantial amounts of RNA co-purifying in complex with TnpB. These nucleic acids were then extracted from the RNP complex and used for small RNA sequencing, which revealed that TnpB binds the right-end element (RE) derived RNA – reRNA. We show that TnpB is an reRNA-guided nuclease capable of cleaving dsDNA targets next to the 5'–TTGAT transposon–associated motif (TAM). Moreover, by changing the 3'-end of reRNA, we demonstrate that TnpB is reprogrammable for target cleavagein bacterial and eukaryotic cells.

Our results reveal that despite its compact size, the TnpB nuclease efficiently hydrolyzed DNA targets ina TAM-dependent manner similarly to much larger Cas12 proteins. Compared to the Cas nucleases, the miniature size of TnpB is suitable for adeno-associated virus-based delivery and opens new horizons fortherapeutic applications. This study also suggests that further characterization of transposon-encoded proteins could expand the genome-editing toolbox by providing a new class of compact non-Cas nucleases with different biochemical requirements for genome-editing applications.

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B.60. UNLOCKING THE POTENTIAL OF *PANTOEA* BACTERIOPHAGES: EXPLORING NEW DNA MODIFYING PROTEINS

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Bacteriophage genomic DNA contains the widest spectrum of nucleotide modifications found in nature. These modifications range from simplistic, such as methylation, to structurally more elaborate nucleotide hypermodifications. Enzymes that modify DNA and the modified nucleotides themselves may be utilized in a variety of disciplines, such as research, medicine, and biotechnology. To deepen our understanding of DNA modifications and DNA-modifying proteins, two *Pantoea agglomerans*-infecting phages vB_PagS_Vid5 (Vid5) and vB_PagS_MED16 (MED16) were chosen to investigate their respective gp34 and gp38 proteins which are potentially involved in DNA modification.

Bacteriophage Vid5 encodes the queuine tRNA-ribosyltransferase (gp34) enzyme within its genome, which catalyzes the switch of the 34th position guanine to 7-aminomehtyl-7-deazaguanine and cyclopentanediol addition to 7-aminomethyl-7-deazaguanine-34 tRNA, forming hypermodified base queuinine. A proposed reaction mechanism is presented in Figure 1 [1]. Additionally, bacteriophage MED16 encodes the preQ$_0$ DNA deoxyribosyltransferase (gp38) protein, which is essential in the Q and G$^+$ tRNA modification pathways, being responsible for changing the target guanines to deazaguanine precursors.

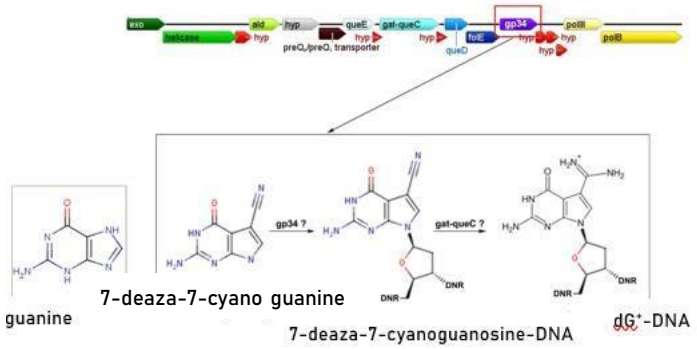

Figure 1. Queuine tRNA-ribosyltransferase potential reaction mechanism.

Recombinant, N and C terminus-histidine tagged proteins corresponding to Vid5 gp34 and MED16 gp38 were successfully expressed in the *E. coli* BL21(DE-3), Rosetta(DE-3) and NovaBlue(DE-3) under varying conditions of 37°C for 3 hours, 30°C for 5 hours and 20°C for 18 hours. Following SDS-PAGE analysis of the recombinant proteins, it was determined that both proteins were present in a soluble, monomeric form with an approximate molecular weight of 30 kDa. The results show that the optimal conditions for protein synthesis is using *E. coli* BL21(DE-3) at 20°C for 18 hours.

The results of this study not only increase our knowledge about hypermodified nucleobases and DNA modifying enzymes in bacteriophages but can also contribute to further potential application of phage DNA modifying enzymes in medicine, biotechnology and other areas.

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B.61. INVESTIGATION OF POTENTIAL CAS4 PROTEIN FROM *BACILLUS* PHAGE VB_BAUM_KLEB27-3

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Unique environments still happen to be very poorly explored, even though they contain a wide variety of microorganisms and viruses that possess potential applications in biotechnology or medicine. An example of such environments are gypsum karst lakes, from which a new *Bacillus* phage vB_BauM_KLEB27-3 (KLEB27-3) has been isolated with a genome of more than 200 kb which classifies the phage as a jumbo one. Because of its large genome this bacteriophage may potentially have a wide variety of genome editing elements, including enzymes, that can disable the CRISPR/Cas system and protect its own DNA during infection of bacteria.

In this study, genome analysis of the newly isolated bacteriophage KLEB27-3 was performed and the gene *g92* was identified for further analysis. Bioinformatic and phylogenetic analyses indicated that gp92 is the closest to CRISPR-associated exonuclease (Cas4 family) of *Bacillus* phage vB_BceM_WH1 (56 % aa identity). Cas4 family proteins are involved in CRISPR adaptation and possess exonuclease activity. As there were no close homologues of gp92 to any bacterial or phage proteins deposited to publicly available databases, it is hypothesized to have unique properties that are worth to investigate in more detail. To studythe potential activity of recombinant gp92 *in vitro*, the gene was cloned into two inducible expression vectors(pET21a and pCDFDuet-1), producing recombinant proteins with a His-Tag fussed to either the N- or C- terminus. Purification of soluble gp92 demonstrated, that gp92 C-His tagged protein is ATP-independent andhas potential endonuclease activity.

Results of this study not only extend our knowledge about bacteriophage genome editing elements, but also give new insights about potential applicability of the aforementioned enzyme in medicine, biotechnology, ecology and other areas.

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B.62. STUDY OF NEW ANTI-PHAGE DEFENSE SYSTEMS

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Bacteriophages are incredibly diverse and widespread viruses that constantly threaten bacteria. Thecontinuing co-evolutionary fight between bacteria and their viral predators has resulted in the developmentof a sophisticated and diverse array of bacterial defense mechanisms, such as the well-known restriction-modification and CRISPR-Cas systems. New anti-phage defense systems and gene editing tools have revolutionized the fields of molecular biology and biotechnology. These tools can target specific genes andsequences for modification or removal, as well as anti-phage defense mechanisms that prevent bacteriophages from infecting bacteria. Identification of these mechanisms led to the development of precise molecular tools used in genetic engineering and genome manipulation, so the identification of novel defense systems may result in the development of new tools for manipulating cells and genomes. On the basis of the discovery that anti-phage defense systems frequently form so-called "defence islands" in bacterial genomes,several groups have employed computational methods to detect new, uncharacterized defense systems [1,2].

Here, we present studies on nine of these newly discovered systems with well-known nuclease,NTPase and DNA/RNA helicase domains. We have shown that these systems are active and show resistanceagainst bacteriophages. Also, we have successfully cloned these systems into high-copy plasmid DNA, as confirmed by the whole plasmid sequencing. Lastly, we have performed protein expression studies on all these systems.

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B.63. THE ROLE OF MIRNA-574 ARMS IN LUNG CANCER PROGRESSION

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Lung cancer is a leading cause of cancer death world-wide. Non-small cell lung cancer (NSCLC) is the most common form accounting for more than 80 % of lung cancer cases (Duma et al., 2019). The early diagnosis and control of cancer metastasis are critical factors in cancer treatment. Discovery of novel biomarkers and therapeutic targets have helped to increase survival rates for many types of cancer, including lung cancer. Over the past decade miRNAs have emerged as potential biomarkers and targets in lung cancer diagnosis and treatment. miRNAs are small 20-25 nucleotide long single-stranded noncoding RNAs that play an important role in tumour initiation and growth. miRNAs function in post-transcriptional regulation of gene expression can act as tumour suppressors, oncogenes or metastasis regulators. Previous studies have revealed miRNA-574 as a potential regulator of many cancers (Zhang et al., 2019). However, a precise role of two miRNA-574 arms (5p and 3p) has not been elucidated.

Thus, further investigations into arms expression signatures in normal and cancerous lung tissue biopsies with real-time quantification of microRNAs by stem-loop RT-PCR method (Chen et al.,2005) are necessary in order to identify novel potential biomarkers for lung cancer.

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B.64. THE ASSOCIATION OF ADVANCED GLYCATION END PRODUCTS (AGEs) WITH DIABETES MANAGEMENT INDICATORS IN PATIENTSWITH DIABETIC RETINOPATHY

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Advanced glycation end-products (AGEs) are heterogeneous s group of molecules that are formed in non-enzymatic reactions. In healthy people, AGEs normally accumulate slowly with natural ageing. Higher AGEs levels are associated with metabolic syndrome, cardiovascular diseases, and diabetes-related complications such as diabetic retinopathy (DR). Good diabetes control helps to lower the risk of diabetic complications, which can be accelerated by AGEs. Monitoring of AGEs could help to reduce and prevent diabetic vascular complications.

The aim of this study was to investigate the relationship between AGEs levels and main diabetes management indicators in patients with DR, including HbA1c, arterial blood pressure and low-density lipoprotein cholesterol (LDL-C).

AGEs concentration in the skin was non-invasively measured using ultra-violet light to excite autofluorescence with AGE Reader in 67 diabetic patients with no DR and in 66 patients with DR (age ≥18). Data on patients' clinical characteristics were collected from medical records. Based on diabetes control indicators (HbA1c < 7.0%, blood pressure < 130/80 mmHg, and low-density lipoprotein cholesterol (LDL) < 2.6mmol/l), four groups were formed depending on the number of achieved diabetes control goals: 0 goals, 1 goal, 2 goals, 3 goals. Collected data was used to investigate associations between diabetes management indicators and the patients' AGEs concentration.

The study cohort consisted of 73 (54.9%) women and 60 (45.1%) men. The median diabetes duration of the patients was 14 years, age – 55 years. The levels of AGEs increased with HbA1c ($r=0.195$, $p<0.05$) and systolic blood pressure ($r=0.265$, $p<0.05$). Patients who had not achieved any of the diabetes management goals had higher concentration of AGEs, indicating higher vascular risk ($p<0.05$). Also, the results showed the association between diabetes management goals achievement and age, DR, and smoking (all $p<0.05$). Patients who achieved higher numbers of diabetes management goals were younger, had lower AGEs levels,lower frequency of DR complications, and smoked less.

AGEs levels were significantly associated with diabetes management goal achievement. Patients with low AGEs levels were more likely to achieve diabetes management goals.

The work was supported by the EEA Financial Mechanism 2014–2021 "The Baltic Research Programme". No.:EEA-RESEARCH-60.

B.65. SYNTHESIS AND EVALUATION OF OPTICAL PROPERTIES OF NEW CONJUGATED 3H-INDOLE DERIVATIVES

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3H-Indole core is a common structural unit in biologically active natural products and often serve as an inspiration to medicinal chemists. Synthetic 3H-indole derivatives are of great importance to their vast applicability as pharmaceuticals [1], fluorescent imaging probes [2], photosensitizers [3], etc. Fluorescent probes are extensively used in in vivo imaging due to their low cost, operational simplicity, high sensitivity and selectivity, non-invasiveness and possibility to conduct real-time experiments [2,4]. Fluorescence sensing technology enables detection and analysis of various ions, organic and inorganic molecules, biomarkers or intracellular pH values, etc. in vivo and could complement or event replace traditional imaging methods in the near future [2].

A wide array of suitable optical and biological properties possessing compounds are frequently based on cyanine dye structure containing 3H-indole moiety [4]. Although many studies have been performed with these compounds, their number in clinical use is scarce. Therefore, synthesis of new 3H-indole derivatives remains of high interest. To fill this gap, a series of new 3H-indole derivatives and their corresponding 3H-indolium salts were synthesized and their optical properties were investigated.

Target compounds were synthesized via Suzuki cross-coupling and Knoevenagel condensation. Subsequent alkylation of the final compounds afforded 3H-indolium iodide salts. Structures of new compounds were confirmed by 1D, 2D NMR, HRMS and FT-IR spectroscopic data.

Optical properties of newly prepared 3H-indole derivatives and their corresponding 3H-indolium salts were investigated in aqueous solutions. It was observed that absorption and emission peaks of 3H-indole derivatives were in the visible part of electromagnetic spectrum, however the fluorescence of the tested compounds was low. Absorption peaks were in the range of 383–477 nm and emission peaks were in the range of 504–616 nm. In addition, the Stokes shifts of the tested compounds were quite large, ranging from 71 to 233 nm. Interestingly, 3H-indolium salts did not produced fluorescence under excitation with UV light.

Overall, presented results show, that new 3H-indole derivatives are easily obtainable, and their fluorescence properties are structure dependent.

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B.66. DEVELOPING HUMAN BIOMIMETIC TETHERED BILAYER LIPID MEMBRANES FOR ASSESSMENT OF THE EFFECTS OF S. AUREUS EXOTOXIN A-HL

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Tethered bilayer lipid membranes (tBLMs) are robust model membrane systems, that mimic the lipid bilayer membrane of eukaryotic cells [1]. tBLMs are increasingly used for membrane-protein interaction studies including functional reconstitution of the pore-forming proteins and peptides. To this day, the tBLMs were made from up to two to three various synthetic lipids, yet they poorly reflect the biological plasma membranes which could contain up to five major lipid compounds.

In this communication, we explored a proof of concept for the possibility to form multi-lipid biomimetic tBLMs that would closely resemble the lipid compositions of Red Blood Cells (RBCs) and Brain Capillary Endothelial Cells apical membrane (BCECs) (Fig. 1). The novel tBLM compositions were assembled via multilamellar vesicle fusion onto 30% molecular anchor WC14 and 70% β-mercaptoethanol functionalized thin film gold surface [2]. This was followed by studying the effect of pH changes (acidic to basic) and the activity of alpha-hemolysin, a common pore-forming toxin. In both cases, the study was performed utilizing the Electrochemical Impedance Spectroscopy (EIS) methodology, and the effect of pH was quantitatively assessed by measuring the impedance phase minimum shift as described in [3]. In addition to this, Atomic Force Microscopy (AFM) was used to visualize the surface roughness of the novel membrane compositions.

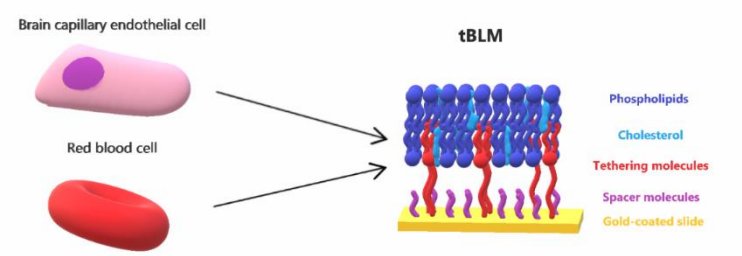

Figure 1. General tBLM structure is based on the compositions of major lipids in the BCEC and RBC cells.

We found that as the pH increases from 4.4 to 7.4 the EIS phase minimum moves towards higher frequencies for all the tested tBLM compositions. The RBC membranes showed the highest shift of 0.649±0.292, followed by positive control DOPC:Chol 60:40 with 0.497±0.122, and in BCEC lowest shift of 0.344±0.290 was observed in log scale, respectively. Studying the effects of alpha-hemolysin activity on the novel tBLMs showed that the RBC membranes were most responsive to the activity of the toxin which is shown by the average frequency logarithmic shift of 2.273±0.342 followed by the BCEC membrane with the average logarithmic shift of 0.402±0.389. We hypothesize that this high activity of alpha-hemolysin towards the RBC membranes could be attributed towards the presence of higher amounts of cholesterol that leads to the presence of more lipid-ordered domains resulting in a decrease of the membrane dielectric properties. These findings could be correlated to the study by [4] which reports an increase in toxin activity with a decrease in the dielectric property of tBLMs. Furthermore, both RBC and BCEC membranes show similar surface roughness (R_a) of 0.45 and 0.40 nm when analysed using AFM.

In conclusion, we demonstrated through EIS and AFM that tBLMs that closely resemble the RBC and BCEC could be formed using multi-lipid composition and this could be used to structurally and functionally interrogate the lipid-protein interactions.

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B.67. ISOLATION AND CHARACTERIZATION OF KLEBSIELLA-INFECTING BACTERIOPHAGE VB_KAEM_F14

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Klebsiella spp. is a causative agent of both healthcare-associated infections and plant diseases such as stem rot of pearl millet and soft rot of E'ves needle cactus. However, the emergence of antibiotic-resistant Klebsiella spp. strains poses a major challenge to effective treatment, necessitating the exploration of alternative methods of controlling the spread of these microorganisms such as phage therapy.

In this study, a novel, ambient-temperature adapted, Klebsiella-infecting phage - vB_KaeM_F14 (F14) - was isolated from a gypsum karst lake in North Lithuania. The results of transmission electron microscopy (TEM) analysis revealed that F14 is a myovirus, possessing an isometric head with a diameter of approximately 76 nm and a contractile, non-flexible tail of about 58 nm (contracted) / 117 nm (noncontracted) in length. Phage F14 was tested against a panel of 80 bacterial strains, revealing that only Klebsiella aerogenes isolate F14 was sensitive to F14. Additionally, plating tests revealed that F14 can form clear plaques with a diameter of 1.9 mm in a temperature range of 4 to 29°C, with 22°C being the most optimal temperature for infection. Lastly, chloroform sensitivity tests did not reveal any sensitivity of F14 to this detergent under investigated conditions.

The findings of this study not only extend our knowledge about Klebsiella infecting viruses but also suggest the potential of phage F14 as a phage-based biocontrol agent for the regulation of Klebsiella- associated plant or human pathogens.

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B.68. NUCLEIC ACID TAGGING BY THE MAMMALIAN N6-ADENINE METHYLTRANSFERASE METTL4

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In eukaryotes, N6-methyladenine is an abundant RNA modification and has also been detected on DNA. However, its function in cells remains unclear. The mammalian protein METTL4 is known to be the main methyltransferase that methylates adenine on eukaryotic DNA as well as some types of RNA. The methyltransferase activity of METTL4 is dependent on the natural cofactor *S*-adenosylmethionine (AdoMet). Importantly, METTL4 has been implicated in cancer development and it is thus essential to characterize its activity further[1]. Recently, a high resolution technique was described for mapping Dnmt1-dependent cytosine methylation sites (Dnmt-TOP-seq)[2,3]. During this process a synthetic cofactor analog, Ado-6-azide, is used to install 6-azide-hex-2-ynyl groups at cytosine methylation sites, where these tags guide subsequent mapping of modified cytosines. We in turn were interested in using the synthetic cofactor analog Ado-6-azide for tagging adenine methylation sites to track METTL4 activity.

In this study, active mouse and human METTL4 proteins were purified from HEK293 cells. Following *in vitro* enzyme activity assays with DNA and RNA substrates, HPLC-MS was used to determine adenine modification levels. METTL4 was shown to methylate RNA substrates when supplied with the natural cofactor AdoMet. Importantly, METTL4 was found to transfer azide tags onto RNA from the synthetic cofactor analog Ado-6-azide. Furthermore, METTL4 was shown to install an azide tag on DNA as well. METTL4 was then sterically engineered to increase its affinity for the synthetic cofactor analog. Importantly, engineered METTL4 variants could tag nucleic acid using the synthetic cofactor analog Ado-6-azide even when the natural cofactor AdoMet was present.

To conclude, it was shown that METTL4 methylates RNA and transfers azide tags onto RNA as well as DNA *in vitro*. Furthermore, METTL4-dependent nucleic acid tagging using the synthetic cofactor analog Ado-6-azide can be achieved even in the presence of the natural cofactor AdoMet.

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B.69. IDENTIFYING MODIFIED LONG NON-CODING RNA TARGETS IN GLIOMAS BY DIRECT RNA-SEQ

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Introduction

Glioblastoma is one of the aggressive brain tumors with an average life expectancy of patients of about 12–15 months [1]. Attempts to develop a diagnostic and targeted approach for the treatment of glioblastoma continue. Since post-transcriptional modifications of RNA are vital for cells, it is important to study their role in human glioblastoma in order to develop both diagnostic signatures and potential therapeutic targets [2]. So far, successful attempts have been made to comprehensively characterize the role of RNA modifications in glioblastoma [1,2].

Aim

The aim of the current study was to evaluate and compare gene expression and m6A modification levelin glioma patient samples at the gene level.

Methods

Following the manufacturer's instructions 24 snap-frozen tumor tissues (n=8 LGG or diffuse astrocytoma and n=16 GBM or glioblastoma) were homogenized in TRIzol for total RNA extraction. The "Dynabeads mRNA DIRECT kit" was used to enrich polyA RNA. Nanopore direct RNA sequencing libraries were created, loaded into R9.4.1 SpotON flow cells, and sequenced on the minION instrument for 72 hours. *Guppy* used the default settings to base-call the raw data in the fast5 format. The pipeline "*nf-pore/nanoseq*" was used to process the sequencing data, while "*m6Anet*" and *Epinano* were used to analyze the predictedm6A alteration. m6A modification (prediction) and gene expression values (in TPM) were calculated from RNA-seq data. The gene was dedicated as modified if at least one RRACH motif were modified per gene.

Results

Using DEseq analysis on RNA-seq data, there were selected 10 genes which were differentially expressed (from 0.48–fold (*SNHG29*) to 2.21–fold change (*LINC00844*)) between LGG and GBM (lncRNAs that passed adjusted p-value threshold) ($p<0.05$). Significant differences ($p<0.05$) between pathologies in single gene expression were determined in 5 analyzed genes *(LINC00844, NEAT1, OTUD6B-AS1, ENSG00000225792, MIR9–1HG)*. Kaplan–Meier survival analysis showed significant differences in patient survival for in *LINC00844, NEAT1, OTUD6B–AS1, MIR9–1HG* and *OIP5–AS1* in glioma patients. The modification level analysis showed significantly lower m6A level ($p<0.05$) in GBM as compared to LGG in *LINC00844* and *SNHG6* genes. Better survival prognosis was observed in patients with m6A–modified *LINC00844*, *OTUD6B–AS1*, and *OIP5–AS1* genes. Correlation analysis showed no association between m6A and the level of gene expression in gliomas.

Conclutions

To summarize, this study revealed differences between LGG and GBM samples in terms of lncRNA gene expression and m6A modification. *LINC00844, OTUD6B-AS1* and *OIP5-AS1* were associated with patient survival at gene expression level and m6A level, showing selected genes involvement in gliomagenesis. However, additional molecular and biological studies are needed to confirm the impact of m6A on the essential glioma gene functions.

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B.70. THE IMPACT OF MEMBRANE MICROENVIRONMENT ON STREPTOLYSIN O-MEMBRANE INTERACTIONS

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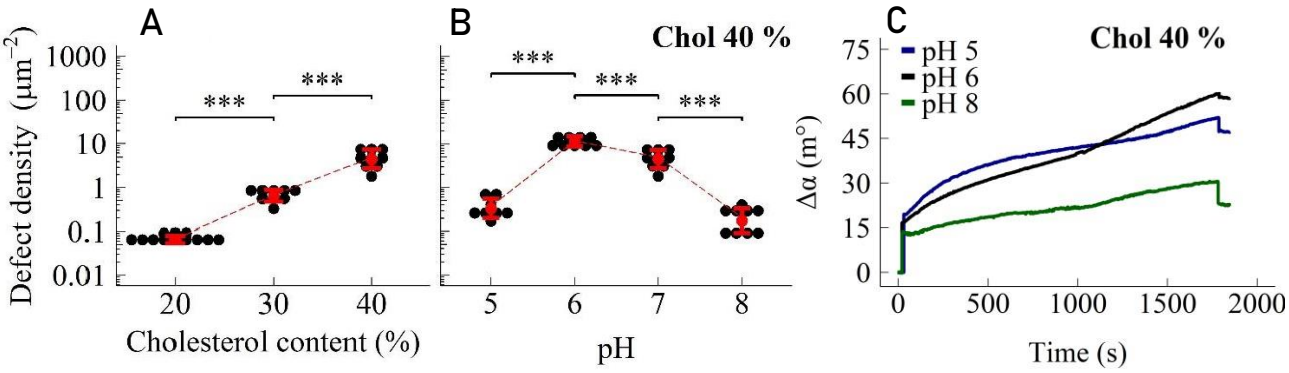
Streptolysin O (SLO) is a bacterial pore forming toxin which belongs to a family of cholesterol dependent cytolysins (CDCs) [1]. As the name suggests all members of this family require cholesterol for their cytolytic activity [2]. Membrane cholesterol binding is the first step of CDC pore formation, followed by oligomerization and membrane perforation which leads to cell death [2]. Streptolysin O is produced as one of the main virulence factors of many strains of *Streptococcus pyogenes* and group B and G streptococci [3]. These human-specific pathogens are a common cause of upper respiratory tract infections such as pharyngitis and scarlet fever [4]. In severe cases streptococcal infections can result in toxic shock syndrome, "flesh-eating" infections and other life-threatening conditions [4]. Invasive cases and higher mortality rates have been correlated with higher levels of streptolysin O production [1]. Although, the main mechanisms of SLO pore formation are well understood there is a lack of detailed knowledge on how membrane composition and its surroundings influence the interplay between protein and membrane.

The aim of this work was to evaluate how membrane microenvironment: cholesterol content and pH, affect streptolysin O-membrane interactions. To evaluate differences in membrane binding we employed hybrid lipid bilayers together with surface plasmon resonance (SPR). Membrane permeability changes caused by streptolysin O were monitored in tethered lipid bilayers using electrochemical impedance spectroscopy (EIS). Our work shows that the number of streptolysin O-induced defects is affected by membrane cholesterol content (Fig. 1A). Toxin induced damage is also pH dependent (Fig. 1B). SLO caused the greatest change in membrane permeability in membranes with highest cholesterol content at acidic pH (pH 6). SPR data indicates that lower toxin activity when pH is increased above and decreased below optimum pH could be due to impaired membrane binding (Fig. 1C). In the light of repeating group A streptococci outbreaks these findings could be used to create biosensors for drug screening and early disease diagnostics.

Figure 1. EIS measurements of density of SLO-induced (5 nM) defects in membranes with increasing cholesterol content (A) and in membranes containing 40 % cholesterol within a pH range of pH 5-8 (B). SPR observations of SLO (25 nM) binding in membranes with 40 % cholesterol at pH 5, 6 and 8.



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B.71. BFERREDOXIN:NADP+ OXIDOREDUCTASE FROM BACILLUS SUBTILIS: REACTIONS WITH NONPHYSIOLOGICAL OXIDANTS AND POTENTIOMETRIC CHARACTERISTICS

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Ferredoxin:NADP⁺ oxidoreductases (FNRs, EC 1.18.1.2) are flavoenzymes classified as dehydrogenases – electrontransferases acting in electron transfer in plastids, mitochondria and bacteria. FNR catalyzed two electron transfer from two equivalents of reduced ferredoxin to NADP⁺ is the final step of electron transfer in photosynthesis, while electron transfer in the opposite direction is implicated in nitrogen fixation or isoprenoid biosynthesis. After the transfer of a single electron to the oxidant a FAD semiquinone forms. *Bacillus subtilis* is a Gram-positive bacterium. *B. subtilis* ferredoxin:NADP⁺ oxidoreductase (*Bs*FNR) belongsto a novel subgroup of glutathione reductase-like FNRs resembling thioredoxin reductase. The key differences from other FNRs are a native homodimeric structure and NADP(H) binding domain being insertedbetween the two segments of an FAD binding domain [1].

By combining steady- and presteady-state kinetics and photoreduction methods, here we present astudy on the *Bs*FNR catalyzed oxidation of NADPH in the presence of nonphysiological electron acceptorscommonly considered to be xenobiotic compounds, often found as environmental pollutants or various drugs. These compounds exhibit redox-cycling behavior under aerobic conditions and this is considered to be the basis of their prooxidant cytotoxicity.

The mechanism of oxidation of *Bs*FNR by several different groups of nonphysiological electron acceptors was examined. Quinone reductase and nitroreductase reactions proceed via a ping-pong mechanism. The k_{cat}/K_m values of quinones and aromatic *N*-oxides towards *Bs*FNR increase with their single- electron reduction midpoint potential with a parabolic dependence. The same is true for nitroaromatic compounds, albeit their reactivity is lower and the increase is linear, owing to their electron self-exchange constants being lower by several orders of magnitude. This then illustrates that the reactivity is governed by the energetics of the compounds and not their structural features. The calculated electron transfer distances in the reaction with quinones and nitroaromatics are close to the ones obtained in the studies of FNRs from *Anabaena*, *Plasmodium falciparum* and *Rhodopseudomonas palustris*, which highlights similar "intrinsic" reactivities [2,3]. Nitroaromatics were reduced entirely in a single-electron way, with the single-electron flux being close to 100 % in the case of trinitrotoluene and *p*-nitrobenzaldehyde while quinones follow mixed one- and two–electron reduction with one–electron flux of up to 20% in the case of 1,4–benzoquinone. The enzymatic reaction is inhibited by the reaction product NADP⁺ which acts competitively towards NADPH with a $K_i \approx 100$ µM and noncompetetively towards the quinone with $K_i \approx 1.5$ mM. 5-deazaFMN and EDTA are used as photosensitizers during the photoreduction of *Bs*FNR with a broad band characteristic to a semiquinone appearing in the 600 nm wavelength region upon a short irradiation under anaerobic conditions.

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B.72. STRUCTURAL VARIABILITY OF PRION PROTEIN AMYLOID FIBRILS UNDER DIFFERENT AGITATION CONDITIONS

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Amyloidogenic peptides and proteins have a property to convert from their native functional states into fibrillar amyloid aggregates. This property is associated with neurodegenerative disorders, such as Alzheimer's or Parkinson's diseases, as well as prionopathies [1]. It has been observed that the environment conditions in which amyloid aggregation takes place have an important effect on fibril polymorphism. Moreover, amyloid aggregates have recently been shown to have the property to adopt more than one different conformation under the same environmental conditions [2].

Since there are countless studies employing different types and intensities of sample agitation, this environmental factor requires additional investigation. In this work, we examined the effect of three different agitation conditions on the aggregation kinetics of mouse prion protein fragment (MoPrP 89-230) and analyzed the secondary structure of the resulting fibrils.

Protein MoPrP 89-230 samples were incubated under three agitation conditions (200, 400 and 600 RPM) at 37°C, under denaturing conditions (2 M guanidinium hydrochloride, 50 mM sodium phosphate, pH 6.0). The kinetics of aggregation were determined by recording the fluorescence intensity of the amyloidophilic dye thioflavin-T (ThT). The secondary structure of fibrils was determined by analyzing each sample's FTIR spectra.

The average kinetic parameters for each agitation condition (lag time and apparent fibril elongation rate) were calculated and these parameters were compared between each other. Interestingly, despite such large variations in agitation intensity, there were no significant differences between the process lag time values. The apparent rate of elongation, however, was significantly different between all three conditions. Surprisingly, the rate was highest at 400 RPM, rather than the expected 600 RPM condition. These results suggest that the intensity of agitation has a minimal influence on primary nuclei formation and that the rate of elongation does to scale with the level of agitation. In all three cases, a diverse collection of secondary structures (at least four structure types) were observed, with the highest variability detected under 200 RPM agitation conditions.

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B.73. ENZYMATIC ELECTROCHEMICAL BIOSENSOR FOR EXTRACELLULAR GLUTAMATE DETECTION IN BIOFLUIDS

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Glutamate (Glu) is the most abundant excitatory neurotransmitter in the human central nervous system (CNS) whichplays a major role in metabolic signalling pathways involved in learning and memory. Whereas excess Glu concentrationsin synapses can excite neurons to the point of cell death in a process referred to as **excitotoxicity**. Powerful uptake systems(glutamate transporters) prevent excessive activation of Glu receptors present on the surface of brain cells by continuouslyremoving Glu from the extracellular brain fluid. Neurodegenerative diseases known to be associated with expressing excess glutamate exciting nerve cells include Parkinson's disease, Alzheimer's disease and Huntington's disease [1]. Whereas insufficient brain glutamate concentration is thought to result in trouble concentrating, mental exhaustion, insomnia, and low energy. Therefor it is essential our CNS maintains moderate extracellular glutamate concentrations. Extracellular glutamate concentration estimates conducted *in vitro* from tissue slices vary around 0.02–20 µM [2].

An option to facilitate real-time, continuous monitoring of analytes such as glutamate is by use of **enzymatic electrochemical sensors.** Enzymatic sensors provide potential for obtaining high measurement sensitivity. However, theyare often expensive and decrease in stability over time, resulting in a signal reduction causing inaccuracies in measurement. **Biosensors** are the biological manipulation of electrochemical sensors, where a biological analyte is detected on a bioreceptor. In order to obtain plausible sensitivity and prove biosensor reliability it is essential to assess itstechnical suitability by accurately optimizing its detection conditions, such as electrolyte solution parameters and providereassurance that the measured analyte is undeniably glutamate.

Therefor the approach to detect extracellular glutamate levels was using first generation biosensors to utilize the reaction enzyme - glutamate oxidase (GluOx). When the analyte (glutamate) is detected a reduction–oxidation (redox) reaction takes place, indirectly producing an electron through the production of hydrogen peroxide [3]. The following reactions occur, Eq (1):

$$Glutamate + H_2O + O_2 \xrightarrow{GluOx} H_2O_2 + \alpha - ketoglutarate + NH_3 \quad (1)$$

$$H_2O_2 \longrightarrow H_2 + O_2 + 2e^-$$

The used three-electrode potentiometric sensor consists of a platinum (Pt) working electrode on which the redox reaction occurs; a silver chloride (AgCl) reference electrode as an electrode which has a stable and well-known electrodepotential (acts as a dynamic baseline to compare the measured potentials of the other electrodes); a titanium (Ti) counterelectrode which provides a means of applying input potential to the working electrode. The electrodes were submerged in different concentration acid/base/salt electrolyte solutions to optimize which buffer pH would ensure electrical neutrality and allow ions to migrate with minimal resistance. Electrodes were connected to a data processor to relay the results.

As a **result,** we present a first generation GluOx Pt electrode biosensor with an adjusted membrane which specificallymeasures glutamate levels in mouse brain tissue. Biosensor results assure reliability by providing high sensitivity measurements and a low limit of detection (LOD) for hydrogen peroxide (down to 5 µM of Glu) while submerging electrodes in a 10 mM, pH 7 electrolyte solution.

Considering Glu is linked to various neurodegenerative diseases and mental health disorders its **significance** as a biological marker and fast methods of sensing [Glu] in biofluids remain perspective and important matters to elaborate infuture research.

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B.74. DETERMINANTS OF DNA REPAIR IN CRISPR NUCLEASE-MEDIATED GENE EDITING

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CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) nucleases transformed our approaches to medicine, diagnostics, agriculture, and research. By programming a CRISPR nuclease with a guide RNA (gRNA), we can direct it to cut a gene with the matching DNA sequence - its target [1]. When CRISPR nucleases induce double stranded breaks (DSBs) or site-specific single-stranded breaks (SSBs), endogenous cellular repair mechanisms resolve these breaks [2]. These mechanisms include non-homologous end-joining repair (NHEJ) and homology directed repair (HDR) [3]. Cellular enzymes repair these cuts, introducing changes to a gene's sequence that can prevent or alter the expression and activity of its product.

CRISPR nucleases tolerate small differences between the gRNA and DNA target sequences. These contribute to 'off-target' activity, where the nuclease cuts additional genes, causing unexpected (and sometimes dangerous) changes. These small differences also affect how the nuclease cuts DNA, producing DNA ends with different shapes and sequences. But how does cellular repair machinery interpret these DNA ends during repair? The study of the 'off-target' activity of CRISPR nucleases via programmed modifications to the gRNA or target DNA sequences could reveal which repair mechanisms different off-targets induce.

Our research focuses on investigating if and how gRNA-DNA sequence parity alters the repair outcomes of editing with CRISPR nucleases. This relies on our lab's expertise in bioinformatics, biochemistry and cell biology. We have elected candidate gRNA and off-target DNA pairs for the CRISPR nuclease Cas12a by searching its cleavage specificity profile. We have validated the *in vitro* performance of each pair and are linking this with their cellular repair outcomes. We anticipate identifying reproducible gRNA manipulations that direct specific gene editing outcomes, making future gene editing safer and more reliable.

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B.75. MODIFICATION AND INVESTIGATION OF PHOSPHOLIPID BILAYER MODIFIED TRANSPARENT SURFACES

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Silanization is a process of coating a surface with self-assembled monolayer (SAM) using alkoxysilane molecules (specifically trichloro(octadecyl)silane in this case). Surfaces of minerals components such as glass, metal oxide can be silanized, since they have a hydroxyl group which attack the alkoxy groups in the silane as a result forming a covalent -Si-O-Si bond. The aim of silanization is forming bonds across the interface of the organic and mineral components. Forming such bonds on a surface (glass in this case) results in vastly increased hydrophobicity, such property is applied when seeking to minimize cell adherence to a glass wall in cell cultures, etc. [1]. Another use for this process in biomedical fields is to anchor DNA onto substrates. In this study we chose to anchor a phospholipid bilayer to silane SAM. The mechanism of silanization itself can be summed up in 4 steps: First, adsorption of silane to the surface through molecular interactions; second, hydrolysis of silane's alkoxy group to form silanol; third, silanol on the surface undergoes condensation with the remaining alkoxy groups on the silane molecule thereby forming Si-O-Si bonds; lastly molecules form cross-linked networks forming a highly durable and stable surface [2]. One of the aims of this research was finding the optimal conditions while silanizing glass which were judged by extent of their hydrophobicity. Silanized glass surfaces may be employed as substrates for assembly of phospholipid bilayers. The study of protein incorporation into cell membranes is made possible by such phospholipid bilayers, which may also have biological benefits.

By measuring contact angles, you can determine the degree of wetting of a liquid on a solid surface. Hydrophilic surfaces give low contact angles which indicates better wetting, one example is glass. The contact angle of a droplet of water usually is around 15–20 degrees. However, once you silanize the aforementioned glass it's contact angle increases to 100 degrees, indicating strongly hydrophobic surface. But surface bound groups are not the only factor altering the contact angle measured, other being fluid's surface tension, ambient temperature, the medium above the drop, etc. [3] To measure the contact angle the sessile drop method using a contact angle goniometer was employed. Goniometers function by measuring the surface-liquid angle, the set up usually consists of a sample stage, a drop dispense mechanism, a camera, and some sort of backlight. When the drop is dispensed, the camera records it and then using specialized software the shape of the drop is analyzed and relevant calculations are made, providing the results. Later on, confocal microscopy is employed to further analyze the phospholipid bilayer formed on silanized glass using lipids with fluorescing dyes mixed in.

Table 1. Comparison of contact angles between 1 cm x 2 cm glass platelets silanized in a 0,1% OTS solutionin heptane at 60 ^{0}C for varying amounts of time.

Silanization time (min)	Contact angle (degrees)	Standard deviation
15	81,42	4.594
30	87,28	5.325
45	95,51	9.150
60	103,72	5.394

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CHROMOGENIC IN SITU HYBRIDIZATION IN AVIAN HAEMOSPORIDIAN RESEARCH: HOW IT FURTHER STRENGHTENS THE RESEARCH ON TISSUE STAGES

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Avian haemosporidian parasites (Haemosporida, Apicomplexa) multiply asexually in the tissues of their hosts before the sexual stages (gametocytes) develop in blood cells. The asexual multiplication takes place in the organs of birds, resulting in the development of exo-erythrocytic stages (EES) or tissue stages called meronts or megalomeronts in the case of the *Haemoproteus* and *Leucocytozoon* species [1]. EES are known for less than 30 species among the 177 described species of *Haemoproteus* [2]. This gap in knowledge in these parasites' life cycle reflects the difficulties to study the EES, requiring the application of innovative approaches for better understanding patterns of their development.

Gametocytes (invasive stages for vectors) are studied by light microscopic examination of blood films, while the EES are studied by light microscopic screening of histological sections of internal organs. Thus, the dissection of infected hosts is necessary to assess the exo-erythrocytic development (EED) of these parasites. Furthermore, as the EES are known for only a few *Haemoproteus* species, it is not possible yet to predict where (which organ), how (the intensity of the infection; development level; small or big), or which stages (meront and/or megalomeronts) would develop in susceptible hosts.

We collected samples of *Emberiza citrinella* (yellowhammer) and *Delichon urbicum* (common house martin) from 2017-2019 and in 2021 at the ornithological station Ventė Cape, Lithuania. Parasite species were identified by microscopic examination of blood films, and cytochrome *b* lineages were obtained by DNA sequencing. The organs were collected from single-infected birds, fixed in formalin, embedded in paraffin blocks. Histological sections were cut, stained with haematoxylin & eosin (H&E), or processed using chromogenic in situ hybridization (CISH) – a diagnostic technique to identify haemosporidian parasites on the genus level by using genus-specific probes which target the parasites' 18S ribosomal RNA.

A new blood parasite - *Haemoproteus dumbbellus* - was found and described from the gametocyte stages. Meronts were detected in six organs in *Emberiza citrinella* birds. *Haemoproteus hirundinis* was found in *Delichon urbicum*, but only megalomeronts were identified in the birds' pectoral muscles. H&E sections were used to describe and characterize the EES of these two parasites, while CISH-treated histological sections allowed the confirmation of the observed stages to belong to *Haemoproteus,* and facilitated the detection of meronts, which ranged from 8 to 44 µm in length (Fig. 1).

The combination of all used techniques - blood smear microscopic examination, DNA sequencing, histology, and CISH - allowed a finer description and recognition of the parasite species and their different stages of development in their avian host.

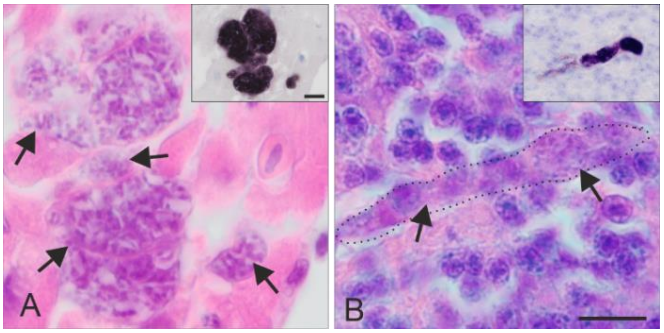

Figure 1. *Haemoproteus dumbbellus* meronts (arrows) found in the heart (A) and brain (B) of *Emberiza citrinella*. The parasites are difficult to recognize in haematoxylin & eosin-stained sections but are readily distinguishable in chromogenic in situ hybridization treated preparations (inserts).

Grant P33480 from FWF Austrian Science Fund is acknowledged.

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ŽUVINTAS LAKE MACROPHYTES DETECTION ALGORITHM USING SATELLITE DATA

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Macrophytes are sensitive to changes in the environment and are a water body health indicator which can be observed by using remote sensing methods [1]. The aim of this study is to define the most significant combination of observation seasonality and wavelength for the macrophytes detection model using machine-learning method based on seasonal dynamics of reflectance of Žuvintas Lake aquatic vegetation.

Google Engine platform was used to collect Sentinel-2 MSI (Level-1C) reflectance data of macrophytes for April 19, May 11, June 18, July 10, September 08, September 26, October 31 in 2021. For testing of algorithm we
used on 2022 March 22, May 09, June 25, July 20, August 24, September 26 data. The clustering and visual analysis of clusters were used to create target labels for supervised learning - the application of machine learning algorithms. The CLARA Clustering using Manhattan distance method was applied to distinguish water surface, two types of emergent macrophytes, floating and submerged macrophytes. The machine learning model trained by using the Recursive Partitioning and Regression Trees (RPART) method [2]. To evaluate the model and its behavior on independent data, the method of repeated cross-validation was applied, and the training set was divided into 10 folds, the test was carried out with 5 repeats. The results of repeated cross-validation were assessed using the accuracy metric. The complexity parameter cp = 0.256 was applied for tuning model which allow to increase accuracy from 0.70 to 0.89.

The final model shows that using only three Sentinel-2 bands data for three dates is enough to determine the type of macrophytes, within lake boundaries: spring reflectance data in near infrared band (842 nm) and vegetation red edge (705 nm and 740 nm) reflectance data in summer and autumn. Spring reflectance data in near infrared separates water surface with submerged macrophytes and emergent macrophytes. Summer and autumn reflectance data in vegetation red edge range distinguish water surface and submerged macrophytes, two types of emergent macrophytes.

The boundaries of macrophyte types cause questions when we compare the results of clustering and applying the model. It is there that the model makes more errors and does not fall into the clustering results, or vice versa.

We applied the model to the 2022 dataset that was not used as a training data. The result of application shows that is it possible to determine the type of vegetation with an accuracy of 84.0%, which is slightly lower than applying the model to the 2021 data. Wrong identified territories in 2022 have a similar distribution and configuration, but they are wider. However, in 2021 and 2022, the largest number of conflicting identification of macrophytes and water surface occurs between submerged macrophytes and water, as well as between emerged macrophytes.

Thus, the three reflectance variables in spring and summer in near infrared and two of vegetation red edge bands respectively are sufficient to classify aquatic vegetation types and a water surface using developed machine-learning model.

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CURRENT STUDIES OF HELMINTH PARASITES FROM UKRAINIAN AMPHIBIANS: FROM MOLECULES TO COMMUNITIES

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Amphibians are essential subjects for parasitological studies due to their extensive range of trophic connections and adaptability to various ecosystems [1]. Their ecological flexibility, which causes a high diversity of helminths and their limited capacity for long-distance migrations, makes their helminths communities suitable for testing environmental hypotheses. However, in Ukraine, comprehensive studies using molecular methods to verify the true helminth diversity in hosts have not yet been conducted. Also, there are only a few studies on helminth communities in amphibians from the region [2].

Due to that, our study aims to characterise the helminth species composition of amphibians in Ukraine, to obtain sequences from helminth species found in various amphibian hosts, and to analyse the helminth infracommunities of Pelophylax ridibundus (marsh frog), which is one of the most common and widely distributed amphibian species in the country.

We examined seven species of frogs and toads and found 40 helminths species, including 13 species of nematodes, 23 species of trematodes, two species of monogeneans, and two species of acanthocephalans.

We extracted DNA from all helminth species and successfully obtained sequences for the 18S and/or cox1 genes of nematodes from genera Oswaldocruzia, Aplectana, and Cosmocerca; trematodes from genera Gorgodera and Gorgoderina; acanthocephalan Pseudoacanthocephalus bufonis; and monogeneans Polystoma viridis and P. integerrinum. Soon, we will generate sequences for the other species and add them to the GenBank database.

Most of amphibians were obtained in small samples (2–18 individuals), which was insufficient for reliable conclusions in statistical analysis of their communities. Nevertheless, we obtained 201 individuals of the marsh frog from 14 localities in Ukraine and studied the helminth communities of this host, considering such a sample size representative.

In each locality, we collected mostly adult frogs with the snout-vent length (SVL) 38–105 mm (mean 74.7±SD 14 mm). On average, each sample comprised 15 frogs. We found 32 helminth species from three taxonomic groups: trematodes (22 species), nematodes (9 species), and acanthocephalans (1 species). Due to the semi-aquatic lifestyle of marsh frog, trematodes were the most abundant group of helminths. 23 recorded helminth species reached maturity in amphibians, while other nine species, represented by trematode metacercariae and nematode third stage juveniles, used amphibians as intermediate hosts. Some helminth species were found in the host body cavity, muscles, lungs, urinary bladder, spinal canal. However, most species were found in the digestive system, including three trematode species infected more than half of the hosts: Prosotocus confusus (prevalence 52.2%), Diplodiscus subclavatus (70.6%), and Opisthioglyphe ranae (74.6%). Besides, the latter species reached the highest total abundance (5343 specimens in the entire sample); D. subclavatus was the most widespread species found in all 14 localities.

Species richness in the helminth infracommunities was larger in male (median: 6) than in female (median: 5) marsh frogs; the difference was significant ($p \approx 0.02$). The study also found a positive correlation between the size of the host (SVL) and both the helminth species richness ($r_S=0.38$; $p<0.01$) and abundance ($r_S=0.26$; $p<0.01$). Thus, the larger frogs had higher diversity and abundance of helminths.

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High School Students

M1. *MC4R* IR *PPARG* GENŲ VIENO NUKLEOTIDO POLIMORFIZMŲ SĄSAJA SU II TIPO CUKRINIU DIABETU

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Įvadas. Antrojo tipo cukrinis diabetas (CD) yra viena iš dažniausių metabolinių ligų pasaulyje. Dėl didelio sergamumo ir mirtingumo ši liga tapo pasauline sveikatos problema [1]. Pacientams, sergantiems II tipo CD, būdinga insulino rezistencija, sutrikusi organizmo gliukozės tolerancija ir jos pasisavinimas bei hiperglikemija [2]. Aukštas gliukozės kiekis kraujyje skatina ir gretutinių ligų bei komplikacijų vystymąsi, tokių kaip aterosklerozė ir miokardo infarktas, hipertenzija bei išeminė širdies liga [3]. Nors CD išsivystymui didelės įtakos turi pasyvus gyvenimo būdas, nesubalansuota dieta ir žalingi įpročiai, mokslininkai vis daugiau dėmesio skiria genetiniams šios ligos tyrimams [4]. Pastebėta, kad už energijos homeostazę, gliukozės ir lipidų metabolizmą atsakingų genų, tokių kaip MC4R ir PPARG, variacijos (polimorfizmai) gali didinti riziką vystytis pagrindiniams II tipo CD rizikos veiksniams – insulino rezistencijai bei nutukimui [5–7]. Šių genų polimorfizmų tyrimai, ieškant sąsajų su II tipo CD, ne tik padėtų geriau suprasti šios metabolinės ligos genetinius veiksnius, bet ir anksčiau numatyti asmens riziką susirgti II tipo CD, įvertinti šios ligos komplikacijų riziką bei geriau valdyti pačią ligą ir jos eigą.

Tikslas. Ištirti MC4R (rs17782313) ir PPARG (rs1801282) genų polimorfizmų paplitimą II tipo cukriniu diabetu sergančiųjų ir sveikų asmenų (kontrolinėje) grupėse bei nustatyti šių polimorfizmų sąsają su II tipo cukriniu diabetu.

Metodai. DNR išskirta iš 110-ies (II tipo CD grupė – 55 ėminiai; kontrolinė grupė – 55 ėminiai) veninio kraujo ėminių. Specifiniai tirtų polimorfizmų DNR fragmentai padauginti ARMS-PGR metu. Rezultatai vizualizuoti taikant agarozės gelio elektroforezę – DNR fragmentai ir gauti genotipai įvertinti ultravioletinėje šviesoje. Rezultatų patikimumui įvertinti atliktas Fisher`s exact statistinis testas (apskaičiuota p-reikšmė).

Rezultatai. Įvertinus MC4R (rs17782313) ir PPARG geno (rs1801282) polimorfizmų paplitimą, nustatyta, kad tirtose II tipo CD ir kontrolinėje grupėse dažniausias buvo TT genotipas (MC4R genas, dažnis grupėse atitinkamai 0,636 ir 0,8), ir CC genotipas (PPARG genas, dažnis grupėse atitinkamai 0,691 ir 0,745). Įvertinus MC4R geno (rs17782313) polimorfizmo sąsajas su II tipo CD nustatyta, jog C alelis statistiškai reikšmingai dažnesnis buvo II tipo CD grupėje (p-reikšmė 0,03). Tuo tarpu statistiškai reikšmingų sąsajų tarp PPARG (rs1801282) geno polimorfizmo ir II tipo CD nebuvo nustatyta.

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M2. KETURIŲ POTENTILLA GENTIES RŪŠIŲ SKIRTINGŲ AUGALO DALIŲ ALKOHOLINIŲ EKSTRAKTŲ ANTIBAKTERINIO AKTYVUMO TYRIMAS IN VITRO

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Jau senovės liaudies medicinoje buvo naudojamos vaistažolės įvairiomis formomis ir įvairioms ligoms gydyti. Tačiau daugumos vaistažolių savybės ir jų veiksmingumas moksliškai yra neištirtas arba ištirtas nepakankamai. [1]

Bakterijų sukeltos infekcijos paprastai yra gydomos antibiotikais, tačiau pastaruoju metu bakterijų atsparumas jiems yra pakilęs iki pavojingai aukšto lygio ir bakterijos išvysto naujus atsparumo antibiotikams mechanizmus [2]. Dėl šios priežasties yra svarbu ieškoti alternatyvių gydymo būdų mikroorganizmų sukeltoms ligoms. Viena iš tokių alternatyvų – augaliniai preparatai.

Sidabražolės (*Potentilla*) – žolinių žydinčių augalų gentis, priklausanti Erškėtinių (*Rosaceae*) šeimai. Liaudies medicinoje sidabražolės jau ilgą laiką naudojamos tradicinėje Azijos, Europos bei Šiaurės Amerikos kultūrų medicinoje. Jų orinių ir požeminių dalių ekstraktai yra naudojami uždegimams, žaizdoms, tam tikroms vėžio formoms, bakterijų, grybelių ir virusų sukeltoms infekcijoms, viduriavimui, cukriniam diabetui ir kitiems negalavimams gydyti. [3]

Didžiąją dalį sidabražolių farmakologinio poveikio galima paaiškinti dideliu taninų kiekiu, o kiek mažiau – triterpenoidų. [3]

Darbo tikslas: Ištirti keturių *Potentilla* genties rūšių skirtingų augalo dalių alkoholinių ekstraktų antibakterinį poveikį in vitro.

Tiriamos keturios sidabražolių rūšys – žąsinė sidabražolė (*Potentilla anserina*), miškinė sidabražolė (*Potentilla erecta*), tikroji sidabražolė (*Potentilla argentea*) ir pelkinė sidabražolė (*Potentilla palustris*). Naudojamos plačiai paplitusios gramteigiamos ir gramneigiamos bakterijos, kad tyrimas būtų kuo aktualesnis – *Hafnia alvei, Escherichia coli, Enterobacter cloacae, Pseudomonas aeruginosa, Salmonella agona, Enterococcus faecalis, Listeria monocytogenes, Staphylococcus aureus.*

Pirmiausia iš išdžiovintos sidabražolių žaliavos buvo išgauti sidabražolių alkoholiniai ekstraktai, o antibakterinio poveikio tyrimui naudotas difuzijos į agarą įdubų metodas. Petri lėkštelese sumaišyta kiekvienos bakterijų rūšies suspensijos ir sterili agarizuota augimo terpė. Agarui sustingus, padarytos įdubos į kurias įlašinta kiekvieno sidabražolių ekstrakto. Lėkštelės inkubuotos ir išmatuotas bakterijų auginimo slopinimo zonos plotis. Yra tam tikra priklausomybė tarp slopinimo zonos pločio ir bakterijų jautrumo, pagal ją yra vertinami gauti rezultatai.

Tyrimo rezultatai rodo, kad visi sidabražolių ekstraktai turėjo antibakterinį poveikį bent vienai tirtai bakterijų rūšiai. Didžiausią antibakterinį poveikį prieš tirtas bakterijas turėjo pelkinės sidabražolės žiedų ir žolinių dalių ekstraktai – visos bakterijos jiems buvo jautrios. Mažiausiai bakterijų augimą veikė pelkinės sidabražolės šakniastiebių ekstraktai. Pagal augalo dalis, bakterijos buvo labiausiai jautrios iš žolinių augalo dalių išgautiems ekstraktams – jie paveikė 82,5% mėginių. Pagal rūšį, iš žąsinės sidabražolės išgauti ekstraktai turėjo didžiausią antibakterinį poveikį – apie 87,5% bakterijų rūšių buvo jautrios ekstraktams.

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M.3. THE SYNTHESIS OF DARROW RED DYE

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Darrow red dye is a simple oxazine dye that can be used to stain neuronal cell bodies [1][2]. In contrast to related compounds, the synthesis pathway of this dye is not precisely described.

The aim of this research is to discover a more efficient way of synthesis of Darrow Red dye and to choose the most suitable method of purification.

The intermediate compound was synthesized from 1.2 g of metacetamol and 0.913 g $NaNO_2$ at 0-4°C in an acidic environment (Fig. 1). To ensure the required compound was obtained, HPLC-PDA-MS chromatography was performed. (Fig. 2) Rt=3.4 min - metacetamol, Rt=3.9 min - an intermediate compound with a molar mass of 181.15. The molar mass of the obtained intermediate agrees with its theoretical molar mass, so it can be said that the desired intermediate was acquired. Further synthesis was carried out with 1 g of the intermediate compound and 0.150 g of 1-naphthylamine dissolved in 20 mL of ethanol and refluxed for 4 h. The reaction mixture in progress was sampled every hour and analyzed by TLC. The final product was purified by column chromatography. The resulting compound was subjected to HPLC-MS analysis to confirm that the target compound had been synthesized. (Fig. 3). The theoretical mass of the compound agrees with the molecular mass of 303 Da obtained during HPLC-MS analysis. It was found that after purification, the purity of the obtained Darrow Red reached 89%.

In the course of further research, we plan to look for a method to improve the yield of this synthesis and an application of staining of nerve cells of various animals with the obtained compound.

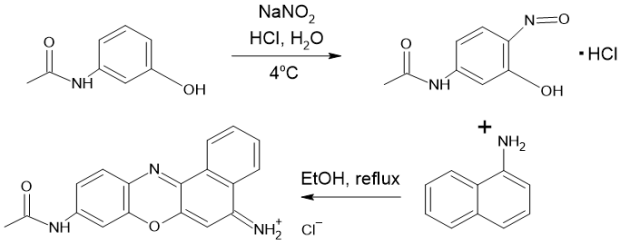

Fig. 1: Darrow Red synthesis pathway

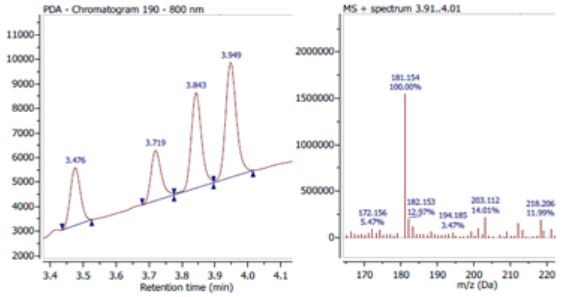

Fig. 2: PDA-MS chromatogram of intermediate compound

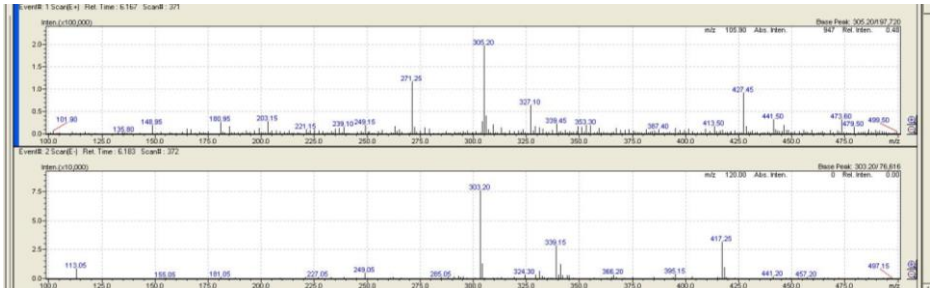

Fig. 3: HPLC-MC analysis of the resulting compound; the positive spectrum shows molar mass of 305 Da (M+1), and the negative spectrum shows molar mass of 303 Da (M-1).

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M.4. TRANSITION METAL ION DETECTION USING AN ORGANIC DYE

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Transition metals, including aluminum, lead, and mercury, are extremely toxic and quantities in an organism can result in organ damage, and disrupt metabolism and the immune system. In addition, these elements are carcinogens. It enters the body primarily through the air, water, soil pollution, and food.[1] Research on the detection of these metals is of great significance. Currently the most widely used are spectrophotometric and electroanalytical methods, such as atomic absorption/emission spectroscopy, and inductively coupled plasma mass spectroscopy (ICP-MS). A significant drawback is that they require complex sample preparation, and these method techniques are costly and have limited availability.[2] However, using an organic dye Brooker's Merocyanine is a cheaper, quicker, and more accessible way of detecting transition metals.

This research aims to create a method of Ni^{2+}, $Cu^{2+,}$ Pb^{2+}, Hg^{2+}, Co^{2+}, Al^{3+} ion detection using an organic dye Brooker's Merocyanine.

Three-stage Brooker's Merocyanine synthesis was carried out. First intermediate compound was synthesized from 3,2 ml 4-methylpyridine, 2,1 ml iodomethane, 3.3 ml isopropanol. Further synthesis was carried out with 4.733 g of intermediate compound, 2.42 g 4-hydroxybenzaldehyde, 2.32 ml triethylamine. Lastly, the second compound was dissolved in 0.5 M KOH solution and then crystalised. Next, to determine the interaction of Brooker's Merocyanine dye with metal ions spectrophotometric analysis was used. Once the dye is in various transition metal solutions, it will shift bathochromicaly or hypsochromicaly. The color intensity of the dye and metal ion complex can help determine the concentration of transition metal ions in the test sample(Figure 1). Investigation of the interaction of Al^{3+}, Pb^{2+}, Hg^{2+} with Brooker's Merocyanine demonstrated that these three metals together with the dye form clear complexes. Brooker's Merocyanine water solution absorption maximum was determined at a wavelength of 374 nm. It was used later in developing the calibration curve of 100-500 ppm concentration aluminum, and 200-1000 ppm lead solutions.

In conclusion, solvatochromic dye Brooker's Merocyanine is a cost-effective technique of transition metal ion detection. In further research, our objective is to validate Al^{3+}, Pb^{2+} detection methods, include other transition metal(Ni^{2+}, Cu^{2+}, Hg^{2+}, Co^{2+}) ion solutions to the study as well as devise test strips for accessible identification of these metal ion concentrations in drinking water.

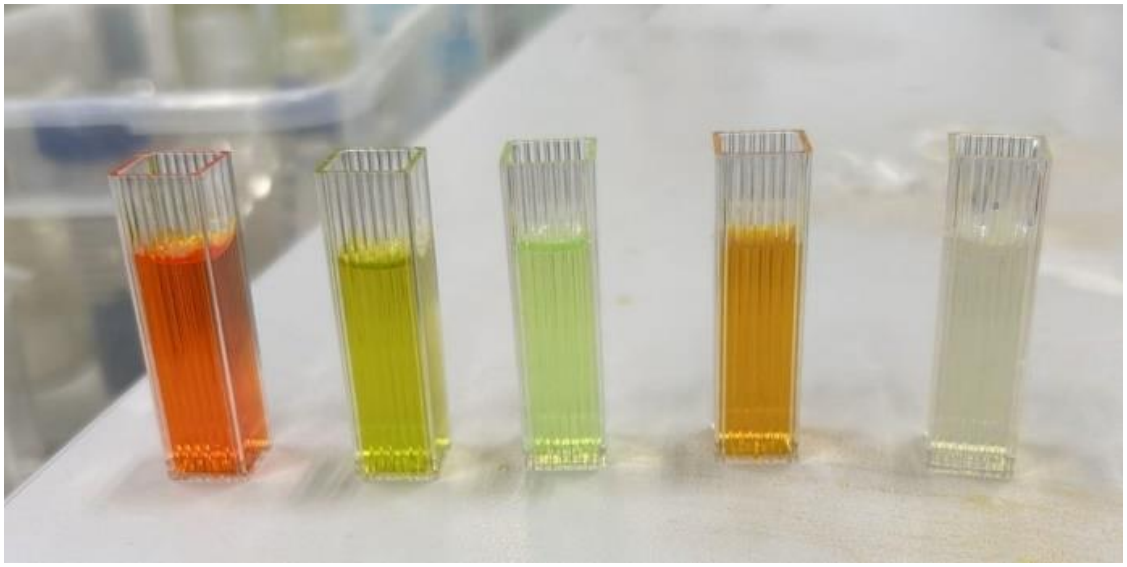

Figure 1. Brooker's Merocyanine complexes with metal solutions. From left to right: 0,1 mmol/l Brooker's Merocyanine solution; 0,1 mmol/l Ni^{2+}, Cu^{2+}, Co^{2+}, Al^{3+} solutions combined with dye 1:1 ratio.

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M.5. FTO IR ALKBH5 DEMETILAZIŲ, METTL1 IR BUD23 METILTRANSFERAZIŲ RAIŠKOS TYRIMAI SKIRTINGO PIKTYBIŠKUMO LAIPSNIO GLIOMOS NAVIKINIUOSE AUDINIUOSE

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Įvadas: Vėžys yra plačiai paplitusi pavojinga liga, kuria serga ir nuo kurios miršta milijonai žmonių visame pasaulyje. Kiekvienais metais priklausomai nuo regiono, tautybės ar žemyno smegenų navikai diagnozuojami nuo 3,21 iki 9,23 iš 100000 žmonių[1]. Gliomos, labiausiai paplitusio smegenų auglio tipas, sudaro 80% piktybinių navikų centrinėje nervų sistemoje, o 50% atvejų diagnozuojama jau vėlyviausioje stadijoje. [2]. IV stadijos astrocitoma vadinama glioblastoma. Tik 36% sergančių pacientų, kuriems diagnozuoti smegenų navikai išgyvena ilgiau nei penkerius metus, o glioblastoma sergančių pacientų penkerių metų išgyvenamumas siekia vos 6,8% [3]. Gydymo galimybės šiuo metu apima chirurgiją, radioterapiją, chemoterapijąbei tolozolmido vaistus arba šių gydymo būdų derinius. Kaip bebūtų šių gydymo būdų poveikis vis dar yra nepakankamas ir glioma sergančių pacientų mirtingumas išlieka didelis.

Dažniausiai vėžio išsivystymo ir progresavimo kaltininkais yra laikomi ląstelių transkriptominiai ir proteominiai pakitimai, tačiau naujas tyrimų objektas kaip vienas iš galimų veiksnių, prisidedančių prie vėžio atsiradimo bei progresavimo, yra RNR metilinimas, kuris įtakoja ląstelių ciklą bei chemiškai modifikuotų RNR molekulių struktūra, kuri gali turėti įtakos RNR stabilumui, nukreipimui ar transliacijai. Manoma, jog šios modifikacijos ir jų reaguliatoriai gali suteikti galimybių ankstyvai diagnostikai ar tikslinei genų terapijai.

Tikslas: Šiuo tyrimu siekiama ištirti m6A ir m7G reguliatorių *FTO* ir *ALKBH5* demetilazių, *BUD23* bei *METTL1* metiltransferazių raiškos skirtumus skirtingo piktybiškumo laipsnio gliomos pacientų pooperaciniuose navykiniuose audiniuose iRNR lygmenyje ir raiškos ryšius su klinikinėmis pacientų charakteristikomis bei įvertinti šių reguliatorių potencialą ankstyvai diagnostikai ir tikslinei genų terapijai.

Metodai: Vykdant tyrimą buvo tiriami baltymus koduojančių *FTO, ALKBH5, BUDS23* bei *METTL1* genų raiška žmonių, sergančių astrocitomomis, pooperaciniuose navikiniuose audiniuose. Tokių mėginių buvo ištirta 54. Genų raiška įvertinta naudojant TL-PGR metodą, o šio metodo optimizavimui buvo atliekamas temperatūrinis gradientas, kalibracinės kreivės bei atsižvelgiama į lydymosi kreives. Gauti rezultatai lyginami su RHB (sveikų smegenų kontrolė, *angl. reference human brain* (RHB)) mėginiais remiantis statistiniais metodais. TL-PGR metu naudojami genų pradmenys sukuriami naudojantis bioinformatikos įrankiais: „Ensemble“ duomenų baze bei „PerlPrimer“ programa. Genų raiškos bei išgyvenamumo rezultatai nagrinėjami naudojantis „Excel“ ir „GraphPad Prism“ programomis, o genų raiškos priklausomybė nuo klinikinių pacientų charakteristikų nagrinėjama „IBM SPSS“ programa.

Rezultatai: Atlikus literatūros analizę pasirinktas TL-PGR metodas genų raiškai nustatyti ir juo sėkmingai ištirta *ALKBH5, FTO*, *BUD23* bei *METTL1* genų raiška 54-iuose pacientų mėginiuose. *ALKBH5*, *FTO* ir *BUD23* raiška buvo tiesiogiai susijusi su naviko piktybiškumo laipsniu bei reikšmingai ilgiau išgyveno astrocitomos pacientai, kurių mėginiuose išmatuota šių genų raiška buvo didesnė arba lygi imties medianai, todėl galima teigti, jog šie genai yra potencialūs gliomos žymenys bei tikslinės terapijos taikiniai. Tuo tarpu ištirta *METTL1* raiška nebuvo susijusi su naviko piktybiškumo laipsniu ir išgyvenamumu.

Įvertinus sąsajas tarp genų raiškos ir klinikinių charakteristikų: piktybiškumo laipsnio, lyties, amžiaus, išgyvenamumo trukmės, *IDH* mutacijos buvimo arba nebuvimo bei *MGMT* promotoriaus metilinimo nustatyti statistiškai reikšmingi ryšiai tarp *ALKBH5* geno raiškos ir piktybiškumo bei *IDH* mutacijos, *FTO* geno raiškos ir piktybiškumo, lyties, amžiaus bei *IDH* mutacijos, *BUD23* geno raiškos ir piktybiškumo, amžiaus, išgyvenamumo trukmės, *IDH* mutacijos bei *METTL1* geno raiškos ir paciento amžiaus. Statistiškai patikimi rezultatai tarp šių genų raiškos ir *MGMT* promotoriaus metilinimo nebuvo nustatyti.

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M.6. PERCHLORATE REDUCTION IN MARTIAN SOIL USING AZOSPIRAORYZAE

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Introduction. With the growing human population, the colonization of Mars is increasingly being considered. One of the problems hindering Mars colonization is perchlorates. Perchlorates are compounds that contain the perchlorate ion (ClO_4^-). Perchlorates are commonly found in groundwater and surface water, as well as on the planet Mars, the concentration of perchlorates in Martian soil ranges from 0.5 to 1% [1]. The removal of perchlorate salts is a complex process, as perchlorates are highly stable and soluble in water. Even trace amounts have a significant negative impact on living organisms. Some microorganisms, such as *A. oryzae*, *D. agitata*, and *D. suillum*, can be used to break down perchlorates [2]. In this study, an *A. oryzae* bacterial culture was used to reduce the concentration of perchlorate ions in Martian soil.

Objective. The objective of this study is to reduce the concentration of perchlorate ions in Martian soil using an *Azospira oryzae* bacterial culture.

Methods. The concentration of perchlorates was determined using the spectrophotometric technique by using chlorobenzene for the extraction. The calibration curve was prepared by creating a series of standard perchlorate solutions with known concentrations (Figure 1). *A. oryzae* was cultivated under aerobic conditions in Reasoner's 2A agar (R2A) medium, with 200 µl of 1 mg/ml perchlorate solution added to each test tube, and incubated at 30°C for 14 days. After the incubation period, the samples with bacteria were centrifuged at 6000 rpm for 5 minutes, and 10 ml of the supernatant was used for sample preparation. The results were compared to the calibration curve values. Moreover, the bean seeds were planted in Martian soil mixed with Murashig Skoog medium to find out how perchlorates affect the growth of beans. Five test tubes were prepared with the mentioned medium alone, five with medium and perchlorate solution, and five with medium, perchlorate solution, and *A. oryzae* bacteria. Into each test tube, sterile bean seeds were placed and their growth was monitored.

Results. The mass of perchlorate after the incubation period with *A. oryzae* decreased in all samples (Figure 2). The average mass of perchlorate was 24.95 ± 8.80 µg. The best growth of the bean seeds, as expected, was observed in the test tubes containing Murashig Skoog medium, perchlorate solution, and *A. oryzae* bacteria. *A. oryzae* not only broke down perchlorates but also formed a symbiotic relationship, that promoted the growth of bean plant.

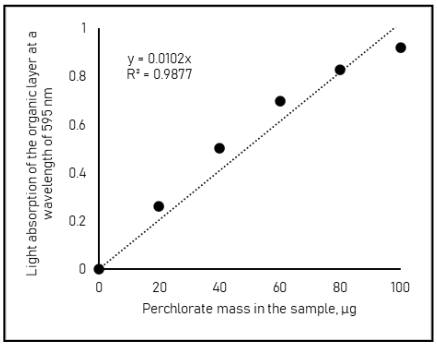

Figure 1. Calibration curve of perchlorate

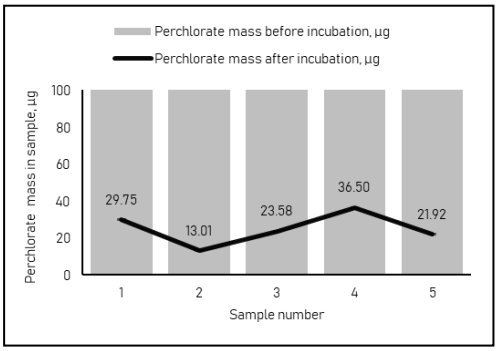

Figure 2. Perchlorates mass before and after incubation with *A. oryzae*, µg

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M.7. TRANSFERRING *VIOA-E* GENES FROM *C.VIOLACEUM* TO *E.COLI* FOR PRODUCTION OF VIOLACEIN AND DEOXYVIOLACEIN

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Violacein is a water-insoluble purple pigment reported to have a wide variety of qualities including potent antibacterial, antiviral, antiprotozoal, and antiparasitic properties [1]. Violacein is not only researched for potential use in medical and biochemistry fields [2] but also in biotechnology as a natural replacement for organic dyes in textile dyeing [3]. Two main methods are currently used for pigment extraction: isolation of naturally produced pigment [4] and expressing pigment synthesizing proteins in heterologous hosts [5]. However, these methods are complex and either not efficient enough or too expensive, as the current market price of violacein and its metabolite deoxyviolacein is 463 €/mg [6]. Therefore, research on the properties of the pigment itself is limited. One of the most common bacterial species that produce this purple-colored pigment is *Chromobacterium violaceum* [7] containing *vioA-E* genes that could be cloned to *Escherichia coli* for the production of violacein and deoxyviolacein.

This work focuses on transferring *vioA-E* genes involved in synthesis of the pigment violacein from *C.violaceum* to *E.coli*. Thereby, PCR was performed on genomic DNA from *C.violaceum* ATCC 12472 with primers introducing restriction sites. The obtained fragment and plasmid vector were cut with the same restriction endonucleases, purified, ligated and transformed to *E.coli* DH5a strain competent cells. Bacterial selection was performed on Petri dish with antibiotic containing medium. The resulting colonies were tested for insert with PCR, but the required length DNA fragment was not amplified suggesting the cloning process was unsuccessful.

Nonetheless, the research will be continued. Each cloning step needs to be optimized to yield best results. After successful cloning the pigment synthesizing genes will be expressed in *E.coli* and obtained pigment further used in textile dyeing.

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C.1. THE EFFECT OF ANAESTHESIA ON THE AUDITORY STEADY-STATERE-SPONSE IN MICE

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The auditory steady-state response (ASSR) is evoked oscillatory activity that is entrained to the frequency and phase of repeated auditory stimulation [1]. It tests the capacity of auditory pathways to generate a synchronous activity. The ASSR is altered in schizophrenia [2], therefore it is used as a biomarker in detecting the pathology [3]. Despite high potential, the mechanisms underlying ASSR are largely unknown. For example, anaesthesia is widely applied in animal studies for immobilization and blocking motor responses to painful stimuli [4], however, it is not known how anaesthesia affects ASSR. The aim of this study was to evaluate the impact of anaesthesia on ASSR.

The experiments were performed on C57BL/6 mice. Electrodes were implanted in the primary auditory cortex (A1) and prefrontal cortex (PFC) for ECoG registration at conscious and anaesthetized (sevoflurane and ketamine/xylazine) conditions. ASSR was induced by 2 ms white noise stimuli (clicks) presented at 40 Hz for 1 s at 70 dBa. The Morlet wavelet transformation was used for signal time-frequency analysis. ASSR parameters (power and phase-locking index (PLI)) were calculated at the 35-45 Hz interval.

The results showed that sevoflurane decreases power of all frequencies except frequencies in low-power range (0-10 Hz) in A1. In contrast, ketamine/xylazine increases power of low frequency oscillations (0-10 Hz) and mostly decreases high frequencies oscillations (50-120 Hz) both in A1 and PFC. Also, ketamine/xylazine and sevoflurane induce a significant decrease of ASSR parameters in A1. In contrast, both anaesthetics enhance ASSR generation in PFC.

In conclusion, ketamine/xylazine and sevoflurane anaesthesia have a different effect on ASSR parameters in A1 and PFC at the 40 Hz stimulation. Further studies are needed to explain the detailed mechanisms of ASSR generation under anaesthesia.

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C.2. CONTROLLING GENE ELECTROTRANSFECTION USING MICROSECOND AND HIGH-FREQUENCY NANOSECOND PULSE SEQUENCES IN COMBINATION WITH GOLD NANOPARTICLES

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Non-viral transfection techniques are used to facilitate the transfer of foreign nucleic acids inside the cell, with electroporation being one of the most common methods. Electroporation involves the application of high-voltage pulses and an external electric field, resulting in transient and reversible permeabilization of the membrane, which can allow the entry of various molecules into the cells [1]. Electrically conductive nanoparticles, such as gold nanoparticles (AuNPs), can be used to increase the efficiency of electroporation. AuNPs are added to locally enhance the electric pulse strength and trigger electroporation at significantly lower threshold [2]. Such an approach enables application of pulses, which in terms of intensity are significantly weaker than the one used in clinical protocols. Potentially, safer and more effective gene transfer can be ensured, with minimized effects of ROS, Joule heating, pain or muscle contractions.

In this study, we used Chinese Hamster Ovary cells (CHO-K1) as a model cell line, to investigate the effect of AuNPs on transfection using long micro-range electric field pulses (ESOPE*) and shorter, nanosecond pulsed electric fields (nsPEFs). For microsecond (µsPEF) 0.6 – 1.4 kV/cm × 100 µs × 8, 1 Hz protocol was used and for nsPEF elected parameters were 3 – 7 kV/cm × 300 ns × 100, 1kHz and 1MHz. Cell permeabilization was analyzed using green fluorescent dye Yo-Pro1 and the efficiency of electrotransfection was evaluated using pEGFP-N1 plasmid (4.7 kbp) encoding the green fluorescent protein (GFP). The fluorescence measurements were performed using a BD Accuri C6 flow cytometer.

First of all, we tested how the selected electrotransfection protocols induce permeabilization with and without the use of AuNPs. Results revealed that AuNPs significantly improved µsPEF permeabilization. While in nsPEF AuNPs improved permeabilization slightly at 1 MHz frequency and did not improve at 1 kHz compared to cells without AuNPs. Triggered high permeabilization of the target cells ensures efficient transfection. The best electrotransfection results were obtained using µsPEF protocol (1, 1.2 and 1.4 kV/cm) when cells were combined with AuNPs. While with nanosecond PEFs, better transfection efficiency was obtained using 7 kV/cm and 1 MHz frequency instead of 1 kHz. However, nsPEF transfection protocol combined with AuNPs did not improve the electrotransfection efficiency.

The study showed that AuNPs had little or no effect on the transfection efficacy of the nanosecond electrotransfection protocol, while AuNPs considerably improved µsPEF protocol. We have also demonstrated that using a higher frequency, i.e. 1 MHz nanosecond pulse sequences is much more beneficial for increasing the transfection efficiency. Results of this study suggest that µsPEFs range protocols together with AuNPs can be applied for gene electrotransfection experiments.

* European Standard Operating Procedures for Electrochemotherapy (1.3 kV/cm x 100 ms x 8, 1 Hz).

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C.3. MODULATION OF THE SIALYLATION OF EXCITATORY NEURONS IN MOUSE ORGANOTYPIC HIPPOCAMPAL SLICE CULTURES

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Synaptic pruning is a fundamental process for healthy neuronal circuit maturation and development.The disbalance between synapse elimination and strengthening may underlie neurodevelopmental disorders,such as autism, schizophrenia, or epilepsy [1]. The phagocytosis of neuronal material is carried out by microglia, the main phagocyte of CNS. One of major signals for neuron-microglia interaction is sialic acid, a monosaccharide, which typically terminates the structures of glycoconjugates on the external surface ofneurons. The presence of neuronal sialic acid at neuron-microglia contacts leads to inhibited microglial phagocytic activity. Meanwhile, desialylated neuronal sialoglycoconjugates can be recognised by complementsystem and promote neuronal phagocytosis by microglia *in vitro* [2]. We hypothesize that neuronal sialic acidmediates local synapse-microglia contacts and regulates developmental synaptic pruning.

We used mouse organotypic hippocampal slice cultures as a platform to define how sialylation provides a selective mechanism for the maintenance and strengthening of a subset of synapses. We applied bioorthogonal CLICK chemistry to metabolically label *de novo* synthesised sialic acid on glycocalyx of neurons [3]. By using chemical inhibitors of sialyltransferases ((1S,2R)-1-((3S,4R,5R,6S)-3-acetamido-4,6- diacetoxy-5-fluoro-6-(methoxycarbonyl)tetrahydro-2H-pyran-2-yl)propane-1,2,3-triyl triacetate, 3FAX) or sialidases (2-deoxy-2,3-didehydro-N-acetylneuraminic acid, DANA) we inhibited sialic acid synthesis or desialylation, respectively, to later neuronal sialylation.

Mouse organotypic hippocampal slice cultures were established and used as an *ex vivo* system allowing to study metabolic processes of neuronal sialylation. The visualization and quantitative analysis of newly synthesized sialic acids on dendrites of excitatory neurons showed specificity of metabolic sialic acid labelling. The inhibition of sialyltransferases by 3FAX significantly reduced neuronal sialylation, while sialidase inhibition by DANA led to increased sialylation levels. Our results indicated that neuronal sialylation can be chemically modulated *ex vivo* allowing the precise assessment of sialic acid abundance on the neuronal structures, such as dendrites and individual synapses.

In conclusion, we demonstrated the applicability of mouse organotypic hippocampal slice cultures asa robust model to investigate *de novo* synthesis and the turnover of sialic acid in the mouse developing brain*ex vivo*. Moreover, our system enables to target and characterise the exact role of the enzymes involved in neuronal sialylation and desialylation in synaptic pruning.

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C.4. A BIENZYMATIC ELECTROCHEMICAL BIOSENSOR FOR GLUCOSE

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Biocatalytic sensors are tools used to identify and quantify specific compounds. These sensors rely on the activity of specific enzymes as a recognition element, allowing them to identify particular analytes. The enzyme's selectivity is provided by a unique active site that binds to a certain substrate molecule. Biocatalytic sensors are often simple to use and inexpensive because they do not require expensive equipment and have a straightforward design. Additionally, they can be modified for automated and clinical lab work [1]. In some cases, challenging bioanalytical issues, including specificity, stability, and selectivity, are resolved by combining nanomaterials with nanotechnological methods. As a result, the electron transfer rate between the active center of enzymes and electrodes has been improved by using gold nanoparticles (AuNPs) as electric wires and mediators for electron transfer. AuNPs increase the efficiency of enzyme immobilization [2]. The advancement of biosensor technology has opened the door to highly sensitive continuous glucose monitoring. The most popular method for monitoring glucose oxidase (GOx) from blood to interstitial fluids is to use electrochemical biosensors. Additionally, constant monitoring of H_2O_2 is necessary to detect oxidative stress in cell and tissue culture.

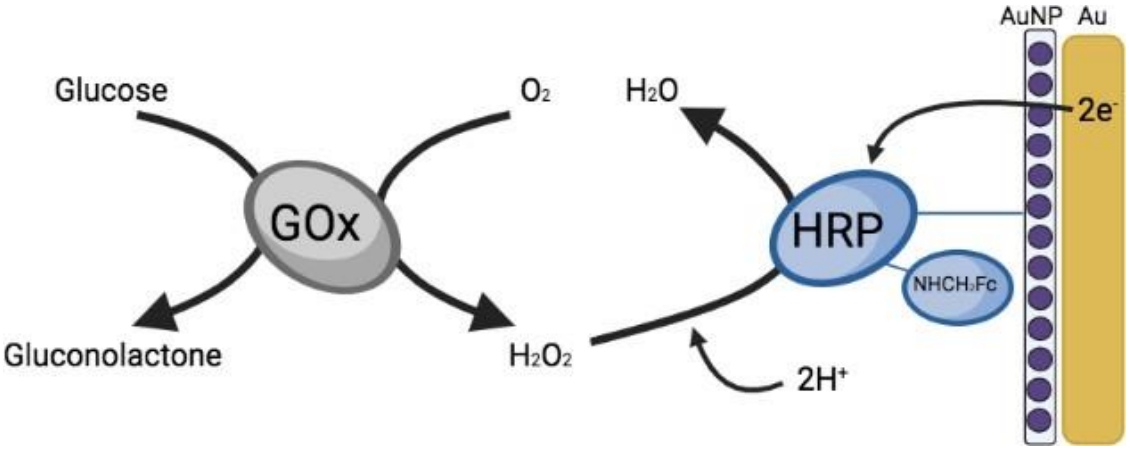

Figure 1. This figure shows a schematic representation of the bioelectrode based on HRP-Fc and GOx. The matrix relies on the oxidation of β-D-glucose catalyzed by GOx, which involves molecular oxygen. The reaction produces gluconic acid and hydrogen peroxide as end products. HRP-Fc acts as a mediator, taking up the hydrogen peroxide along with two protons and two electrons from a gold electrode.

The goal of this study is to develop a versatile matrix for H_2O_2-releasing enzymes suitable for biosensors (Fig. 1). Therefore, the optimal conditions for modifying horseradish peroxidase (HRP) in an organic solvent with a redox-active ferrocene group and immobilizing it on an electrode decorated with AuNPs were investigated. After characterizing and comparing the bioelectrodes based on the original and modified enzymes, a biosensor for the detection of glucose based on GOx and HRP-Fc was developed, and its sensitivity, selectivity, and stability were evaluated.

Acknowledgments: This work was supported by the European Regional Development Fund under "Promotion of Centers of Excellence and Innovation and Technology Transfer Centers" program No. 01.2.2- CPVA-K-703 (grant No. 01.2.2-CPVA-K-703-03-0010).

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C.5. ELECTROPHYSIOLOGICAL INVESTIGATION OF THE EFFECT OF GLUTAMATE ON THE PROPAGATION OF ACTION POTENTIALS IN A SINGLE PLANT CELL

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Introduction: action potentials (APs) in plants are evoked when the membrane potential exceeds a threshold value, above which voltage-gated ion channels open causing an abrupt membrane depolarization and later, repolarization. These signals propagate along the membrane of the cell and the plant tissues. It is known that exposure of *Characean* macroalgae internodal cells to glutamate hyperpolarizes the AP's excitation threshold and prolongs repolarization phase duration [1]. Yet, it is to be determined whether these glutamate- induced alterations of AP affect its propagation velocity.

Materials and Methods: internodal cells of macroalgae *Nitellopsis obtusa* were used as a model system. The morphology and physiology of these cells offer a robust experimental system to explore plant electrophysiology using intracellular microelectrode techniques [2]. Two-pair current clamp method was utilized in two separate setups. The schematic representation of the registration chamber arrangement is shown in Fig.1. The internodal cell was placed in the registration chamber filled with control sotution and. Intracellular glass microelectrodes (tip diameter 1 µm, filled with 3 M KCl) were impaled using micromanipulators in two sites of the cell 3 cm from each other. The impalement areas were electrically separated using vaseline. The reference electrodes were immersed in the vicinity of the cell in two different compartments. Direct current for stimulation was injected via extracellular Ag/AgCl wires in two separate circuits. After 60 min from impalement, 3 APs in one of the microelectrode sites were evoked in 5 min. intervals by current stimulus ramp - once AP threshold was reached, stimulating current was ceased. After 10 minutes of rest, the same procedure was repeated on the other site of impalement, thus, AP propagation velocity and the parameters in apical and basal sites were assessed. The described protocol was repeated when the cell was incubated in 1 mM glutamate solution (APW +1 mM L-Glutamic acid hydrochloride) for 30 min. This way propagation of APs apically and distally can be investigated and compared between both control and experimental conditions.

Results: we successfully investigated propagation of APs along internodal cell of *Nitellopsis obtusa* in control conditions (n=6). Results indicate that the propagation velocity of APs is higher from basal to apical site (22±6%) in comparison to the opposite direction (1.4±0.2 cm/s). Externally applied glutamate increases propagation velocity of electrically induced APs in both apical (32±10%) and basal (23±7%) directions. Further research will investigate the correlations between Glu-induced alterations of AP parameters and the presented changes of AP propagation velocity.

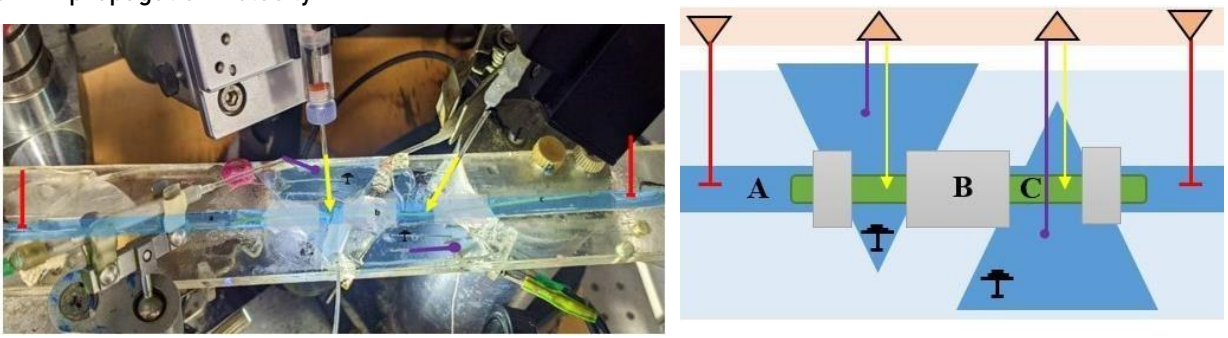

Fig. 1. Schematic representation of multielectrodal chamber: a – plexiglass chamber, b – vaseline isolation, c – internodal cell of macroalgae *Nitellopsis obtusa*, ⊥– stimulation Ag/AgCl electrode, ⌋ - extracellular reference electrode, ↓ – intracellular recording glass microelectrode

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C.6. ELECTROPHYSIOLOGICAL PROPERTIES OF HIPPOCAMPAL PYRAMIDAL NEURONS IN WILD-TYPE AND XKR8-DEFICIENT MICEDURING POSTNATAL DEVELOPMENT

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Postnatal brain maturation involves neural circuitry formation and changes in dynamics of synapticconnections. During the first weeks of postnatal development hippocampal pyramidal neurons undergo microglia-dependent synaptic pruning [1]. Disruptions of this process lead to various neurodevelopmental disorders, including autism spectrum disorder, schizophrenia and epilepsy [2]. The key "eat me" signal for microglia-dependent synaptic pruning is phosphatidylserine (PtdSer) which is known to be translocated across the membrane bilayer by Xk-related protein 8 (Xkr8) scramblase [3]. We investigated the effect ofXkr8 scramblase deficiency on electrophysiological properties of hippocampal pyramidal neurons in different sex mice during early postnatal development.

Wild-type and Xkr8 knock-out mice of different sex and age (5 to 21 postnatal days) were used in this study. Electrical activity of hippocampal CA1 pyramidal neurons in acute mouse brain slices was recordedusing patch-clamp whole cell configuration method. Analysis of neuron action potential parameters wasperformed.

Results showed that electrophysiological properties of wild-type and Xkr8 knock-out mice hippocampal CA1 pyramidal neurons changed with age: in both male and female groups rheobase, actionpotential amplitude, maximum upstroke and downstroke velocities increased, action potential width decreased and threshold hyperpolarised. While changes of most of these properties from 8th to 15th postnatal days occurred in male group of both wild-type and Xkr8 knock-out mice, in female group on day 15 theyappeared in Xkr8 knock-out mice only and in wild-type females emerged on postnatal day 21.

Our study indicates that electrophysiological properties of mouse hippocampal pyramidal neuronschange during early postnatal brain maturation. Furthermore, results suggest that defective PtdSer scrambling affects neuronal circuitry formation leading to earlier brain maturation changes in female mice occurring on 15th postnatal day rather than 21st.

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C.7. THE CONSEQUENCES OF MATERNAL HIGH-FA DIET ON OFFSPRING RETINA

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Aim: Today Western diet has increasing content of fats, and this leads to increasing obesity rates. Many studies show that maternal high-fat diet causes chronic inflammation which can lead to neurodevelopmental disorders of the offspring [1, 2]. It has been shown that the retina, as a part of central nervous system, is also affected in the individuals consuming high-fat diet [3, 4]. However, there are no studies investigating the effect of maternal high-fat diet on the retina of the offspring. In this study we aim to determine whether maternal high-
fat diet leads to retinal changes in the offspring.

Methods: We fed female C57Bl/6J mice with a control diet (10% fat) or high-fat diet (60% fat) from weaning to lactation. The offspring were weaned to CD. The eyeballs of the offspring were collected, fixed with 4% PFA, cryoprotected and sliced using cryotome. Retinal ganglion cells, Müller cells and microglia cells were labeled immunohistochemically using anti-RBPMS, anti-GFAP and Iba1 antibodies, respectively.

Results: We evaluated the thickness of retinal layers, density of retinal ganglion cells and microglia cells, GFAP and Iba1 signal area. The measurements were compared between the groups of offspring. No significant changes were found in the layer thicknesses of the peripheral retina. Outer nuclear layer of the central retina was significantly thicker in male offspring of maternal high-fat diet group compared to the male offspring of maternal control diet group. Density of the retinal ganglion cell did not differ significantly between the groups. There were no significant differences in the GFAP signal area and Iba1 signal area between the groups. Microglia density was significantly higher in maternal high-fat diet female offspring compared to maternal control diet female offspring in peripheral and central retina. However, in the male offspring the microglia density was lower in the maternal high-fat diet group in the peripheral retina. Iba1 signal area was significantly larger in the peripheral retina of maternal high-fat diet female offspring and significantly lower in peripheral retina of maternal high-fat diet male offspring compared to maternal control diet offspring. No significant differences in Iba1 signal area were found on central retina of female and male offspring.

Conclusions: Our findings showed that maternal high-fat diet had a gender-specific effect on the morphology of offspring retina and activation of microglia in the retina.

Funding: This work was supported by the Science Promotion Fund of Vilnius University.

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C.8. ACCUMULATION AND EFFECTS OF CURCUMIN LIPOSOMES ON CANCER CELLS

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Cancer is one of the most devastating diseases worldwide, accounting near 10 million deaths in 2020 [1]. In response to this issue, not only do therapeutic options are aimed to be improved but also prevention programs are being developed. For example, a chemopreventive substances are considered to lower a person's risk of (re)developing cancer [2]. However, minority of people, even if they are acknowledged of genetic, age related or other risk factors leading to predisposition to cancer, choose to use chemotherapy drugs because of severe side effects. Natural substances are thought to be free of severe side effects and would therefore be more accepted and used by the target group.

Curcumin is one of the naturally occurring substances that is considered to have anti-cancerproperties [3], and therefore, having potential as a cancer chemopreventive agent. Nevertheless, curcumin is a hydrophobic compound and is poorly absorbed by a living organism. Encapsulation of curcumin in phospholipid liposomes could facilitate absorption since liposomes have a desired bioavailability and are biocompatible as well as biodegradable. However, the comparison of encapsulation techniques in terms of cellular uptake and localization in biological models is still limited.

The aim of our study was to evaluate the effect of liposomal curcumin on breast cancer cell line (MCF-7) and to identify liposomal curcumin localization in the cell. In our research we synthesized liposomal curcumin solution applying a thin-film and pH-differences methods. MCF-7 breast cancer cell line was used to evaluate liposomal curcumin effects on cancer cells. As a control, liposomes without curcumin were synthesized to eliminate the possible effect of liposomes. For the assessment of accumulation of liposomal curcumin in cancer cells, confocal microscopy imaging with fluorescent dyes labeling were applied.

The cell morphology, viability and proliferation test results showed toxicity of curcumin to breast cancer cell line and preliminary results from confocal microscopy images indicated an accumulation of curcumin in the cytoplasm at the lysosomal sites. No uptake of liposomal curcumin into the nucleus or mitochondria was observed. When it comes to comparison of synthesis methods, we found that liposomal curcumin solutions of both syntheses had similar effect on MCF-7 cells morphology, viability and proliferation, although thin-film synthesis resulted in higher yield. In conclusion, preliminary results suggest that liposomal curcumin has a potential as a chemopreventive agent, and a thin-film synthesis method is advantageous over the pH difference synthesis method.

ACKNOWLEDGEMENTS: We thank Vilius Poderys for technical assistance and Migle Ragelytė for refinement of synthesis protocols.

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C.9. RARE EARTH DOPED NANOPARTICLES FOR CANCER THERANOSTICS

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Every year cancer continues to be one of the most affectious diseases and takes away hundreds of thousands of lives. Common methods for diagnosing and/or treating cancer, such as surgery, chemotherapy, radiotherapy, or a combination of these methods, are not always effective or do not provide long-term efficient results, thus the development of more precise, rapid, and novel technologies is still required [1]. Regarding the fight with cancer, biomedicine-focused research creates the concept of combining two main aspects – diagnostics, and therapy, so-called theranostics. While the area of nanoscience exists longer than the theranostics itself, the nanoparticles which can be modified, and their physicochemical properties adjusted made it possible to create multifunctional theranostic nanoplatforms. The perfect example is rare-earth doped upconverting nanoparticles (UCNPs), with exceptional optical properties and ability to convert low energy light to high energy light, or in other words, NIR-to-VIS conversion which is important for diagnostics. When UCNPs are combined with photosensitizer, prepared complexes become potential multifunctional tool for cancer theranostics [2].

This study aimed to investigate the surface modification of $NaGdF4:Yb^{3+},Er^{3+}@NaGdF4:Yb^{3+},Nd^{3+}$ UCNPs with different types of phospholipids and photosensitizer chlorin e6 (Ce6). Ce6, is a second-generation photosensitizer with a high extinction coefficient and light absorbance in VIS light range, including absorption at the red side of the spectrum. Firstly, several UCNPs modifications were performed using unsaturated and saturated phospholipids, combined with polyethylene glycol group, and were evaluated spectroscopically. Furthermore, the complex between UCNPs and Ce6 was formed and the photosensitizing properties of the complex was evaluated using Singlet Oxygen Sensor Green.

Obtained results revealed that 3 out of 5 modifications of UCNPs led to successful UCNP-Ce6 complex formation manifesting in generation of singlet oxygen upon excitation of a 980 nm laser. Although more study is needed to indicate best concentrations of phospholipids for UCNPs' surface modification as well as Ce6 amounts for efficient complex formation. These results encourage for further development of the theranostic complex.

In conclusion, the modified UCNPs-Ce6 complex has a lot of potential as a theranostic nanoplatform for cancer and can be further developed and used in biomedical research.

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C.10. INVESTIGATION OF CELLS PLASMA MEMBRANE REPAIR AFTER ELECTROPORATION

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Electroporation (EP) is the process when cell plasma membrane gets permeabilized due to applied short high amplitude electrical pulses. It is believed, that increased cell plasma membrane permeability is the result of electropore formation [1]. However, the actual mechanism of electropermeabilization is still unclear. Through electropermeabilized membrane can be transferred substances as ions, drugs, dyes, markers, antibodies, oligonucleotides, RNA or even DNA. The outcome of transfer of various molecules after EP is applied in areas as medicine, biotechnology and industry. There are several main parameters that affect electroporation efficiency: electric field intensity and duration, number of electric pulses, period of electric pulses. If any of parameter is too high, the outcome leads to a decrease in cell viability [2]. In case for the cells to remain vital after EP, the wounded plasma membrane sites must be restored because cells can suffer the loss of vital substances. In cells exist annexin family proteins that are fundamental proteins for injured plasma membrane repair. One of the most abundant proteins from the annexins family is the annexin A4. Activity of annexins is excited by increased intracellular calcium concentration in the cells leading to an increased affinity of excited annexins to plasma membrane phospholipids. Binding to cell plasma membrane annexins initiate the restoration of damaged plasma membrane sites [3]. Interesting, that over 40 years in which electroporation phenomenon is intensively under investigation, but data about intracellular proteins in cell plasma membrane damage repair nearly found. In this work we aimed to figure out how calcium affects wild type MCF7 cells, in which annexin A4 gene is fully functional compared with MCF7-ANXA4- knock out (KO) cells in which this gene expression was disturbed, after electroporation.

We used MTS method to determine cell viability. Cell plasma membrane repair and electropermeabilization dynamics were evaluated using flow cytometry after staining cells with propidium iodide. Electroporation was carried out between parallel stainless-steel electrodes separated with 2 mm gap. $CaCl_2$ concentration was 2 mM. Cells were electroporated with 1 of 100 µs duration electric pulses at varying pulse intensities.

Collected results showed that wild type MCF7 and MCF7-ANXA4- KO cells respond to electroporation at the same or very similar pattern which differed insignificantly. On the other hand, we determined that calcium did not affect propidium iodide electrotransfer, but decreased cell viability and disrupted cell plasma membrane repair process leading to higher amounts of electropermeabilized cell amounts using calcium in EP medium. However, these results are preliminary and do not prove or deny of the activation of annexin A4 protein after electroporation. These results suggest that annexin A4 might be not that crucial to cell plasma membrane repair after EP and that other annexins, or their combinations might play more important role.

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C.11. DEVELOPMENT OF A BIOANODE USING A BRACKET SYSTEM FOR OPERATION IN MICROBIAL BIOFUEL CELL

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Straight and healthy teeth are especially relevant today. The teeth straightening bracket system is a tool used in orthodontics. When straightening teeth with these systems, there are many risks associated with other dental problems such as cavities or tooth decay. The evolving 3D technique of orthodontic treatment, which includes smart orthodontics such as the implantation of lithium batteries [1] and microbial biofuel cells in the bracket system, and the specific integration of indirect bonding techniques into the digital system will be much more effective.

In this research, an efficient microbial biofuel cell was developed using a bracket system with immobilized *Streptococcus mutans* bacteria as an anode. A biofilm of bacterial culture of *Streptococcus mutans* was grown on the contour of the bracket and the quantitative composition was evaluated using a crystal violet dye for work with a microbial biofuel cell. The results showed that after 24 h of incubation, a higher biofilm biomass was formed on the contours of the bracket compared to ones grown in the microplate wells. The thickness of the biofilm depended on the concentration of sucrose. The influence of the mediator PQ (9,10-phenanthroline quinone) on the viability of bacterial *Streptococcus mutans* cells was evaluated [2]. Bacteria growth was most inhibited by 0.3 mM PQ solution and at least 0.04 mM PQ solution.

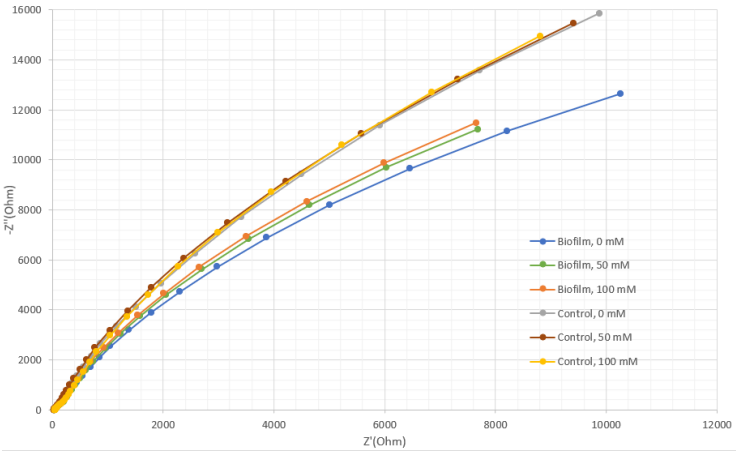

Figure 1. Electrochemical impedance spectroscopy measurements at different glucose concentrations.

Electrochemical impedance spectroscopy was applied to investigate *Streptococcus mutans* biofilm on a bracket system immersed in PBS with different glucose concentrations. Brackets without biofilm were used as control samples. The charge transfer resistance was increased with increasing glucose concentrations (Figure 1). No significant change in results with different glucose concentrations was detected in control samples.

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C.12. SINGLE MOLECULE DNA FORCE MEASUREMENTS WITH CUSTOM-BUILT MAGNETIC TWEEZERS MICROSCOPE

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Traditional methods for studying the behavior of molecules in a given reaction are based on the assumption that all molecules of the same type are synchronous, but in fact, in most cases, they behave stochastically. Single-molecule research methods allow direct observation of target molecule action trajectories, including static and dynamic components of this process. From this data, intermediate reaction chains can be determined, which show the true heterogeneity of biomolecules [1]. Force manipulation-based single-molecule techniques, such as magnetic tweezers, are used to simulate and quantify force-dependent processes in vitro. Using this method, it is possible to precisely control the reaction conditions and reveal the mechanisms of action of macromolecules, such as myosin pulling against actin, kinesin walking along the microtubule, the folding of proteins, DNA and RNA, protein-protein and protein-DNA/RNA interactions [2].

In this work, we are developing a custom-built microscope, based on the Warwick Open Source Microscope (WOSM) project [3], with integrated magnetic tweezers for force-based single-molecule measurements. The microscope consists of an aluminium base with most other parts 3D-printed. The microscope stands out with its stability, compactness and simplicity of implementation. By using this microscope we hope to push the boundaries of the measurement limits of single-molecule magnetic tweezers experiments.

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C.13. KETAMINE CHANGES SPONTANEOUS AND INDUCED BRAIN ACTIVITY IN AWAKE MICE

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Schizophrenia is a complex neuropsychiatric disorder characterized by a range of cognitive, emotional, and perceptual symptoms. Individuals with schizophrenia exhibit deficits in auditory processing, including impaired ability to filter out irrelevant sounds and decreased sensitivity to certain frequencies [1]. The development of multiple animal models resembling symptoms of schizophrenia have been instrumental in advancing our understanding of the disease and its underlying neural mechanisms. Since changes in NMDA neurotransmission are thought to play a critical role in the pathophysiology of schizophrenia, the systemic suppression of NMDA neurotransmission is probably the most popular schizophrenia model used in rodents. Here, NMDA antagonists, such as ketamine, PCP or MK-801, induces schizophrenia-like symptoms i.e., hyperlocomotion, social withdrawal, cognitive deficits etc [2]. Two types of NMDA suppression models are typically used – acute and chronic. Acute model test schizophrenia-like symptoms after single injection of NMDA antagonists, while chronic model uses daily injections of these NMDA antagonists for couple weeks and tests cumulative NMDA suppression effect. However, there is lack of comparative studies using both models to examine if and how they compare.

Auditory steady-state responses (ASSR) are brain responses to repetitive auditory stimuli presented at a specific frequency, typically between 20-80 Hz. These responses can be measured by means of electrophysiology and are thought to reflect the synchronization of neural activity in the auditory cortex [3]. Studies have shown that individuals with schizophrenia exhibit abnormalities in ASSR. Specifically, they have reduced power and phase locking of ASSR at various frequencies, especially 40 Hz, indicating deficits in auditory processing. These deficits may contribute to the characteristic symptoms of schizophrenia, such as auditory hallucinations and disorganized speech. The relationship between ASSR and schizophrenia suggests that deficits in auditory processing may play a role in the pathophysiology of schizophrenia [4]. Changes in ASSRs are one of the primary biomarkers for animal models of schizophrenia.

In this study, we evaluate the effect of acute and chronic NMDA suppression by means of ketamine on both spontaneous brain activity and ASSRs. Brain activity was measured chronically through implanted electrode in the primary auditory cortex. We found that chronic ketamine model has larger effect on spontaneous brain activity while changes of induced brain activity (ASSRs) were similar after acute and chronic ketamine application.

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C.14. NEURAL CORRELATES OF IMPULSIVE BEHAVIOR IN MOUSE CEREBRAL CORTEX

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Impulsive behaviour is defined as quick, premature and inappropriate action that often results in negative outcomes [1]. Impulsiveness can be subdivided into impulsive actions and impulsive choices. Not properly managed excessive impulsive behaviour is common to some type of brain disorders, such as attention deficit hyperactivity disorder (ADHD), addictions, emotional disorders [2] or even Parkinson's disease [3]. Unfortunately, we still don't have a clear understanding of the brain mechanisms that governimpulsive behaviour. The aim of the study is to find neural correlates of the brain activity during impulsivebehaviour.

Here, we addressed this by training mice to perform a cued sensory-association task which was designed to highlight mice impulsive behaviour. During the cued sensory-association task mice were presented with an auditory cue and in order to receive a reward mice had to respond right after the cue and refrain from responding during the cue. Even though mice were not rewarded when responding during the cue (impulsive responses, IR), they continued to show this impulsive behaviour throughout the study. Tobetter understand this impulsive behaviour, we evaluated rates and timing of the IR. The rate of IR does not change over multiple training days, but its timing does. At the start of the training mice tend to respond to the given cue right at its onset while the response time gets more delayed over the period of the training.These differences in the timing of the IR throughout the training reflect different responding strategy anddemonstrates impulsive behaviour all the way throughout the animal training.

The brain activity was monitored by chronic ECoG (electrocorticogram) recordings through implanted electrodes at various sites of the cerebral cortex. ECoG activity was evaluated both as event-related potentials and spectral intensity at different frequencies. We found cue associated activity in multiple cortical areas. Our findings suggest that neural correlates of impulsive behaviour are globally distributed throughout the cortex.

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C.15. ELECTROCHEMICAL INVESTIGATION OF PHOSPHOLIPID MEMBRANE DAMAGE INDUCED BY S100A9 PROTEIN

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Increased accumulation of misfolded proteins in the brain is a hallmark of several neurodegenerative disorders. In Alzheimer's disease (AD) abnormal levels of naturally occurring proteins clump together to form plaques that accumulate in the brain and disrupt functions of neurons [1]. Pro-inflammatory S100A9 protein is increasingly recognized as an important contributor to inflammation-related neurodegeneration. Due to its inherent amyloidogenicity S100A9 contributes to amyloid plaques formation together with Aβ [2]. AD brains contain soluble (neurotoxic oligomers) and insoluble assemblies (aggregates) of proteins, both of which are the focus of intensive research with the aim of using them as targets for potential therapeutic intervention and raising the possibility that their manipulation will lead to an AD cure. However, toxicity mechanism of amyloid proteins is not well established. One prevailing hypothesis suggests that toxic effect of amyloids is accomplished through a membrane disruption mechanism, which leads to loss of synaptic efficiency, neuronal dysfunction, and degeneration [3].

Biomimetic lipid bilayer systems are a useful tool for modelling specific properties of cellular membranes. In this work the interaction between S100A9 protein and tethered bilayer lipid membranes (tBLMs) was studied. Tethered lipid bilayers represent one of the most promising classes of model membranes and are based on the immobilization of a planar lipid bilayer on a solid support that enables characterization by a wide range of surface-sensitive analytical techniques.

The aim of our work was to use tBLMs as a membrane model for studying S100A9 protein-induced membrane damage. Here we formed tBLMs and optimised their properties. Atomic force microscopy (AFM) was used for characterization of S100A9 aggregates. By employing electrochemical impedance spectroscopy (EIS) we monitored membrane damage caused by protein. We observed that S100A9 aggregates at different stages of oligomerization exhibit different ability to impair integrity of phospholipid membranes. In addition, the effect of S100A9 protein depends on the lipid composition of the membrane.

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C.16. IMMORTALIZATION OF AGED MOUSE MICROGLIA

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Neuroinflammation is a common hallmark of many neurodegenerative diseases and is associated with disease onset and further progression. Microglia, the resident macrophages of the central nervous system (CNS), play a biphasic role in pathological conditions due to their phenotypic plasticity. Under normal conditions, these cells maintain tissue homeostasis, play a role in brain development and synaptic pruning. However, in the presence of CNS damage or other pathological insults, activated microglia cells produce pro-inflammatory factors, that released over time damage the surrounding healthy brain tissue [1]. In addition, it is essential to note that microglia exhibit increased activation and enhanced release of pro- inflammatory factors during aging, further losing neuroprotective potential [2]. However, most current *in vitro* microglia cell models include immortalized microglia cell lines derived from newborns or embryonic CNS origin and likely do not represent the phenotype of adult or aged microglia [3]. Therefore, it is crucial to study the role of microglia in neuroinflammation using aged animals, ensuring the phenotypic response of aged cells.

The aim of this study was to develop an immortalized aged mouse microglia cell line. The generated cells could therefore be further employed in *in vitro* research of neuroinflammatory processes. Firstly, to achieve this, lentiviral plasmids containing SV40 (SV40 large T antigen) and hTERT (human telomerase reverse transcriptase) transgenes were constructed. Secondly, aged mouse microglia cells were isolated and stably transduced with either SV40 or hTERT-containing plasmids. In addition, functional assays and gene expression analysis were performed to compare immortalized microglia phenotype resemblance and key microglia marker expression with non-immortalized primary microglia and to ensure the cell line's possible application in future studies. The generated cells were first assessed for the expression of Iba1, a specific microglia marker, using an immunocytochemistry assay. Furthermore, the phagocytic activity of microglia cells was determined by the number of cells that were able to engulf fluorescent latex beads. Analysis of ROS was carried out with CellROX™ Green Reagent. Cell senescence was determined by a β-Galactosidase assay to confirm that the cells were not undergoing senescence. Finally, qPCR was performed to estimate gene expression associated with pro- and anti-inflammatory microglia phenotypes.

The results show that microglia immortalization with SV40 can be used to develop a stable aged mouse microglia cell line that resembles primary aged mouse microglia, while transduction with hTERT alone does not appear to cause efficient cell proliferation. Our results suggest that immortalized microglia cells can be further used in future studies on the role of microglia in neuroinflammatory processes.

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C.17. IMAGING MICROVISCOSITY OF LIPID DROPLETS IN HUMAN BREAST CANCER CELLS USING A VISCOSITY-SENSITIVE FLUOROPHORE

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Lipid droplets (LDs) are cytoplasmic organelles involved in energy storage, metabolism, and protection against lipotoxicity within cells. An increased number of LDs and their aberrant metabolism are hallmarks of several diseases, including obesity and cancer [1]. Investigating the biophysical properties of LDs, particularly microviscosity, in cancer cells can provide an enhanced understanding of LD functions in tumorigenesis and cancer aggressiveness. Furthermore, imaging the microviscosity of LDs may help identify different cancer cell subtypes, therapy-resistant tumor cells, and cancer stem cells based on their LD microviscosity.

Intracellular microviscosity can be determined by measuring the fluorescence lifetimes of viscosity-sensitive fluorophores (molecular rotors) [2]. In our study, we employed BDP-H molecular rotor (Fig. 1), whose sensitivity to viscosity was previously confirmed [3]. The intramolecular rotation of BDP-H depends on the microviscosity of the surrounding micro-environment: in a less viscous cellular compartment (e.g., cytoplasm), the rotation is unhindered, resulting in faster non-radiative relaxation from the excited state, thus shorter fluorescence lifetime, and vice versa in micro-environments with higher microviscosity [2-3].

The aim of our study was to measure and quantitatively compare the microviscosity of LDs in MCF-7 and MDA-MB-231 human breast cancer cells of different malignancy.

Our findings indicate that BDP-H accumulates in LDs and the cytosol of MCF-7 and MDA-MB-231 cells and exhibits monoexponential fluorescence decays. We assigned microviscosity values of 120 ± 9 cP and 195 ± 48 cP to the LDs in MCF-7 and MDA-MB-231 cells, respectively. The differences in LD microviscosity in human breast cancer cell lines of different malignancy were statistically significant ($p < 0.001$) [4]. Collectively, our study highlights the potential of LD microviscosity as a biomarker for cancer cell malignancy and proposes the use of the BDP-H molecular rotor for this purpose.

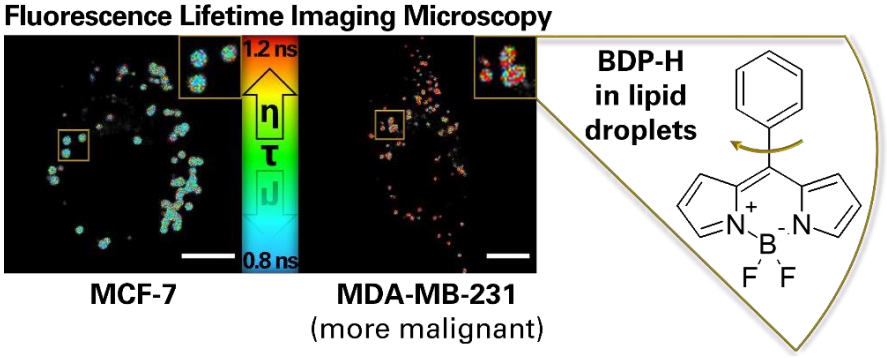

Figure 1. Fluorescence lifetime imaging microscopy images of live MCF-7 and MDA-MB-231 human breast cancer cells and their lipid droplets, where BDP-H molecular rotor accumulates. Scale bars: 10 µm. Pixel colours indicate BDP-H fluorescence lifetimes and microviscosity (η) values [4].

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C.18. INVESTIGATION OF THE MOTILE CELL WITH MONTE CARLO METHOD

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Motile cell has been the subject of intense study during the recent decades [1]. Motility and shape change of the cell play role in many biological processes such the development [2], the wound healing [3], the metastasis [4], and inflammation [5]. Using the Monte Carlo method, we theoretically investigate the behavior of a motile cell inside a microchannel. Monte Carlo method is very powerful computer simulation method for studying the shape change and the movements of the motile cell in different environments. Here, using the Monte Carlo method, we examine the spatio-temporal behavior of the biological cell inside a one dimensional microchannel. The surface of the microchannel is tiled with adhesive nano-scale or micro-scale topographical features. We consider two patterns of topographical features namely the symmetric pattern and the asymmetric pattern. We give a quantitative description of how cell size changes and how it migrates within the one dimensional microchannel as we vary the parameters of the model. We show that the cell stays at the point of symmetry on average in the case of symmetric pattern of topographical features. In the case of the asymmetric pattern of topographical features, the cell moves to the equilibrium site on average. The position of the equilibrium site depends on the parameters of the model. The directed migration of the of a living cell due to asymmetric pattern of the adhesive topographical features in two-dimensional substrate is seen in other experimental works and it is called *Topotaxis* [6]. Topo comes from topographical features of the environment, and taxis means directional migration. The shape change and the size change happen continuously in directional migration of the cell. Our theoretical study confirms that the asymmetric pattern of the adhesive topographical features results in the directed migration which is in agreement with the experimental results for the topotaxis.

Figure 1. The upper panel: behavior of the cell in presence of the symmetric pattern of the topographicalfeatures, and the lower panel: behavior of the cell in presence of the asymmetric pattern of the topographical features.

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C.19. CONCENTRATION AND PH-DEPENDENT SPECTROSCOPIC STUDIES OF PHOTOSENSITIZER $TPPS_4$ AGGREGATION

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It is widely known that traditional cancer treatment approaches have many limitations, chemotherapy usually leads to systemic side effects, surgical resection has a high recurrence rate, and radiotherapy is restricted by cumulative doses. Photodynamic cancer therapy (PDT) has been considered as a promising treatment for cancer [1]. Porphyrins and their derivatives are a large group of aromatic pigments, that can be used for PDT and diagnostics. It is known that some porphyrins tend to self- assemble in to aggregates if suitable media conditions are reached. This aggregation is pH and concentration dependent. Various cells and media in human body has different pH values - while blood is mostly neutral, the cancerous cells tend to be more acidic - this pH difference can influence the properties of $TPPS_4$ molecules. Molecule aggregation depends on pH and photosensitizer concentration, Changes in pH and concentration can lead to changes in diagnostic and therapeutic properties of 5,10,15,20-Tetrakis(4- sulfonatophenyl)porphyrin ($TPPS_4$) molecule. In this research we studied influence of pH and concentration of $TPPS_4$ on spectral properties of $TPPS_4$.

Fig.1 shows the absorption spectra of $TPPS_4$ monomer at neutral pH. It corresponds to typical free base porphyrins spectra with D_{2h} symmetry, characterized by an intense Soret band in the blue region and four less intense Q bands in the visible light region. In acidic pH , the $TPPS_4$ porphyrin core and the meso-substituents are protonated by H^+ (pKa=4.9), which in turn changes the symmetry of the molecule from D_{2h} to D_{4h}. At extremely low pH (-1), the Soret band intensity matches the one present at neutral pH, but with a noticeable red shift, while the remaining three Q bands undergo a shift to the longer wavelengths. At low pH values (~1), $TPPS_4$ molecules can form J-aggregates at relatively low pigment concentrations [2], while at the pH values that are neutral or higher J-aggregates were not detected. However, at high concentrations at neutral pH, the change to the absorption spectra was detected (Fig.2,). At millimolar $TPPS_4$ concentrations (c=1x10^{-3}M and above): relative Q_3 band intensity increases, Q_2 band disappears and Q_4 undergoes a significant red-shift. Corresponding spectral changes according to literature are referred as formation of aggregates. J-aggregates with characteristic bands at 490nm and 710nm are well known and documented, they are formed when a negatively charged SO_3^- group slots in the center of a positively charged porphyrin ring [2]. However, at neutral pH $TPPS_4$ molecules can't assemble to J-aggregates, so we presume, that at high concentration $TPPS_4$ molecules forms sandwich-type aggregates.

If significant quantities of photosensitizer are introduced to the neutral pH in the human body, the $TPPS_4$ molecules would be able to form H-aggregates, that could potentially harm PDT and influence the relaxation of singlet oxygen. However further research is needed to estimate influence of TPPS4 aggregation on PDT efficiency.

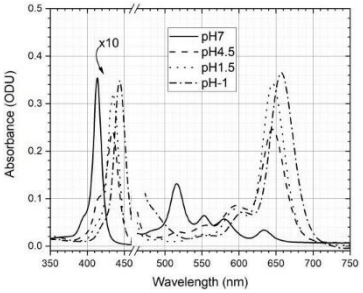

Figure 1. $TPPS_4$ absorption spectra dependence on pH value (c=1x10^{-5} M).

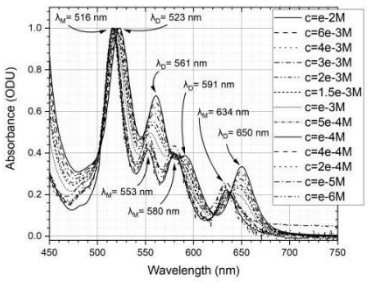

Figure 2. $TPPS_4$ absorption spectra dependence on concentration (pH=7). λ_M - $TPPS_4$ monomers, λ_D - dimers/aggregates.

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C.20. TAMOXIFEN DOSE-DEPENDENT tdTOMATO EXPRESSION IN MICROGLIA INDUCIBLE Cre-ER TRANSGENIC MICE

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This template shows how your Microglia are highly motile glia cells that are proposed to mediate synaptic pruning during neuronal circuit formation through phagocytosis of synaptic connections. Studies have shown that during mice visual cortex critical plasticity period (from the 21th to the 35th postnatal day) microglia plays an important role in synaptic pruning and network development. High resolution fluorescent microscopy enable us to thoroughly investigate microglia and neurons in the visual cortex but an efficient and stable cell labeling system is needed. To get fluorescent labeling in microglia cells we used transgenic mice line with genetically modified estrogen receptors (ER) that are connected to Cre recombinase as a transgene forming Cre-ER complex. Cre recombinase allow cell type-specific contributions in respective animal models making it conditionally active in microglia. Cre-ER complex by binding to tamoxifen instead of estradiol, activates and is transferred to the nucleus where recombinase specifically cuts out DNA STOP codon that is located before designed target tdTomato gene. In that manner with promotor Cx3cr1 only found in microglia we observe tdTomato expression. Tamoxifen is widely used for genomic recombination induction for transgenic genes, in this case it is specific to modified estrogen receptors (ER) and do not bind to natural estradiol, but multiple tamoxifen injections are needed to get most of the cells labeled. To evaluate the accuracy of this system we analyzed tdTomato expressing microglia in visual cortex depending on tamoxifen doses by calculating the average cell count per area. As it is mentioned in previous researches occasionally in mice without tamoxifen injections we spotted fluorescent tdTomato signal that indicates that the system is “leaky”. We found that tdTomato expression is tamoxifen dose dependent and that 3 tamoxifen injections gave the highest number of induced tdTomato expressing microglia cells which is 86% higher compared to the control group without tamoxifen injections.

C.21. INVESTIGATION OF HERBICIDE DCMU EFFECT ON AUTOFLUORESCENCE OF *NITELLOPSIS OBTUSA* CELLS

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Measuring the fluorescence emitted by chlorophyll molecules is a commonly used technique to study the effect of stresses on photosynthetic performance in algae and plants, as emission and photochemical reactions are in direct competition for excitation energy. Therefore, an increase in the intensity of the emitted fluorescence indicates a disruption in the photosynthetic process [1]. However, further investigation of emission signals is needed to improve their diagnostic potential in practical applications, as different factors have similar effects on the recorded fluorescence.

Pairing an optical fiber system with a controlled stepper motor enabled registration of autofluorescence signals along Characean alga *Nitellopsis obtusa* internodes in 1 mm intervals. A low intensity (< 1 mW) LED light source emitting at 405 nm was used for excitation. To assess whether the sensitivity of the chosen method is sufficient to detect the effect of an external stress, algae were immersedovernight in a 100 µM solution of herbicide 3–(3,4–dichlorophenyl)–1,1–dimethylurea) (DCMU). This compound inhibits photosynthetic electron transport [2] and should therefore affect the autofluorescence spectra. Experiments were carried out under different lighting conditions: darkness, natural daylight, intense (photosynthetic photon flux density of 829 µmol m^{-2} s^{-1}) white LED light, as it is unknown whether DCMU alters the effect of other environmental factors on emission signals. In addition, the data obtained after DCMU treatment were compared with spectra recorded in untreated internodes, and a principal component analysis (PCA) algorithm was used to visualise the results.

The autofluorescence spectrum of *N. obtusa* exhibited two peaks at 680 nm and 739 nm (Fig. 1A). Fluorescence intensity values varied along the cell and these variations were observed regardless of lighting conditions. Emission spectra of algae treated with 100 µM DCMU displayed an altered shape, but the recorded intensity remained similar to that of untreated cells. With the exception of internodes irradiated for 30 min, visualisation of spectral data in PCA space showed a clear separation of DCMU-poisoned cells (Fig. 1B). These findings confirm that DCMU interferes with photosynthetic performance. Hence, the sensitivity of the chosen optical non-invasive method is sufficient to detect the effect of external stresses in algal cells. Nevertheless, more data on the effects of other environmental factors are needed to better evaluate the sensitivity and selectivity of the chosen method.

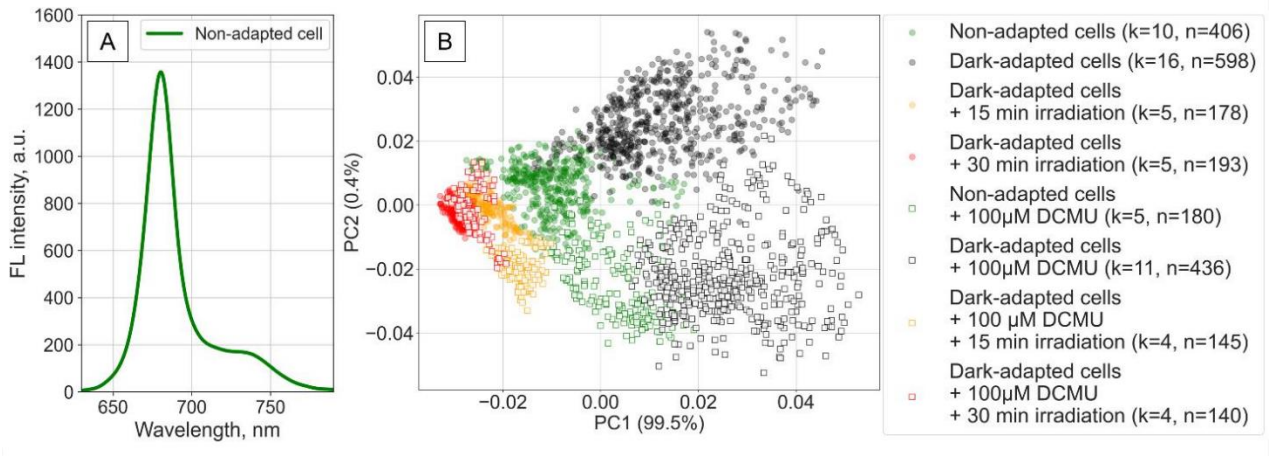

Figure 1. *N. obtusa* autofluorescence (FL) spectrum (A) and the score plot of PCA analysis for algae keptunder different conditions (B); k – number of cells, n – number of registered spectra.

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C.22. MATURITY ASSESSMENT OF HUMAN-INDUCED PLURIPOTENT STEM CELLS DERIVED CARDIOMYOCYTES

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Cardiomyocytes derived from human induced pluripotent stem cells (hiPSC-CMs) have become the most promising and powerful tool for heart regenerative medicine, disease research, drug development, and the reduction of animal experimentation. However, it is still challenging to obtain fully differentiated and functional cardiomyocytes [1]. It is known that the electrophysiological properties of hiPSCs differentiated into CMs differ from those of mature cardiomyocytes [2], making the electrophysiological properties a potential markers forassessment of functional maturity. Therefore, this study aimed to establish the electrophysiological evaluation of the maturity of hiPSC-CMs.

Intracellular recordings of hiPSC-CMs were performed in the whole-cell current clamp mode. From 48cells recorded, only 16 generated APs (6 cells generated APs spontaneously, and 10 required a current step stimulus for AP generation). We analyzed electrophysiological properties such as the maximum rate of actionpotential (AP) depolarization, the lowest potential between APs, the amplitude of AP, the duration of AP at 50% repolarization, and capacitance. The evaluated parameters of these properties were compared with the parameters of mature cardiomyocytes from the current literature.

The evaluated parameters of electrophysiological properties of hiPSC-CMs significantly differ from theparameters of human adult cardiomyocytes. Specifically, the maximum rate of AP depolarization and capacitance were significantly lower than in mature cardiomyocytes.

In conclusion, we successfully implemented the electrophysiological technique for evaluating the maturity of hiPSC-CMs and confirmed that hiPSC-CMs we used did not possess the electrophysiological properties offully matured cardiomyocytes.

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C.23. L-TYPE CALCIUM CURRENT IN HUMAN CARDIOMYOCYTE LINE AC16

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Cardiac cell lines derived from Human Induced Pluripotent Stem Cell (hiPSC) or cardiomyocytes opens new prospects in personalized medicine areas such as disease modelling, drug screening or new cell therapy possibilities [1]. AC16 is human cardiomyocyte cell line with good proliferation capability, biochemical markers, however it lacks contractility and ability to generate action potentials (AP). Number of ion channels contribute to AP generation in native cardiomyocytes, including L-type Ca^{2+} channels responsible for plateau phase of cardiac AP. While it is already known that AC16 cells cannot generate action potentials [2], we aimed to investigate whether this cell line still contains functional L-type calcium channels.

Electrical activity of AC16 cells was recorded using patch-clamp in whole cell voltage clamp mode. L-type calcium currents were measured as voltage-dependent inward currents at +10mV. Additionally, Bay K was used for activation and Nifedipine was used for inhibition of L-type calcium channels.

We observed inward current at +10mV, which was inhibited by Nifedipine. This is compatible with activity of functional L-type Ca^{2+} channels in AC16 cells. However, instead of activation, this inward currentwas reduced by Bay K.

Our study indicates the presence calcium currents in AC16 cells. However, the effect of Bay-K on inward currents in AC16 cells remains to be explained.

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C.24. INVESTIGATION OF TITANIUM VS STAINLESS-STEEL ELECTRODE EFFECT ON CELL TRANSFECTION, PERMEABILITY, PORE RESEALING AND VIABILITY

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During electroporation (EP), short electric pulses increase cells permeability; therefore, materials could be transferred to cells like drugs and genes or intracellular molecules extracted from cells. Daily EP is used for biotechnology, food industry and medicine, however the real potential of EP is still not revealed. One of many topics that researchers investigate is the effect of electrode materials to cells after EP. During pulsing iron ions get released from anode electrode and cause reduction EP medium pH subsequently leading to cell viability decrease, reduction in fluorescence intensity of fluorophores and change in medium conductivity [1]. Scientist demonstrated that electrodes made from alloys, like aluminum, copper, contaminate samples with ions more significantly compared to stainless steel electrodes and that more inert electrodes release less ions [2]. Moreover, it was determined that release of aluminum ions depend on pulse parameters [3]. Taking all into consideration, we were unable to find if titanium electrodes were used for EP. For this we compared stainless steel to titanium electrodes impact on cell electropermeabilization, gene electrotransfer, viability and pore resealing. We increased pulse duration by decreasing pulse intensity accordingly to maintain the same pulse power output.

Experiments were carried out by electroporating Chinese hamster ovarian (CHO) cells in low conductivity electroporation medium (0.1 S/m, 270 mOsmol, 7.2 pH). EP was performed between titanium or stainless-steel electrodes. Cells were electroporated with 0,1, 0,5 and 1 ms duration high voltage (HV) electric pulses. Strength of electric pulses varied from 0,445 kV/cm to 1,8 kV/cm and delivered the same pulse energy. Flow cytometry was used to measure the amounts of cells that were permeabilized or irreversibly electroporated by determining intracellular Propidium iodide (PI) fluorescence intensity and number of PI positive cells (PI^+). Transfection was performed using pEGFP-N1 plasmid that express green fluorescent protein (EGFP) which was measured using flow cytometry 24 hours after EP. Cell viability was evaluated using colorimetric MTS assay 24 hours after treatments.

Conducted research results revealed that usage of titan and stainless-steel electrodes give nearly identical amount of permeable CHO cells after EP using same pulse parameters. Similar tendencies were noticed measuring gene electrotransfer and pore resealing efficacies and cell viability- cells electroporated between titanium electrodes gave similar results as cells electroporated between stainless-steel electrodes. However, an unexpected result was that the levels of electropermeabilized cells decreased with decreasing pulse intensity and increasing pulse duration (same pulse energy), while cell death results gave the opposite and increased with increasing pulse duration. This phenomenon was observed using both material electrodes.

Overall, EP of CHO cells with different duration of 0.1 ms, 0.5 ms and 1 ms HV pulses bearing the same power output revealed no significant difference in results using titan either stainless-steel electrodes concerning electropermeabilization, gene electrotransfer, pore resealing efficacies and cell viability. Our data emphasize that using titanium electrodes for EP one may expect the same results as using stainless-steel electrodes.

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C.25. DOUBLE CONES IN AVIAN RETINA

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Double cones are the most abundant photoreceptor type in most avian retinas [1]. They constitute up to 40-55% of all photoreceptors [2], however, the main function of double cones is still unclear. Typically, it is assumed that in photopic conditions avian single cones are responsible for chromatic vision and double cones are responsible for achromatic vision.

Previous studies have shown that in birds of prey [3] and some other foveate species [4] a double cone-free zone exists in the fovea. This contradicts the theory that only double cones are responsible for achromatic vision in bright light, as fovea is the retinal area, in which the highest resolution vision occurs. However, double cone distribution in high resolution areas of birds without a fovea have not yet been investigated. The aim of this study was to compare distribution of double cones between foveate and afoveate bird species.

We investigated the retinae of common starling (*Sturnus vulgaris*), which possesses a central fovea, and domestic chicken (*Gallus gallus domesticus*), which does not have a fovea. We took one eye from each species, removed cornea and lens and embedded the eye-cups in paraffin to prepare retinal tissue for histological analysis. The eyes were sectioned in 6 µm slices using a microtome and samples were prepared for staining with a Periodic Acid-Shiff (PAS) reaction. The PAS reaction is commonly used to visualize carbohydrates and glycoproteins in various tissue samples, however, it is rarely used to investigate avian retinae. The PAS reaction can be used to stain photoreceptor outer segments and paraboloids of double cone accessory members. Light microscopy was used to count the double cones and measure double cone-free zone.

We found that in the retina of starling, the double cone-free zone has ellipsoidal shape and is approximately 400-450µm wide. In the retina of domestic chicken, we found no double cone-free zone, although the number of double cones in the high spatial resolution area in the centre of the retina is lower compared to the mid-peripheral retina.

Our preliminary results on afoveate domestic chicken retina suggest, that absence of double cones might be related not only to the presence of fovea, because double cones are wider than single cones, it would be beneficial to have fewer or no double cones in the high-resolution area, as is the case in raptors - birds with deep central fovea and highest spatial resolution among animals in general [3]. It is known that achromatic vision is responsible for best resolution [5], thus presence of double cone-free zone in the fovea of common starling (a passeriform species) and reduced number of double cones in the area centralis of domestic chicken (a galliform species) further support the idea that avian single cones might be responsible for both functions – colour and non-colour vision. This work provides more insight into mysterious function of double cones and other (than just chromatic vision) functions of single cones, and calls for more investigation of double cones in other foveate and afoveate bird species.

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C.26. EFFECT OF LEVEL OF AROUSAL ON THE LOUDNESS DEPENDENCE OF THE AUDITORY EVOKED POTENTIAL (LDAEP) IN HEALTHY SUBJECTS

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The effect of the alertness level or the condition of eyes closed versus eyes open during recording on the LDAEP was evaluated. LDAEP is considered as non-invasive indicator of central serotonin function (O'Neill et al., 2008). Disorders of serotonin metabolism are strongly associated with the onset of such disorders as depression (Lee K.S. et al., 2012). Usually LDAEP is recorded with eyes open. However there is no data about the brain responses to auditory stimuli when subject's eyes are closed.

The aim of this work was to record LDAEP with eyes closed and eyes open and to compare corresponding brain responses in order to evaluate optimal conditions for LDAEP recording.

Methods. 20 university students took part in this study. Prior to the LDAEP recording they performed General Anxiety Disorder test (GAD-7) and Patient Health Questionnaire (PHQ-9). The recording was repeated 2 times - with eyes closed and eyes open. Auditory stimuli of 5 different intensities (60, 70, 80, 90,
100 dB) were used. Auditory evoked potentials were recorded with EEG electrodes at the Fz, Cz, Pz positions. Comparisons of amplitude means between two different alert states with increasing sound intensity were performed.

Results. The amplitudes of the brain auditory evoked potentials (AEPs) with eyes closed were higher than with eyes open. However, there were no statistically significant differences (Fig. 1). LDAEP calculated from AEPs also didn't differed significantly. Correlation analysis revealed a positive correlation between the amplitude of AEP at Cz electrode and the anxiety scale (GAD-7) measures in EC condition.

Conclusion. There were no significant differences in LDAEP evaluation results in both condition of level of arousal. However, in our opinion, the recording of AEPs when eyes are closed is preferable, because lower state of arousal allows evaluate AEPs better and there are less problems with eyes movement artifacts.

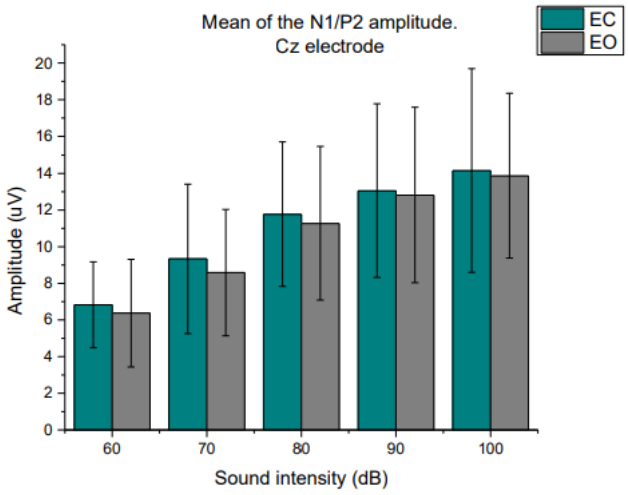

Fig. 1. Amplitudes of auditory evoked potentials recorded in conditions of eyes closed (EC) and eyes open (EO). Presented are mean values +/- SD.

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C.27. PROTEIN STABILIZED GOLD NANOCLUSTERS AS BIOSENSORS FOR CANCER CELLS DETECTION

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Ultrasmall photoluminescent gold nanoclusters (Au NCs), composed of several atoms with sizes up to a few nanometers have recently stimulated extensive interest. Unique molecule-like behaviors, low toxicity, and facile synthesis, which can be performed under mild, economical and eco-friendly conditions, make Au NCs very promising photoluminescent probes. Protein stabilized Au NCs are especially promising due to their biocompatibility. Since Xie et al. demonstrated Au NCs stabilization with bovine serum albumin (BSA) [1], various proteins were used for Au NCs synthesis, although BSA-Au NCs still remain one of the most common choice. BSA-Au NCs are investigated as potential photoluminescent biosensors for cancer cells labeling [2] and as photodrug for photosensitized cancer therapy [3].

However, due to limitation of excitation light penetration into deeper tissues and low PL quantum yield, BSA-Au NCs are more suitable for superficial imaging as optical tracking of such NCs *in vivo* is complex and requires extra efforts. The combination of optical imaging with other methods could result in significant diagnostic advancements. Such a system's goal would be to integrate the unique benefits of each imaging technique in order to offer accurate information at the illness site while getting beyond each technique's limitations.

The present study's objective was to assess the biodistribution of biocompatible technetium-99m-labeled bovine serum albumin-gold nanoclusters (^{99m}Tc-BSA-Au NCs) used as a SPECT/CT biosensor in experimental animals. The radiolabeling with ^{99m}Tc did not affect the optical characteristics of BSA-Au NCs inside the produced ^{99m}Tc-BSA-Au NCs bioconjugates, as was confirmed spectroscopically. A clinical SPECT/CT system was used to image the biodistribution of the ^{99m}Tc-BSA-Au NCs in Wistar rats. Wistar rats' *in vivo* imaging revealed intense cardiac blood pool activity as well as rapid blood clearance and accumulation in the kidneys, liver, and urinary bladder. The absence of visible uptake in kidney, liver, and spleen tissues was revealed by confocal images, indicating that the circulation lifetime of ^{99m}Tc-BSA-Au NCs in the bloodstream may be too short for accumulation in these tissues. The cellular uptake of ^{99m}Tc-BSA-Au NCs in kidney cells was also delayed, with significant accumulation only observed after a 24-hour incubation period.

Based on our findings, ^{99m}Tc-BSA-Au NCs could be used as a contrast agent and show promise as potential diagnostic agents for *in vivo* bloodstream imaging of excretory organs [4].

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C.28. IMPACT OF MICROSECOND PULSED ELECTRIC FIELDS ON ENDOPLASMIC RETICULUM MEMBRANE PERMEABILIZATION

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Nanosecond pulses have traditionally been employed for the permeabilization of intercellular organelle membranes, however, the application of this technique can be challenging. An alternative approach is the use of microsecond pulsed electric fields (µsPEF) which provide the capability to regulate cytosolic signaling molecules such as calcium (Ca^{2+}) concentrations. The increase in cytosolic Ca^{2+} has been shown to alter gene expression through the activation of Ca^{2+}-responsive transcription factors [1]. For example, Ca^{2+} influx into cytosol activates the calmodulin/calcineurin pathway, which leads to dephosphorylation of the nuclear factor of activated T-cells (NFAT). Dephosrylated NFAT can be translocated to the nucleus, where it activates the NFAT-sensitive promoter and triggers transgene expression [2]. Thus, µsPEF may serve as an external gene expression inducer in gene therapy by inducing Ca^{2+} influx.

In this study, we aimed to achieve ion influx through electropermeabilization of the endoplasmic reticulum (ER). Previous literature has indicated that the ER can be permeabilized by 100 µs PEF without adverse effects on cell viability [3]. To facilitate electropermeabilization, the extracellular conductivity was altered by modifying the extracellular medium composition. 100 µs and 1 Hz electric pulses were generated to disrupt intracellular organelle membranes. Cytosolic Ca^{2+} concentration changes were used as a marker for ER permeabilization. The increase in cytosolic Ca^{2+} concentration was observed in the HEK 293 cell line using fluorescence microscopy with the fluorescent calcium detection reagent Fluo-4. Furthermore, live cell impermeant SYTOX™ Green, a fluorescent nucleic acid stain, indicated that 0.6 kV/cm µsPEF caused plasma membrane damage.

Our results demonstrate that µsPEF treatment leads to an increase in cytosolic Ca^{2+} concentration, however, further studies are required to confirm the source of this increase by blocking specific Ca^{2+} transport system components. This study highlights the potential of easily applied microsecond pulses as a modulator of signaling molecule concentrations in gene therapy.

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C.29. THE PHYLOGENY, MORPHOLOGY AND TOXIGENICITY OF ALIEN CYANOBACTERIA RAPHIDIOPSIS RACIBORSKII OF LAKE JIEZNAS (LITHUANIA)

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Raphidiopsis raciborskii (Nostocales) is known as an alien species to Europe (Fig. 1). It is a harmful species that is able to produce saxitoxin and cylindrospermopsin. Although it originated in tropical latitudes, is currently reported to be spreading worldwide likely due to factors related to climate change. Lithuania is one of the northernmost distribution points. *R. raciborskii* was detected in Lake Jieznas thirty years ago and it is the only lake in Lithuania where this species occurs [1]. Therefore, it was important to investigate isolates from this lake.

The aim of this study was to determine seasonal dynamics of *R. raciborskii* in Lake Jieznas and to characterize the local isolates based on morphology and molecular analysis both of phylogenesis and toxigenicity.

In this study, we found that Lake Jieznas had the following characteristics: low transparency (0.5-0.7 m Secchi depth), high temperature (<26.3 °C), alkaline pH (7.94-9.02) and conductivity (432-450 µS/cm), which are favorable for the development of the species. However, *R. raciborskii* occurred in Lake Jieznas only in the warmest months – July and August with a low biomass of 0,07 mg L^{-1}. The morphological characteristics of the isolates were similar: the shape of the trichomes was straight and the variation in length and width was insignificant. Based on the morphology and phylogenetic analysis, the isolates were confirmed as *R. raciborskii* even though this species is very closely related to *R. mediteranea* and *R. curvata*. Genes responsible for the production of cyanotoxins, such as *cyrA, cyrJ* and *sxtA* were not found, revealing that they are not toxin producing isolates. Based on these data, *R. raciborskii* found in Lake Jieznas corresponds to the description of the European ecotype of the species.

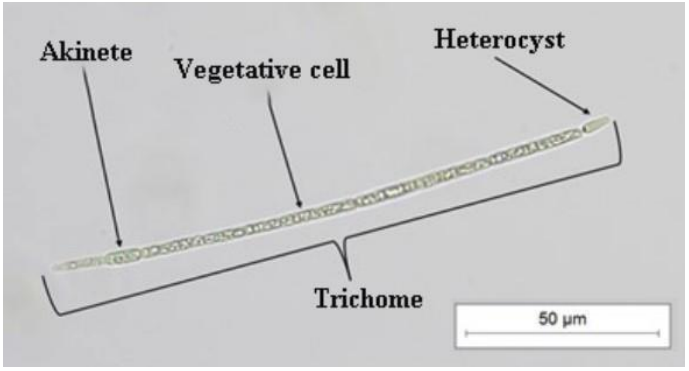

Figure 1. Morphology of *Raphidiopsis raciborskii* found in Lake Jieznas

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C.30. STAT3 SIGNALING PATHWAY AS A POTENTIAL PREDICTIVE TARGET IN TRIPLE-NEGATIVE BREAST CANCER

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Breast cancer is the most common neoplasm worldwide and is the leading cause of cancer deaths among women. Triple-negative breast cancer (TNBC) is the most aggressive subtype with the lowest 5-year survival rates accounting for 12-18% of all breast cancers. TNBCs have a much higher recurrence and metastasis as a result of not being eligible for current treatment options due to the lack of estrogen, progesterone receptor, and human epidermal growth factor receptor 2 [1]. This aggressive cancer contributes to the overall shortened survival of patients diagnosed with TNBC [2].

Considering the absence of molecular targets, neoadjuvant chemotherapy (NAC) remains the standard of care for patient treatment. **However, the effectiveness of treatment is unpredictable, as** patients frequently develop resistance. For this reason, there is a growing need to develop novel non-invasive molecular predictive approaches for this disease [3]. It has become evident that the development of TNBC chemoresistance is based on the elaborate interplay of the tumor microenvironment, drug efflux, and cancer stem cells. Alterations of multiple signaling pathways govern these interactions [2]. Recent evidence from clinical trials and preclinical studies have demonstrated a pivotal role of the STAT3 (signal transducer and activator of transcription 3) signaling pathway in the initiation, progression, and metastasis of TNBC [3].

The aim of this study was to evaluate the expression of *STAT3*, *ALDH1A1*, *NFIB, UPF3A*, *BCL-2*, and *R-RAS-2* genes in serial plasma samples before and after NAC. Gene expression changes were used to determine associations with clinical features of TNBC patients and to analyze *STAT3*, *ALDH1A1*, *NFIB, UPF3A*, *BCL-2*, and *R-RAS-2* expression as potential biomarkers for predictive purposes.

In this study, we used reverse transcription quantitative PCR to determine *STAT3*, *ALDH1A1*, *NFIB*, *UPF3A*, *BCL-2*, and *R-RAS-2* expression in paired 122 blood plasma samples of TNBC patients before and after NAC. We determined that a combination of expression changes of *STAT3*, *BCL-2*, and *R-RAS-2* could be used as a predictive biomarker panel to determine TNBC patients that show a more positive response to NAC ($p=0.0012$, $p=0.0098$ and $p<0.0001$, respectively).

Overall, understanding how the expression changes of these genes influence the course response to NAC in TNBC patient plasma potentially could be used as non-invasive molecular biomarkers for treatment effectiveness.

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C.31. DISCOVERY OF A NOVEL POLYOMAVIRUS IN EUROPEAN HAMSTER

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Our research subjects are circular dsDNA viruses of the *Polyomaviridae* family. Most polyomaviruses are host-species specific and display a distinct tissue tropism. Although these small non-enveloped viruses can infect a vast range of mammalian, avian, and fish species, our work focuses on rodent infecting polyomaviruses. The *Rodentia* is the largest order of mammals, however, according to ICTV (International Committee on Taxonomy of Viruses) only 16 out of 117 confirmed polyomaviruses infect rodents and only one of them infects hamsters (subfamily *Cricetinae,* 19 species) - Syrian hamster infecting HaPyV [1]. Considering that as many as 14 polyomaviruses are found in humans, it is reasonable to think that there are still a lot of undetected polyomaviruses in different wild rodents. In the past, polyomaviruses were used as model organisms in tumorigenesis, eukaryotic gene regulation, and expression. Nowadays the major capsid protein VP1 has been studied for its property to form virus-like particles (VLPs). VLPs can be carriers for other peptides or DNA, therefore, VLPs have potential in vaccine, cancer, and molecular mechanism studies [2]. Further polyomavirus discovery can broaden the understanding of evolutionary and biological importance of these viruses and offer new tools in biotechnology and medicine.

In this work we tested 21 kidney samples of wild European hamsters, caught in Germany, for polyomaviruses. DNA was extracted from kidney samples and amplified by EquiPhi29 DNA polymerase to increase viral DNA concentration. Nested PCR with degenerate primers was performed to find polyomavirus- like sequences in extracted DNA. Amplified DNA fragments were ligated into a cloning vector and sequenced. Sequencing revealed polyomavirus-like DNA with 81 % similarity to the mouse infecting MPtV LTAg coding sequence. A 4963 bp long genome was amplified using PCR with novel polyomavirus-specific primers and later sequenced by primer walking method. The analysed genome has 72 % sequence identity to MPtV and 54 % to HaPyV. In addition, the novel polyomavirus genome structure matches all the characteristics of a standard polyomavirus genome. Novel polyomavirus infection was detected in 33 % of kidney samples by diagnostic PCR. Furthermore, we synthesized VLPs of a novel polyomavirus VP1 protein in *Saccharomyces cerevisiae.* VP1 gene was amplified and ligated into a yeast expression vector. VP1 synthesis was induced by galactose. Saccharose and CsCl gradients were used to purify VLPs. The formation of VLPs, ranging between 45-55 nm in diameter, was confirmed with electron microscopy.

Novel polyomavirus LTAg coding sequence differs by 24 % from the closest polyomavirus MPtV LTAg gene, which is considerably more than minimal ICTV criteria of 15 % difference, thus, the discovered polyomavirus can be considered a new species [3]. Although HaPyV belongs to *Alphapolyomavirus* genus, the novel polyomavirus should be considered as a *Betapolyomavirus* as its closest relative MPtV. This novel polyomavirus is the first polyomavirus discovered in European hamster and the second polyomavirus which infects hamsters. VLPs of this novel polyomavirus could be used for further investigation of polyomavirus biology, as well as adapted as a molecular tool for foreign antigen exposition, as HaPyV.

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C.32. IMPACT OF BREX SYSTEM PROTEINS ON CELL VIABILITY

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Comprehension about the ways in which bacteria and bacteriophages interact can be useful in areas such as biotechnology, molecular biology, genetics, medicine, or food industry. To be able to apply suchknowledge, it is requisite to understand the course of action by which bacteria are capable to protect themselves from bacteriophages that infect them. Bacteriophages are the most abundant biological entity in the biosphere, and they are responsible for the destruction of 20-40 % of bacterial cells each day [1]. Constant coevolution and coadaptation of these organisms has resulted in the evolution of bacterial immune systems and myriad of improving ways for bacteriophages to overcome them. One of bacterial immune systems isBREX (Bacteriophage Exclusion). This system is spread in about 10 % of prokaryotic genomes, yet its detailed defence mechanism remains to be elucidated [2].

Our research object is the type I BREX (BREX1) system, which is encoded in the six gene cluster: *brxA- brxB-brxC-pglZ-brxL-pglX*. System protects bacteria from diverse lytic and lysogenic bacteriophages. BREX1methylates specific sites which allows to overcome its protection. Infection of bacteriophages which do not carry this epigenetic modification is restricted but their genome is not degraded. It is surmised that afterBREX1 system recognizes its target one of the vital cell processes is suppressed and phage can no longerproliferate. This work studied whether cells of Escherichia coli are affected by disruption of the natural system or expression of individual proteins (Fig. 1). We have previously showed that deletion of certain BREX1genes resulted in cytotoxicity [3]. This suggests that some BREX1 proteins participate in autoregulation of theimmune response, while other might act as effectors interfering with the vital process of the cell. Here, weassess cell viability by expressing various combinations of BREX1 genes thus seeking to identify the effector and regulator proteins of the BREX1 immunity.

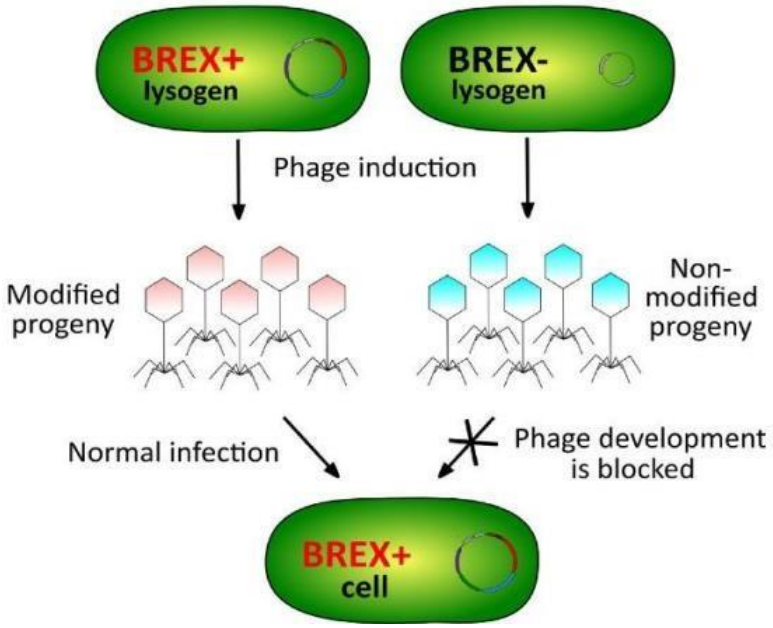

Figure 1. Graphical representation of BREX1 mechanism of action. Adapted from [3].

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C.33. MICRORNA EXPRESSION IN 4-NITROQUINOLINE 1-OXIDE-TREATED LEUKOCYTES OF PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Type 2 diabetes mellitus (T2DM) is a chronic metabolic disease due to tissue insulin resistance (IR) and insufficient compensatory insulin secretion from pancreatic β-cells. Previous studies have revealed that reactive oxygen species (ROS) play a critical role in the development of IR and T2DM. Negative effects of ROS on insulin secretion are mediated by a decrease in pancreatic blood flow as well as the induction of islet fibrosis, oxidative stress, and inflammation [1]. However, the precise cellular and molecular changes caused by ROS in T2DM pathogenesis are not entirely clear. MicroRNAs (miRNAs) are involved in many physiological processes and have been linked to metabolic disorders such as T2DM and obesity [2]. The role of miRNAs as potential biomarkers for the assessment of T2DM complications is well recognized, but the currently available data are contradictory.

The present study aimed to evaluate the expression of four selected miRNAs (miR-16-5p, miR-17-5p, miR-106a-5p, and miR-223-3p) in leukocyte samples treated with 4-nitroquinoline 1-oxide (4NQO), imitating ROS effects on cells. The samples were collected from 38 patients diagnosed with advanced T2DM and 21 non- diabetic patients (NDP). The expression levels of the selected miRNAs were quantified using real-time PCR with TaqMan-based assays.

MiR-106a-5p in T2DM leukocytes was expressed at lower levels than in NDP ($p = 0.0476$), but no differences in expression was observed for the other miRNAs (all $p > 0.05$). Nevertheless, further analysis revealed various associations between miRNA expression and T2DM-associated complications and clinicopathological patient parameters. MiR-17-5p expression was higher in cases with primary arterial hypertension (PAH+) that in those without the complication (PAH-) both before and after 4NQO treatment ($p = 0.0213$ and $p = 0.0291$), however, 4NQO did not affect the miRNA levels directly ($p > 0.05$; Fig. 1). Baseline expression levels of miR-106a-5p also differed between the PAH+ and PAH- cases ($p = 0.0183$), but the difference was not retained after 4NQO treatment ($p > 0.05$), whereas miR-16-5p analysis revealed the opposite tendency. Besides, after 4NQO treatment, miR-16-5p expression correlated negatively with glycated hemoglobin levels ($p = 0.0425$, $r = -0.36$).

In conclusion, the preliminary results of our study indicate a potential role of the analysed miRNAs in the ROS-dependent development of cardiovascular T2DM complications. Nonetheless, functional (gain / loss) analysis is required to confirm the results.

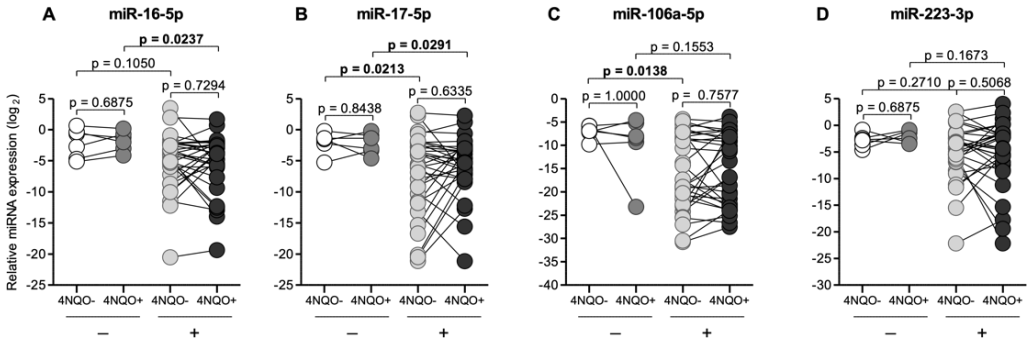

Figure 1. MiRNA expression in leukocytes of T2DM patients according to the manifestation of primary arterial hypertension (+/–) and 4-nitroquinoline 1-oxide (4NQO) treatment. A-D – miR-16-5p, miR-17-5p, miR-106a-5p, and miR-223-3p, respectively. Circles and connecting lines indicate sample pairs.

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C.34. PLASMA MIRNAS AS POTENTIAL BIOMARKERS FOR PREDICTING RADIOTHERAPY TREATMENT-ASSOCIATED DEVELOPMENT OF RIHD IN LUNG CANCER PATIENTS

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Background: Lung cancer is the second most common malignancy and is the leading cause of cancer-related deaths in the world [1]. Improving cancer diagnostic and treatment methods would help to improve survivability. Currently, both non-small cell and small-cell lung cancer have various types of treatment, including radiotherapy as one of the main therapy options. Despite of that, thoracic radiotherapy for lung cancer has been linked to an increased risk of cardiac-related morbidity and mortality [2][3]. Currently available methods that predict radiation-induced heart disease (RIHD) are suboptimal. Identifying and developing biomarkers such as circulating plasma microRNAs (miRNAs) could improve the prediction of RIHD. Changes in miRNA expression levels could be a useful, non-invasive liquid biopsy tool for improved risk stratification and precise treatment planning to reduce the cardiac toxicity in lung cancer radiotherapy.

The aim of this study was to identify miRNAs expression changes and their association with clinical features in the plasma, taken from Lithuanian lung cancer patients, pre-and post-ionizing radiation treatment, in order to evaluate the effects of treatment on the heart and identify miRNAs, which might be used to predict response to treatment.

Methods: 12 pairs of lung cancer patients' plasma samples before and after radiotherapy were included in this study. MiR-1-3p, miR-21-5p, miR-24-3p, miR-29a-3p, miR-34a-5p and miR-222-3p were analyzed. MiRNA expression was examined by reverse transcription quantitative PCR. Results were normalized with exogenous cel-miR-39-3p control and relative expression was acquired by calculating comparative $C_T(2^{-\Delta\Delta CT})$.

Results: The fold change analysis showed that miR-1-3p, miR-24-3p, miR-29a-3p and miR-222-3p were downregulated, while miR-21-5p and miR-34a-5p were upregulated in lung cancer patients' plasma after radiotherapy. Moreover, after treatment, patients with higher natriuretic peptide serum concentration and troponin values showed significant differences in miRNA relative abundance compared to the norm of these indicators. Furthermore, miR-1-3p indicated possible tendencies of cardiac comorbidities.

In conclusion, our study suggests that the identification of microRNA expression level changes in lung cancer patients' plasma before and after radiation therapy could be used for the early diagnosis of RIHD. However, further analysis is needed to validate these miRNAs as potential biomarkers for radiation-induced cardiac toxicity.

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C.35. EVALUATION OF IN VITRO GENOTOXICITY OF DIFFERENTLY SIZED NOBEL METAL NANOPARTICLES BY COMET ASSAY

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Over the past few decades, noble metal nanoparticles (NPs), in particular, gold nanoparticles (AuNPs) and silver nanoparticles (AgNPs) received great interest in the field of biomedicine. Due to their unique characteristics such as electrical, mechanical, thermal, and antibacterial properties, which can be easily altered by changing particle size, shape, or surface coating, noble metal nanoparticles have been of great importance in personalized healthcare and diagnostics [1,2]. However, one of the main concerns of AuNPs and AgNPs exposure is their genotoxic potential.

In this study, lymphocytes of 5 healthy donors were treated with differently sized gold (40 nm and 5 nm) and silver (35 nm and 13 nm) nanoparticles for 1-3 and 24 hours, and cytotoxicity, as well as genotoxicity, was assessed. Cytotoxicity was evaluated using an acridine orange/ethidium bromide stain test. Results revealed that none of the tested AuNP concentrations reduced cell viability by more than 20 %, regardless of the size of the NPs. However, the AgNPs were shown to have cytotoxic potential. All tested 13 nm NPs concentrations and concentrations higher than 40 µg/ml (35 nm) significantly reduced cell viability after 24-hour treatment, which led to excluding these doses from further studies. Genotoxicity was assessed using the alkaline comet assay, which allows evaluation of the induction of primary DNA damage, such as DNA strand breaks and alkali-labile sites. As expected, long-term incubation with NPs induced more DNA damage compared to short-term incubation. Besides, after 24-hour treatment with AgNPs, the amount of induced DNA damage was concentration dependant. Also, it was shown, that 5 nm size AuNPs were significantly more genotoxic than larger, 40 nm AuNPs.

Overall, it was shown, that AgNPs are more cytotoxic, compared to AuNPs. Besides, it was revealed that the size of nanoparticles might affect their reactivity, with smaller particles being more cytotoxic and genotoxic than larger ones.

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C.36. GENE EXPRESSION PATTERNS OF HISTONE METHYLATION-MODIFYING GENES IN PROSTATE CANCER

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Prostate adenocarcinoma (PCa) is one of the leading causes of cancer-associated death in men and the most dominant male malignancy worldwide. Despite the innovative molecular tests already available on the market, the critical need for more accurate prognostic tools is not met, yet. In the last decade, epigenetic alterations (e.g., histone methylation, HM) have been shown to be of great importance during prostate carcinogenesis, making them a potential source of new diagnostic and prognostic biomarkers for PCa.

In this study, expression of HM-associated genes was analysed in two cohorts aiming to evaluate their potential clinical applications. Next-generation sequencing data was obtained for the analysis from the Cancer Genome Atlas dataset (TCGA, PRAD cohort N = 333) while quantitative PCR-based methods were used for quantification of the selected genes in the Lithuanian cohort (N = 121). Of the 58 currently known HM genesin the human genome, i.e. lysine demethylase (KDM) and lysine methyltransferase (KMT) genes, expressiondata of 55 genes were extracted from the TCGA PRAD dataset. In this cohort, 61 % of KDM and 81 % of KMTgenes showed differential expression in PCa compared to noncancerous prostate tissues. In the Lithuanian cohort, several screening steps including RT^2 Profiler PCR Arrays (Qiagen) and Custom TaqMan Array plates(Thermo Fisher Scientific) led to selection of seven HM genes for more thorough analysis by single-gene assay experiments. Expression of *KMT1E*, *KDM5A* and *KMT5A* differed among PCa and non-tumor tissues (all $P < 0.0500$), with diagnostic specificity for PCa reaching up to 87.2% ($P < 0.0001$) in the Lithuanian cohort. Inaddition, *KDM5A* and *KMT1E* emerged as predictors of disease progression-free survival, adding to the prognostic potential of clinicopathological parameters. The observed associations were supported by the analysis of the TCGA cohort.

In conclusion, the present study showed common deregulation of HM-associated genes *KDM5A* and *KMT1E*, revealing their potential clinical application as prognostic PCa biomarkers. However, further validation is required in independent cohorts with extended patients' follow-up data.

C.37. EVALUATION OF RNA SAMPLE PREPARATION METHODOLOGY FOR CIRCULAR RNA QUANTIFICATION *IN VITRO*

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Noncoding circular RNAs (circRNAs), initially thought to be splicing errors, now are recognized as important gene expression regulators. Currently studied circRNAs are involved in various pathologies, including carcinogenesis [1]. This makes circRNAs attractive research targets. However, methodologies for circRNA detection and functional analyses are only developing, without any standardized workflows, yet [2]. The aim of this study was to evaluate several steps of RNA sample preparation for targeted circRNA analysis. Firstly, the yield and quality of the purified RNA were compared among three different RNA extraction kits (coded *M*, *P*, and *Z*). The selected circular and linear transcripts of the same genes (13 transcripts in total) were quantified in both nuclear and cytoplasmic cellular fractions. Quantities of linear transcripts were also compared with and without RNase R treatment. The selected circRNA and linear targets were analyzed by means of real-time PCR using the QuantStudio™ 7 Pro Real-Time PCR System (Applied
Biosystems™, Thermo Fisher Scientific). All analyses were performed using cancer cell lines.

Our preliminary results showed that even though the yield and quality of purified RNA were comparable among the kits, relatively higher quantities of the same circRNA targets were obtained with the *P* kit. The comparison of the cytoplasmic and nuclear RNA fractions revealed generally higher relative circRNA amounts in the former. However, some circRNAs were found in similar quantities in both cellular fractions (e.g. *circNRIP1*). After RNase R treatment the majority of the circRNA targets were detected in higher relative amounts (e.g. *circUBAP2* and *circCSNK1G3*), while the linear transcripts were detected at reduced levels. However, quantities of several other circRNAs showed the opposite tendency (e.g. *circFAT1* and *circNRIP1*).

In conclusion, our preliminary data indicate similar performance of the tested RNA extraction kits from different suppliers, with slightly higher circRNA expression levels obtained when using the *P* kit. The RNase R treatment revealed that the analyzed circRNAs were not enriched uniformly, raising concern for biased data. Therefore, further optimization steps are needed for the development of circRNA analysis methodology.

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C.38. QUANTITATIVE MICRORNA ANALYSIS IN BLOOD PLASMA OF PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder associated with hyperglycemia caused by impaired insulin resistance and pancreatic β-cell dysfunction [1]. Diabetic hyperglycemia results in long-term damage, dysfunction, and failure of many organs, including the eyes, kidneys, nerves, heart, and blood vessels [2]. In order to assess the risk of complications related to T2DM, molecular biomarkers should be investigated for their possible diagnostic or prognostic value. Small regulatory RNAs (miRNAs) are among the most promising liquid biopsy biomarkers related to diabetes.

The aim of this study was to evaluate eight selected miRNAs – miR-16-5p, miR-27a-3p, miR-144-3p, miR-146a-5p, miR-152-3p, miR-155-5p, miR-222-3p, and miR-223-3p – in blood plasma samples of patients with T2DM and to assess their association with the risk of DM complications. Plasma samples from 70 T2DM patients and 26 non-diabetic patients (NDP) were analyzed. The miRNAs were quantified by means of real-time PCR, using TaqMan-based assays, after nonspecific reverse transcription of all miRNAs.

Expression levels of miR-16-5p, miR-144-3p, miR-146a-5p, miR-152-3p, and miR-222-3p in T2DM were lower than in NDP (all $p < 0.050$). The levels of miR-16-5p, miR-27a-3p, miR-144-3p, miR-146a-5p, miR-152-3p, and miR-223-3p were higher in T2DM cases without a history of acute myocardial infarction (MI) compared to T2DM individuals with previous MI (all $p < 0.050$). Moreover, miR-27a-3p, miR-144-3p, and miR- 222-3p levels were lower in the T2DM group with diagnosed primary arterial hypertension (PAH) in comparison to T2DM patients without PAH (all $p < 0.050$). In addition, lower plasma miR-222-3p levels were detected in patients that have been hospitalized for heart failure (HF) compared to those without this condition ($p = 0.010$). No significant associations were found between plasma miRNA levels and other diabetic complications (retinopathy, nephropathy, chronic kidney disease, gout; all $p > 0.050$).

In conclusion, our preliminary data indicated differences of particular miRNA levels in plasma of T2DM patients, which were associated with specific cardiovascular complications. Further large-scale studies are needed for the more detailed assessment of their clinical potential as prognostic T2DM biomarkers.

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C.39. GENOME-WIDE EXPRESSION PROFILING AND FUNCTIONALANALYSIS OF MIRNAS IN PERIODONTAL DISEASES

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Periodontal diseases comprise a wide range of inflammatory conditions that affect the surrounding and supporting structures of the teeth. Up to 50 % of the global population is affected by the periodontal disease of which periodontitis (PD) is the most severe form and often leads to tooth loss. Dental implants are the primary choice to replace missing teeth. Despite technological advances in implantology, the failure rate of implants can reach up to 23 %, with peri-implantitis (PI) being the most common cause [1]. Studies have shown that active PD of adjacent teeth is considered one of the risk factors for PI [2]. Moreover, people with systemic progressive PD have a 5-fold higher risk of implant failure and a 14-fold higher risk of PI [3]. This suggests that these diseases are linked by similar mechanisms of pathogenesis that may be epigenetically regulated. MicroRNAs (miRNAs) are considered one of the critical modulators that influence periodontal homeostasis [4]. MiRNAs are extensively studied in a wide range of diseases, but research is still significantly lacking in the case of PD and PI. This study aimed to reveal PD-associated miRNAs in the gingival tissue and evaluate the therapeutic potential of miRNA inhibition technology in PD and PI.

Microarray analysis of 16 gingival tissue samples revealed 140 upregulated miRNAs in inflamed gingival tissues as compared to healthy controls. Fifteen selected miRNAs were further analyzed by performing RT-qPCR in an extended cohort of gingival tissue samples (N=80). Analysis revealed that the levels of miR-146a-5p were significantly lower in PD-affected individuals as compared to periodontally healthy participants. Severe forms of PD were associated with increased levels of miR-140-3p and -145-5p and decreased levels of miR-125a-3p. Moreover, the correlation between periodontal outcome parameters indicating the worse clinical status of PD and increased levels of miR-140-3p, -145-5p, and decreased levels of miR-125a-3p, was observed. MiRNAs abundantly expressed in gingival tissues, namely, miR-140-3p, -145- 5p, -146a-5p, and -195-5p, were selected for further functional analysis.

Functional analysis of PD-specific miRNAs was performed in human bone marrow mesenchymal stem cells cultivated on surfaces of cell culture plastic and medical titanium by transfection with inhibitors of selected miRNAs (antagomiRs). The efficacy of antagomiR treatment was evaluated under various transfection conditions by analyzing the expression levels of the selected miRNAs. Analysis revealed that inhibitors of miR-140-3p significantly decrease the expression levels of miR-140-3p by 1.7-fold in cells cultured on plastic 2 days after transfection. While in cells cultured on medical titanium expression of miR- 140-3p and -145-5p was most decreased 3 and 2 days after antagomiR transfection, respectively, although the expression differences were statistically non-significant (P > 0.050).

This study revealed that miRNAs play an important role in the pathogenesis of periodontal diseases. However, further studies are essential to evaluate the potential of miRNA inhibition technology in treating periodontal diseases.

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C.40. MIRNA ANALYSIS IN BLOOD OF WOMEN DIAGNOSED WITH GESTATIONAL DIABETES MELLITUS

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Gestational diabetes mellitus (GDM) is a serious complication of pregnancy that develops due to chronic hyperglycemia during pregnancy [1]. Although GDM usually resolves after giving birth, it significantly increases the risk of type 2 diabetes mellitus (T2DM) for both the mother and her offspring. Therefore, investigation of potential molecular biomarkers for the early risk assessment of both GDM and T2DM development is important [2]. Epigenetic alterations, like expression differences of small regulatory RNAs(miRNAs), have received lots of attention during the last decade due to their potential applicability as promising liquid-biopsy biomarkers.

In the present study, several miRNAs, namely miR-16-5p, miR-27a-3p, miR-152-3p, miR-155-5p, miR-222-3p, miR-17-5p, miR-29a-3p, miR-195-5p and miR-499a-5p, were selected for the quantitative analysis. Whole blood samples (N = 256 in total) were obtained from 90 women diagnosed with GDM (GMD+), whereas7 non-GDM pregnant women (GDM-) and 5 healthy non-pregnant women (NP) were included as control groups. For GDM+ group, samples were collected at 24-28 weeks of gestation (N = 90), at 6-12 weeks postpartum (N = 53), at 1 yr postpartum (N = 9) and at 2 yrs postpartum (N = 6). The selected miRNAs were quantified as single assays by means of real-time PCR, after non-specific reverse transcription of allmiRNAs.

In the GMD+ group, higher levels of miR-16-5p, miR-152-3p, miR-155-5p, miR-222-3p, miR-17-5p and miR-195-5p were detected in blood collected after delivery compared to pregnancy. Also, miR-29a-3p Levels was higher in GDM+ during pregnancy than in GDM- or NP, whereas miR-195-5p showed the opposite tendency.

In conclusion, the present study revealed various associations of the analyzed miRNAs and GDM. This indicates that selected miRNAs might become potential biomarkers for GDM diagnosis and prognosis. However, further studies in larger independent cohorts are needed.

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C.41. EPIGENETIC MODIFICATION OF SPECIFIC GENES AS BIOMARKERS FOR RENALCLEAR CELL CARCINOMA

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In 2020, Lithuania was number one in kidney cancer incidence, as well as in the top ten for kidney cancer mortality, ranked globally for both sexes and all ages [1]. These statistics demonstrate the need for more resources to be invested in developing new methods of treatment and novel ways of diagnosis. Kidney cancer when diagnosed early, has a significantly higher chance of being treated successfully [2]. The methods currently used for diagnosis range from medical imaging to biopsies. The goal of this research is to investigate potential molecular biomarkers that could be used as a non-invasive form of diagnosis. These biomarkers are the epigenetic modifications of genes in, DNA extracted from the cancerous renal tissues.

Out of the currently known epigenetic phenomena of human DNA, gene methylation as a biomarker has been explored by our group before by using Agilent Human DNA Methylation 1 × 244K Microarrays [3]. Our current research looks at the methylation of a few specific genes using quantitative methylation-specific PCR. To assess how reliable selected genes are, methylation status is examined in DNA samples, obtained from cancerous and paired non-cancerous renal tissue samples from patients diagnosed with renal clear cell carcinoma included patients who underwent partial or radical nephrectomy without any neoadjuvant therapy at the Urology Centre of Vilnius University Hospital "Santaros Klinikos" (Lithuania) between 2013 and 2016.

Genes with the potential for being used as biomarkers have been selected based on a combination of factors, including the number of gene-associated probes showing significant methylation differences, the localization of the particular probes, where preference is given to those whoare located in the annotated CpG islands and regulatory regions (especially promoters) of the gene. Simultaneously, previous research and current knowledge of the function of the particular gene were considered.

All in all, the effectiveness of treatments for renal clear cell carcinoma can be improved by early diagnosis. By investigating our selected genes, we seek to expand the list of biomarkers that could be used as a non-invasive way of diagnosis.

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C.42. STUDY OF POTENTIAL NEW BACTERIAL DEFENSE SYSTEMS TARGETING NUCLEIC ACIDS

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Bacteria, the most abundant form of life on Earth, face many opponents. The main opponents of bacteria include bacteriophages and foreign plasmids. Bacteria have numerous different molecular mechanisms of defense against these invaders: the immunity can be adaptive like CRISPR-Cas systems, orDNA modification based, like Pgl systems, defense system may also work by inducing programmed cell death.

A variety of defense systems in the bacterial genome are found together in clusters called defense islands. Bioinformatic analysis of one of these prokaryotic genome regions lead to a discovery of a new potential defense system. We are currently working with three types of this new potential bacterial defense system. This system which potentially has significant activity during the invasion of a bacterial cell includes domains having translocase activity and nuclease activity. Two types of this newly found potential system also have a phosphatase domain which occurs in other known defense systems [1].

The main goal of this study is to determine the effectiveness, or lack thereof, of this potential defense system against bacteriophages and plasmids. To test our hypothesis, we performed experiments on transformed *E. coli* cells with three types of native potential defense system. Small drop plaque assays on double-layer plates have been evaluated to examine anti-viral activity, although no significant activity has been shown with various phages. However, experiments that included measuring the effectiveness of the growth of bacteria under viral attack in liquid media exhibit promising results.

Previous studies show that some defense systems target invading plasmids, such as the pAgo system against multi-copy plasmids [2] or DdmABC and DdmDE systems [3]. Initial data show that there is a possible effect against some of the invading plasmids. This has been shown by performing plasmid interference assays. Bacteria were challenged with different plasmids to determine if the potential defense system blocks foreign plasmid transformation into the cell. Potential activity against invading DNA was shown in one of the types of the system. Subsequently, experiments with mutant constructs of this system will be performed. Our results are promising but unclear – further experiments will be performed to assess our hypothesis.

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C.43. EVALUATION OF POLYGENIC PROFILE FOR ATHLETIC PERFORMANCE IN LITHUANIAN ELITE ATHLETES

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Genetic endowment or complex gene–gene and gene–environment interactions and other contributors influence the "complex trait" of being an athletic champion [1]. Total genotype score (TGS) reflects additive effect of genotypes on predicting a complex trait such as endurance, sprint or power performance [2]. The Williams & Folland [3] statistical model of TGS provides a quantitative way of combining existing genotype data to predict a complex phenotype of athletic performance. In the present study, we used the six single nucleotide polymorphisms (SNPs: *ACE* [rs4341 I/D], *MB* [rs7293, G/A], *BDNF* [rs6265, G/A], *VEGF* [rs2010963, C/G], *IL6* [rs1800795, G/C], *HIF1A* [rs11549465, C/T]) that we believed to be more important at present (at least in Lithuanians) for explaining individual variations in elite sports performance (applying TGS analysis, with a maximum value of "100" for the theoretically optimal polygenic score). The aim of the study was to investigate a polygenic profile that combined 6 sports-related SNPs among elite athletes and nonathletic healthy control from Lithuanian population.

A total of 59 elite athletes (from different sports: endurance, mixed, sprint/power) and 67 nonathlete controls (healthy unrelated Lithuanian citizens) were genotyped for six SNPs by polymerase chain reaction (PCR) and restriction fragment length polymorphism method (for *ACE* I/D, *MB* G/A and *HIF1A* C/T) and Real-Time PCR (for *BDNF* G/A, *VEGF* C/G and *IL6* G/C). Using the model originally developed by Williams & Folland [3] we determined TGS (from the accumulated combination of the six SNPs) in a group of Lithuanian elite athletes and nonathletic controls. Statistical analysis was performed using Rv3.2.

It was found that the determined TGS of six SNPs differed significantly between the groups. The mean TGS was significantly higher in sprint/power athletes (60.2 ± 11.5) compared to endurance athletes (54.2 ± 9.2; P = 0.04) and controls (53.4 ± 12.7; P = 0.02), whereas it did not differ between the latter two groups (P = 0.73). A total of 7 athletes had a theoretically "optimal" TGS of 66.7 and one athlete – highest TGS of 75 in sprint/power group, whereas 6 athletes with highest TGS value 58.3 in endurance group. Genotypes of the *ACE* (DD), *BDNF* (CT) and *HIF* (CT) genes were noted as promising genetic markers for sprint/power, *MB* (GG), *ACE* (DD), *IL6* (CC) – for mixed athletes, and *MB* (AA) – for endurance.

In conclusion, we have characterized polygenic profile (of six SNPs) for athletic performance in Lithuanian elite athletes. The TGS was higher in elite sprint/power athletes than in the endurance group orcontrols. Only one of the best Lithuanian sprint/power athletes (who are also amongst the best in the world) had the best polygenic profile (TGS 75) for up to six SNPs and none of all athletes or controls had the optimalprofile (TGS 100). We have identified a polygenic profile that allows us, at least partly, to distinguish elitesprint/power athletes from both endurance athletes and nonathletic population.

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C.44. THE ROLE OF POST-TRANSCRIPTIONAL REGULATION AND EMT RELATED GENES EXPRESSION IN PANCREATIC CANCER TISSUES

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Pancreatic cancer is the fourth highest cause of cancer fatalities, with a 5-year survival rate of less than 10% [1]. The ability of tumor cells to resist treatment and invade surrounding tissues is influenced by a reversible developmental process known as epithelial-mesenchymal transition (EMT). EMT is triggered by transcription factors including SNAIL, SLUG, ZEB1/2 and TWIST [2]. ZEB1 is regarded as the most important EMT–TF, as it promotes pancreatic cancer stemness, invasion, and metastasis [3]. HuR, an RNA–binding protein, is a member of the ELAV family and is found in nearly all carcinomas, including pancreatic cancer. This protein is considered to be a key factor in cancer-related gene expression and EMT regulation, although its specific mechanisms in EMT are not yet fully understood [4]. To develop a new treatment method for pancreatic cancer it is necessary to determine the expression changes of EMT-TFs and HuR in pancreatic cancer pathogenesis.

The aim of this study was to evaluate the expression of mRNA molecules responsible for post- transcriptional regulation and EMT in pancreatic cancer tissues.

Postoperative pancreatic cancerous and precancerous tissues (n=30) were frozen at -80°C and used for this research. The RNA from the tissues was isolated using TRIzol as instructed by "Abexa" manufacturer. cDNAs were synthesized using a Reverse Transcription Kit and converted to 1 µg. The gene expressions of the tissues were measured using TaqMan® Fast Universal PCR Master Mix and TaqMan probes. The data was standardized using GAPDH and the relative change in expression was computed using the $2^{-\Delta\Delta Cq}$ (Livak) method. The statistical analysis was conducted using GraphPad Prism and the Wilcoxon test with a p-value of <0.05 considered significant. All data was presented as median +/- interquartile range.

Our results showed that all genes were down-regulated in comparison between the patient's cancerous and precancerous tissue. ZEB1 was found to have a median expression level of 0.2 with a p-valueof <0.001. ZEB2 had a median of 0.3 with a p-value of 0.00. SNAIL1 had a median of 0.1 with a p-value of <0.005, while TWIST had a median expression level of 0.4 with a p-value of <0.008. The post-transcriptional markerHuR and another marker that plays a role in EMT, known as SLUG, did not show any significant statistical difference as their p-value was greater than 0.05. Although the median was lower, 7 out of 30 patients showed higher ZEB1 expression and 5 of same patients has higher HuR expression. This enables us to categorize patients based on their ZEB1 and HuR expression and analyze clinical features within each group separately.

In conclusion, our results suggest that the expression of EMT-TFs has been decreased, indicating EMT has already occurred in cancerous tissues. However, expression of ZEB1 along with HuR were increased in 5 pancreatic cancer patients. This would allow us to conclude that ZEB1 could be regulated by HuR. Grouping patients according to ZEB1 and HuR expression will let us evaluate clinical aspects in one or the other study group for future analysis.

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C.45. GENERATION OF TRANSGENIC ZEBRAFISH WITH INDUCIBLE EXPRESSION OF TRIPLE HA TAGGED TCF21

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Cardiovascular diseases are the leading cause of death worldwide. In recent years zebrafish (*Danio rerio*) have risen to prominence as a model organism in heart disease research, owing to their incredible regenerative capabilities. Unlike their mammalian counterparts, these tiny fish are able to regenerate heart tissue lost after a cardiac injury [1]. Transcription factor 21 (Tcf21) is expressed in the epicardial layer of the adult zebrafish heart. The epicardium has been shown to play a crucial part in heart regeneration, however its exact role is still not clear [2]. Identification of Tcf21 DNA binding sites would allow researchers to better understand the underlying regulatory networks governing this process. Chromatin immunoprecipitation followed by sequencing (ChIP-seq) is a popular technique used to identify regions of protein bound DNA. This powerful method relies on the use of high quality, target protein specific antibodies. In cases where such antibodies are not available, epitope tagged proteins can be used [3].

In this work, we have introduced a triple human influenza hemagglutinin (3×HA) epitope tag coding sequence into the 3' end of the *tcf21* gene coding sequence. The fusion protein was successfully recognized by two commercially available anti-HA antibodies in transfected 293T cell lysates analyzed by Western blotting. Afterwards, we generated a miniTol2 vector containing the *ubb:loxP-mRFP-STOP-loxP-tcf21-3×HA* construct (Fig. 1), which allows us to conditionally control the expression of Tcf21-3×HA. The transgene was integrated into the zebrafish genome by injecting single-cell stage embryos with a mix of the created plasmid and Tol2 transposase mRNA. F0 embryos were raised to adulthood and screened for germline transmission. RFP positive F1 fish were analyzed for the copy number of integrated transgene. qPCR results allowed us to identify fish with single copy integration of the *ubb:loxP-mRFP-STOP-loxP-tcf21-3×HA* cassette. Conditional activation of ubiquitous Tcf21-3×HA expression was tested by injecting Cre recombinase mRNA into single-cell stage *tcf21*$^{v1n18/v1n18}$, *Tg(ubb:loxP-mRFP-STOP-loxP-tcf21-3×HA)*^{v1n14} embryos. The results indicate the presence of a semi-rescue phenotype, confirming that the generated 3xHA tagged Tcf21 is functional.

In conclusion – we have successfully generated a single-copy zebrafish line *Tg(ubb:loxP-mRFP-STOP-loxP-tcf21-3×HA)*^{v1n14} that allows inducible expression of the Tcf21-3xHA protein. In the future we hope to use these fish to uncover Tcf21 targets during zebrafish heart regeneration, which would provide further insight into the molecular mechanisms of this phenomenon.

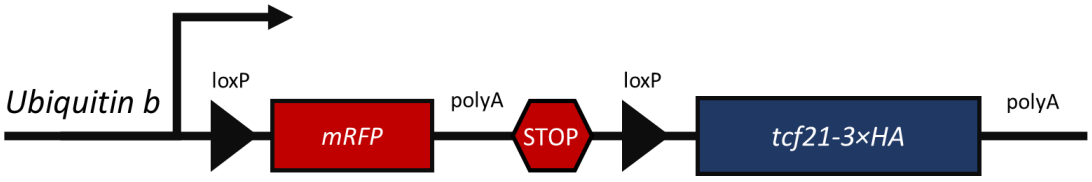

Figure 1. Scheme of the *ubb: loxP-mRFP-STOP-loxP-tcf21-3×HA* transgene.

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C.46. MIR-24-3P, MIR-101-3P AND MIR-132-3P EXPRESSION IN BLOOD SAMPLES OF PATIENTS WITH PARKINSON'S DISEASE

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Parkinson's disease (PD) is the second most common neurodegenerative syndrome and the main clinical symptoms of this disease are bradykinesia and tremor, which are explained by the loss of dopaminergic neurons from the substantia nigra. Most cases of PD are sporadic with no known cause, but it is thought that much of the unknown heritability of the disease lies in understudied variants, including miRNAs. Dysregulation of miRNAs can lead to various pathological phenomena that are important in the development of neurodegenerative diseases such as PD. Studies show that miRNAs are strongly related to the pathogenesis of PD, and their detailed investigation could significantly expand the possibilities of treating Parkinson's disease.

The aim of this study was to determine the expression profile of miR-24-3p, miR-101-3p, miR-132-3p and miR-105-5p molecules in the extracellular vesicles of blood serum of patients with PD. MiRNA expression was evaluated by age, sex, onset of the disease, its duration, severity of symptoms and selected method of treatment for 69 individuals with PD. Exosomal miRNAs were isolated from collected samples of blood serum, transcribed into cDNA and its expression was measured by RT-PCR. Statistical analysis was performed using Student's t test, ANOVA criteria and Pearson correlation coefficient in GraphPad Software Inc. „Prism 9“. The results showed that the expression of the miR-24-3p molecule is the highest and miR- 105-5p is the lowest in the extracellular vesicles of the blood serum between the subjects. It was found that the expression of miR-101-3p molecule decreased as the level of both tremor and bradykinesia increased from moderate to severe, while the expression of miR-24-3p molecule decreased only as the level of bradykinesia increased from moderate to severe. Gender did not influence the expression of the studied miRNA molecules. The expression of the miR-101-3p molecule is higher in PD patients assigned to medical treatment than in patients assigned to surgical treatment. It was also found that only the expression of the miR-24-3p molecule increases statistically significantly with increasing age of PD patients and age at diagnosis. The expression of miR-132-3p molecule after GK treatment was statistically significantly lower than before treatment, and no changes in the expression of miR-24-3p, miR-101-3p and miR-132-p molecules were found before and after deep brain stimulation treatment.

C.47. CHARACTERIZATION OF DRUG-RESISTANT HUMAN COLORECTAL CANCER CELL SUBLINES

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Colorectal cancer is the third most common cancer in the world among both men and women [1]. 5-fluorouracil (5-FU) and oxaliplatin (OxaPt) are the main chemotherapeutics for colorectal cancer, but the development of chemoresistance is almost inevitable. Different mechanisms mediate resistance to 5-FU and OxaPt, including an upregulated drug efflux, inhibition of apoptosis, an increase of cell stemness and an enhanced DNA repair [2].

Cell lines have proved to be a useful *in vitro* model for preclinical cancer research. The most widely used colorectal cancer cell lines are HCT116, SW48, HT-29 and DLD-1. DLD-1 is a colorectal adenocarcinoma line that was established in 1977-1979 by D. L. Dexter [3]. It is an epithelial cell line that harbors *KRAS*, *TP53*, *APC*, *PIK3CA*, *TGFBR2*, *ACVR2A*, *B2M* and *EP300* gene mutations.

One of the ways to study the mechanisms of cancer resistance to chemotherapeutics is by comparing transcriptome and proteome between drug-resistant cancer cell sublines and the parental cell line. In this work, 5-FU and OxaPt-resistant sublines were established by culturing parental human colorectal adenocarcinoma cells DLD-1 in the medium containing 5-FU or OxaPt. The sublines were named DLD/FU and DLD/Oxa, respectively. DLD/FU and DLD/Oxa cells were further characterized by assessing their growth rate, optimal seeding density for *in vitro* experiments and cell sensitivity to chemotherapeutic drugs. The results indicated a decreased proliferation rate of DLD/Oxa, but not DLD/FU cells in comparison to the parental cell line DLD-1. This predetermined a higher optimal seeding density of OxaPt-resistant subline. Cell sensitivity to drug treatment was assayed by two methods, colony forming assay and crystal violet method. Both methods indicated and increased DLD/Oxa cell resistance to OxaPt, but only colony forming assay showed an increased DLD/FU resistance to 5-FU.

In conclusion, we believe that 5-FU and OxaPt-resistant DLD-1 cell line sublines DLD/FU and DLD/Oxa will be a valuable tool for colorectal cancer studies in the future.

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C.48. ENDOMETRIUM-DERIVED STEM CELLS AS A POTENTIAL SOURCE FOR CARDIOMYOGENIC DIFFERENTIATION

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Various types of stem cells have demonstrated the ability to differentiate towards the cardiac lineage, including pluripotent stem cells as well as somatic stem cells, found in many adult tissues. The latter, however, happens to be considered as a more controversial source for cardiomyogenic differentiation due to their limited plasticity compared to pluripotent stem cells [1]. Despite that, adult stem cells are still widely studied for this purpose because of the existing abundant sources and promising features that they show.

Human endometrium is one of the tissues that contain somatic stem cells, where they appear to be vital for the regenerative qualities of this tissue during the pregnancy and menstrual cycle [2]. Endometrium-derived stem cells can also be harvested from a woman's menstrual blood this way providing an easily accessible way for their isolation and cultivation [3]. It has also been previously shown that endometrial stemcells malfunctions could lead to various pathologies, including the tissue expansion outside the uterine cavity,which is also known as endometriosis [2]. In this study, we aim to show that stromal cells isolated from human endometrium, menstrual blood, and endometriosis tissue have the potential to differentiate in a cardiomyogenic direction.

We examined the ability of endometrium-derived stromal cells to differentiate into cardiomyocyte-like cells by inducing them with three different cardiomyogenic differentiation compounds – decitabine, angiotensin II and transforming growth factor-beta 1 (TGF-β1). To prove the successful differentiation, we took three types of endometrium-derived stem cells – endometrial, menstrual, and endometriosis stromal cells and evaluated their cardiomyocyte-like properties using various approaches. Firstly, we noticed that differentiated cells were more similar to cardiomyocytes morphologically and were positive for cardiac- specific cell surface markers (CD36, CD106, CD172a). Secondly, we performed RT-qPCR and confirmed the upregulation of cardiac genes-markers - *MESP1, ISL1, KDR, DES, CNN1, NKX-2.5, MEF2C, ACTN1, cTNI, cTNT*, changes in the expression of genes that encode cardiac ion channels – *CACNA1D, KCND3, SCN5A, HCN2,KCNJ12* and genes related to various signaling pathways important in cardiomyogenesis (MAPK/ERK, Ras/Rho, PI3/Akt, Wnt). Also, differentiated cells highly expressed early cardiac transcription factor, Nkx2.5, as was revealed by Western blot and immunofluorescence analysis. Finally, chromatin immunoprecipitation assay (ChIP) led us to understand the importance of H3K9Ac epigenetic modification during differentiation as it is known to be responsible for the active cardiac chromatin state. These results expand the knowledge oncardiomyogenic differentiation at the gene and protein levels and draw more attention to endometrium- derived stem cells as an alternative source for cardiomyogenic differentiation in future studies.

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C.49. ASSESSEMENT OF CRYOPRESERVED PLACENTA DERIVED MESENCHYMAL STROMAL CELLS CHARACTERISTICS

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Cryopreservation is an approach to preparing and storing biological material for an extended period at low temperatures. As cryopreservation became a general procedure for the long-term storage of cells and tissues for different applications, especially in reproductive medicine, the safety and impact of cryopreservation on cellular properties and functions have to be very carefully investigated [1]. Investigation of regenerative properties of placental tissue revealed its relevance in tissue repair therapy as the placenta promotes epithelialization and angiogenesis and reduces inflammation and scarring [2]. In our study, we compared characteristics of human placental mesenchymal stromal cells isolated from fresh and cryopreserved placenta tissue. Firstly, we analyzed general parameters such as morphology, metabolic and differentiation potential, expression of surface markers, and inflammatory factors. Furthermore, we investigated epigenetic modifications, including miRNA levels, DNA methylation, and histone modifications. Our study's results revealed no significant changes in morphology, proliferative rate, metabolic activity, immunophenotype, and differentiation capacity between cells isolated from fresh and cryopreserved placenta, only slight variations in analyzed mRNA (miR34a-3p, miR29b-3p, miR145-5p) and histone modification (H3K27Ac, H3K9me3) levels. To summarize our findings, cryopreservation of placental tissue is suitable for storage of placenta for future applications, and later placental mesenchymal stromal cells can be successfully isolated from cryopreserved tissue, as it does not significantly affect their molecular characteristics.

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C.50. THE ROLE OF AUTOPHAGY FOR CHEMORESISTANCE AND THE LEVEL OF CELL SURFACE PROTEIN

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Autophagy is a cellular catabolic pathway, which is essential to ensure the quantity and quality of cellular components, mostly by degrading unfolded proteins and dysfunctional organelles. In normal cells, autophagy exerts oncosuppressive functions by degrading potentially oncogenic proteins, playing a part in immunosurveillance, genomic stability preservation, intracellular antibacterial and antiviral defense, maintenance of normal metabolism by removing aggregates, dysfunctional organelles, preventing increased ROS production. However, if a malignant transformation has occurred, autophagy promotes tumor progression and resistance to therapy by providing resistance to hypoxia, starvation, a programmed cell death anoikis, therapy-induced cell death and enhances the survival of senescent cancer cells [1]. More intense autophagy may lead to increased rates of pro-apoptotic protein degradation, thus enabling cancer cells to adapt to cellular stress and evade apoptosis. Moreover, it helps to evade cell death caused by a metabolic stress because more cellular components are recycled and cells adapt to increased metabolic demands [2].

In the context of anticancer treatment, autophagy functions as a cell survival mechanism and the silencing of key autophagy genes emerge as a potent strategy to reduce chemoresistance. Besides its role in cell survival, autophagy impacts molecular pathways regulating the exposition of cell surface molecules: surface delivery, internalization, and degradation [2,3].

We will determine the impact of autophagy in the cellular model of acquired chemoresistance of colorectal cancer cell line DLD-1, induced by 5-fluorouracil or oxaliplatin treatment. In this study, we are aiming to evaluate the role of autophagy on chemoresistance and the level of immunomodulatory cell surface protein PD-L1 in the DLD-1 cell line and its chemoresistant sublines. For this purpose, we will select ATG protein whose silencing using shRNA most efficiently inhibits autophagy. The chosen strategy will be applied to evaluate the impact of autophagy silencing on chemoresistance and the level of cell surface protein PD-L1.

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C.51. LIPID-POLYMER HYBRID NANOPARTICLES OVERCOME DOXORUBICIN TRANSPORTATION CHALLENGES IN TRIPLE- NEGATIVE BREAST CANCER CELLS

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Triple-negative breast cancer, also known as TNBC, is one of the most aggressive forms of breast cancer. Unlike other types of breast cancer, TNBC lacks estrogen, progesterone, and human epidermal growth factor 2 receptors (HER2), making it difficult to treat with traditional hormone therapies or HER2-targeting drugs [1]. However, TNBC cells are over-expressing neuropilin-1 (NRP-1) receptors that could be used as promising targets for treatment [2]. Doxorubicin (DOX) is a commonly used anticancer drug in oncology that faces some transportation challenges in the acidic tumor microenvironment [3]. For this reason, scientists are constantly seeking new DOX transportation approaches like nanocarriers as they may increase DOX selectivity for cancer cells and enhance its therapeutic effect. Moreover, due to insufficient vascularization and low levels of oxygen, some hypoxic tumor areas can contain the most resistant cells for treatment. For this reason, it is important to evaluate the effects of nanosystems in a hypoxic environment [4]. The aim of our study was to synthesize peptide-conjugated doxorubicin (DOX) loaded lipid-polymer hybrid nanoparticles (LPHN) and to evaluate their cell-uptake effect in TNBC cells in normoxia and hypoxia. Prostate cancer cell line (PPC-1), which is characterized by high expression of NRP-1 receptors, was used as a control. Poly(lactic-co-glycolic acid), lecithin and 1,2-distearoyl-*sn-glycero*-3-phosphoethanolamine-*N*- [maleimide (polyethylene glycol)-2000] were used for the synthesis of LPHN. NRP-1 receptors binding peptide FAM-Cys-RPARPAR (P) was conjugated to the surface of nanoparticles. The size, polydispersity index (PdI) and zeta potential of nanoparticles were evaluated using the dynamic light scattering method. DOX entrapment in LPHN was measured by fluorescence spectroscopy. The colloidal stability of LPHN-P-DOX was determined in human plasma, phosphate-buffered saline (PBS) and cell growth medium. For the cell-uptake study, MDA-MB-231 cells were incubated with 5 µg/ml of doxorubicin (DOX), and LPHN-DOX and LPHN-P-DOX at a concentration corresponding to 5 µg/ml of doxorubicin for 1 and 4 hours in normoxia and
hypoxia. For nanoparticle uptake investigation in cells, the confocal microscopy was used.

The LPHN-DOX size was 162 ± 2 nm, PdI was 0.127 ± 0.054, and the zeta potential was -42.63 mV. The LPHN-P-DOX were larger (size was 201.1 ± 5 nm), their PdI was 0.17 ± 0.02, and the zeta potential was -20.4
± 1 mV. The internalization of LPHN-P-DOX was 1.8 times higher in MDA-MB-231 than in PPC-1 cells, which could suggest the rational application of nanoparticles with anchored NRP-1 receptor binding peptide for DOX transport in this type of cancer. After 1 hour in normoxia, DOX and LPHN-DOX internalization in the MDA-MB-231 cell nucleus was significantly ($p < 0.05$) higher compared with LPHN-P-DOX. However, in normoxia conditions after 4 hours, the internalization of LPHN-DOX and LPHN-P-DOX was significantly higher in the MDA-MB-231 cell nucleus compared with free DOX. In hypoxia, LPHN-P-DOX were significantly less internalized in the nucleus after 4 hours compared to DOX and LPHN-DOX.

To conclude, as LPHN-P-DOX have the highest internalization in the nucleus in normoxia and LPHN-DOX have the highest internalization in hypoxia they could be both used for triple-negative breast cancer treatment.

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C.52. CRISPR/CAS9 MEDIATED KNOCKOUT OF MIR-30A POTENTIALLYRELATED TO LUNG CANCER METASTASIS

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Lung cancer is ranked as the most common cause of cancer-related mortality in 2020, and according to the GLOBOCAN database, the number of cases is estimated to increase 64 % by 2040 [1]. This insidious disease, which has a high mortality rate, has prompted research on associated molecular pathways for effective prevention and early diagnosis. Recent studies revealed that microRNAs (miRNAs) can regulate complex regulatory pathways through target genes, whose expression in cancerous tissue is altered compared to healthy tissue. The role of microRNA in cancer has been observed to be complex, acting as either an oncogene or a tumor suppressor and affecting cell differentiation, proliferation, and metastasis. Previous studies in the laboratory have compiled a list of miRNAs potentially associated with LLC1 cell metastasis, which includes miR-30a [2].

The aim of this study was to delete genomic sequence encoding miR-30a that is potentially related to cancer metastasis in *Mus musculus* Lewis lung carcinoma (LLC1) cells. The knockout was performed using the CRISPR/Cas9 genome editing tool. The CRISPR/Cas9 system is composed of the Cas9 nuclease and single guide RNAs (sgRNAs), which were designed to target miR-30a on both sides of the encoding sequence. Oligonucleotides encoding sgRNA sequences with the highest probability of directing the Cas9 protein to target regions were cloned into plasmids containing the CRISPR/Cas9 system. In this study, we transfected CRISPR/Cas9 constructs into LLC1 cells, derived clonal cell lines through the serial dilution method and evaluated DNA editing using PCR. Results demonstrated that genome editing using CRISPR/Cas9 system generated LLC1 cell lines with monoallelic and biallelic deletions of sequence encoding miR-30a. For further research, we will evaluate the expression levels of miR-30a in modified LLC1 cells and assess cell proliferation and migration in monolayer cultures.

To further understand the significance of miR-30a, we performed the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis using the database DIANA-miRPath v.3 [3]. Results showed that targets of miR-30a enrich 37 different signaling pathway categories, including pathways related to cell proliferation, differentiation, migration, metastasis, cell survival, and apoptosis.

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C.53. THERAPEUTIC EFFECTS OF HUMAN MENSTRUAL BLOOD MESENCHYMAL STEM CELL PARACRINE FACTORS ON CARTILAGETISSUE IN VITRO

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Human articular cartilage has a weak ability to restore its lesions leading the tissue to highly progressive diseases such as osteoarthritis (OA) [1]. Cell-based therapies such as mesenchymal stem cells (MSCs) seem promising therapeutic approach for cartilage engineering and regeneration [2]. MSCs can be isolated from almost all human tissues, including menstrual blood (MenSCs) [3]. Although MenSCs isolation has many advantages as compared to classical bone marrow MSCs (BMMSCs), due to low cost and ease of access, their use for cartilage tissue regeneration is still under consideration and research. This study was focused on the potential of MenSC paracrine factors to protect cartilage from degradation under inflammatory conditions in vitro.

The aim of the study is to evaluate the effects of MenSCs paracrine factors on cartilage explants in co-culture conditions, under stimulation with inflammatory cytokines.

Cells were isolated from healthy human menstrual blood (n=3) samples, while cartilage explants were obtained from OA patients after joint replacement surgeries (n=3). The study was performed in compliance with all bioethical requirements. Human cartilage explants were co-cultured in 0.4 µm membrane inserts with MenSCs seeded on the bottom of 6-well plates for 21 days in a chondrogenic medium without growth factors. An inflammatory cytokine Interleukin-1β (IL-1β) was additionally added in order to stimulate cartilage degradation in vitro. Cartilage degradation was evaluated after 1, 3 and 21 days by measuring released glycosaminoglycans in the medium, using Blyscan Sulfated Glycosaminoglycan (GAG) colorimetric assay. Results revealed that levels of released glycosaminoglycans were reduced in co-culture conditions with MenSCs, in both presence or absence of IL-1β, as compared to single explant cultures. Also, collagen type II and metalloproteinases 13 and 3 (MMP13, MMP3) gene expression were analyzed in cartilage explants after7 days of co-culturing with MenSCs under inflammatory conditions (RT-qPCR). It was observed that MenSCs tend to downregulate MMP13 and MMP3 gene expression in chondrocytes after stimulation with IL-1β, as compared to IL-1β stimulated samples, while upregulate collagen type II gene expression. Histological and immunohistochemical cartilage explant sample analysis also showed that the cartilage extracellular matrix was less damaged in co-cultures with MenSCs (Safranin O, Toluidin, collagen type II labeling).

In conclusion, MenSCs may turn out to be a promising population of stem cells for the development of cell-based therapies with the capacity to prevent cartilage tissue from degradation.

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C.54. THE EFFECT OF HYPOXIA ON HUMAN MESENCHYMAL STEM CELL CHONDROGENIC DIFFERENTIATION AS POTENTIAL APPLICATION FOR CARTILAGE TISSUE REGENERATION

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Human articular cartilage is known to possess a weak ability of self-regeneration after being damaged, making it highly susceptible to progressive degeneration and diseases such as osteoarthritis. Although recent in vitro research into cartilage regeneration is promising, no effective treatments for cartilage lesions currently exist. Cell based therapies using mesenchymal stem cells (MSCs) have shown potential in cartilage repair due to their effective chondrogenic differentiation and innate ability to stimulate tissue regeneration. However, despite their promising use in in vitro research, practical applications in vivo are not efficient enough due to obstacles in transplantation and immunogenicity of commonly used tissue scaffolds [1].

Modern methods of MSC growth for tissue regeneration include cell sheet technology, a method developed in Japan by Okano et al. [2], which uses culture dishes coated in a temperature-responsive polymer poly(N-isopropylacrylamide). This polymer allows the temperature-controlled detachment of cell layers as a structure with a fully intact extracellular matrix (ECM), surface proteins and cell junctions. Transplanting these intact sheets directly onto degraded tissue has been shown to result in improved integration with host tissue and promoted cell signalling during regeneration, compared to conventional cell therapy treatments [3,4]. However, a significant aspect of chondrocyte well-being in the cartilage is the naturally occurring hypoxic environment, due to the avascular nature of cartilage. In hypoxic conditions, hypoxia-inducible factors (HIFs) are transcription factors, known to promote cell survival in the absence of oxygen, and in this case, cell differentiation and gene transcription related to the upkeep, formation of cartilage tissue and its surrounding ECM.

The aim of this study is to evaluate chondrogenic potential of human bone marrow derived mesenchymal stem cells (HBMMSCs) and chondrocytes (used as a positive control) grown in cell sheets under hypoxic conditions using the HIF-1α inhibitor LW6. Cell proliferation, differentiation capacity (RT-qPCR), mitochondrial oxygen metabolism (metabolism analyser) and formation of ECM (ELISA) in normoxic and hypoxic environments were evaluated.

Results suggest that hypoxic conditions increased the proliferation rate of HBMMSCs and chondrocytes after 7 days in culture but decreased mitochondrial respiration after 3 days in culture. LW6 reduced mitochondrial respiration in both cell types under hypoxic conditions. Chondrogenic differentiation in cell sheets was similar to the classic 3D pellet model, according to histological staining for cartilage ECM proteins with toluidine blue and Safranin O. Simultaneously, hypoxic conditions increased chondrogenic gene expression in both HBMMSC and chondrocyte cell sheets.

In conclusion, this study demonstrates the importance of environmental oxygen for in vitro studies in simulating natural in vivo conditions, while exploring cell sheet technology as a promising application for cartilage tissue repair in vitro under hypoxic conditions.

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C.55. SKIN CELLS WITH PSORIATIC PHENOTYPE HAVE ALTERED METABOLIC RESPONSE

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Mitochondria are critical players in immune-metabolic responses, and recent research has suggested that they have a crucial function in the pathophysiology of psoriasis [1]. Although it is considered a T-cell-mediated disorder, it has been shown that keratinocytes and fibroblasts can act as an executors in the early psoriasis stages [2,3]. Moreover, the inflammatory state is related to metabolic reprogramming of the cells characterized by decreased mitochondrial and increased glycolytic activity [4]. However, the energetic metabolism changes in keratinocytes and fibroblasts during psoriasis development are not studied.

Thus, our research aimed to investigate mitochondrial and glycolytic activity in human HaCaT keratinocytes and dermal fibroblasts with psoriasis-like phenotype induced by cytokines IL-17, IL-22, and TNF-α. The model was characterized by gene expression (qRT-PCR) and secreted protein analysis (Luminex xMAP), and real-time measurement of cellular bioenergetic activity was assessed using XFp (Seahorse) Extracellular Flux Analyzer.

After 24-hour treatment, cells exhibited an increased expression of the psoriasis-related gene PI3. Its product SKALP/Elafin secretion was also increased in both types of cells. Moreover, the release of IL-6, IFN-β, IL-1α, IL-18, and CCL5 was augmented in supernatants from both cell types after treatment, except IFN-γ and IL-1β secretion only increased by keratinocytes. Investigation of metabolic activity by the Seahorse platform revealed a significant decrease in maximal respiratory capacity in psoriatic keratinocytes, indicating electron transport chain disturbance. Moreover, glycolysis activity, assessed as extracellular acidification rate, decreased after cytokine treatment in keratinocytes. Interestingly, there was no effect on fibroblasts' oxygen consumption and extracellular acidification rates.

Although both fibroblasts and keratinocytes under psoriatic conditions exhibited a plentitude of released pro-inflammatory proteins, only keratinocytes demonstrated altered mitochondrial functions and glycolysis efficiency. Therefore, our results show a distinct metabolic reaction to psoriatic-like inflammation. Further investigation of mitochondrial changes under inflammatory conditions can shed light on the pathogenesis of psoriasis and reveal new mitochondria and glycolysis-related pharmacological targets.

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C.56. TP53 GENE KNOCKOUT IN TRIPLE-NEGATIVE BREAST CANCER CELLS MDA-MB-231

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Gene TP53 and its product protein p53 are well known for their role in anticancer drug treatment. Protein p53 is activated in case of cellular stress, which includes DNA damage, oxidative stress or anaerobicconditions. Tumor suppressor protein p53 by itself is a transcription factor that is responsible for the transcription of genes that regulate cell death and survival. In particular, p53 is involved in the regulation of processes related to chemotherapy drug sensitivity such as DNA reparation, cell cycle arrest, treatment- induced senescence or programmed cell death [1]. Any changes in the regulation of these cellular processesusually lead to resistance to chemotherapy drug treatment which remains a major problem in cancer therapyand limits treatment options leading to worse treatment outcomes.

We investigate chemotherapy resistance to third-generation platinum-based chemotherapy compoundoxaliplatin, which is known for being less mutagenic than other platinum-based drugs such as cisplatin orcarboplatin [2]. Oxaliplatin shows antitumor activity in ovarian cancer, non-small-cell lung cancer, and colon cancer. However, therapeutic options for metastatic breast cancer and one of the most aggressive breastcancer types - triple-negative breast cancer (TNBC) - are yet to be established for first-line and/or second- line treatment [3].

As an object of study resistance to oxaliplatin in breast cancer cells, we have chosen triple-negative breast cancer cells MDA–MB–231. This cell line bears TP53 gene mutation which changes arginine to lysine in the 280 amino acid position of the DNA binding domain thus potentially disabling interaction with DNA andp53 function as a transcription factor. We found out that MDA–MB–231 cells are resistant to oxaliplatin– mediated cell cycle arrest and oxaliplatin-induced cell death altogether. To elucidate the significance of p53 mutational status for anticancer drug resistance, we genetically manipulated MDA–MB–231 cells using CRISPR/Cas9 system to knock out the TP53 gene. Next, we used inducible expression vector that allowed usto switch the mutated gene version into the wild-type upon doxycycline treatment and examine its contribution to the cell cycle arrest and apoptosis after oxaliplatin treatment. Data will be presented in theposter.

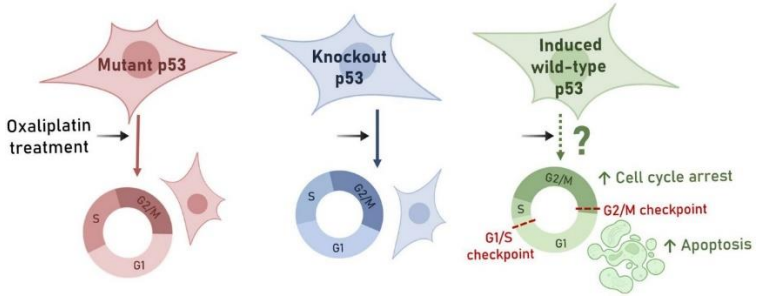

Figure 1. The concept of TP53-R280L switching into inducible wild-type TP53 andstudy its role to oxaliplatin resistance

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Oral presentations

DETERMINANTS OF DNA REPAIR IN CRISPR NUCLEASE-MEDIATED GENE EDITING

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CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) nucleases transformed our approaches to medicine, diagnostics, agriculture, and research. By programming a CRISPR nuclease with a guide RNA (gRNA), we can direct it to cut a gene with the matching DNA sequence - its target [1]. When CRISPR nucleases induce double stranded breaks (DSBs) or site-specific single-stranded breaks (SSBs), endogenous cellular repair mechanisms resolve these breaks [2]. These mechanisms include non-homologous end-joining repair (NHEJ) and homology directed repair (HDR) [3]. Cellular enzymes repair these cuts, introducing changes to a gene's sequence that can prevent or alter the expression and activity of its product.

CRISPR nucleases tolerate small differences between the gRNA and DNA target sequences. These contribute to 'off-target' activity, where the nuclease cuts additional genes, causing unexpected (and sometimes dangerous) changes. These small differences also affect how the nuclease cuts DNA, producing DNA ends with different shapes and sequences. But how does cellular repair machinery interpret these DNA ends during repair? The study of the 'off-target' activity of CRISPR nucleases via programmed modifications to the gRNA or target DNA sequences could reveal which repair mechanisms different off-targets induce.

Our research focuses on investigating if and how gRNA-DNA sequence parity alters the repair outcomes of editing with CRISPR nucleases. This relies on our lab's expertise in bioinformatics, biochemistry and cell biology. We have elected candidate gRNA and off-target DNA pairs for the CRISPR nuclease Cas12a by searching its cleavage specificity profile. We have validated the *in vitro* performance of each pair and are linking this with their cellular repair outcomes. We anticipate identifying reproducible gRNA manipulations that direct specific gene editing outcomes, making future gene editing safer and more reliable.

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TICK BORNE ENCEPHALITIS (TBE) VIRUS PREVALENCE IN WILD RODENTS CAPTURED IN TBE FOCI IN LITHUANIA

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Tick-borne encephalitis (TBE) is one of the most important tick-borne diseases in Europe and the prevalence rate of human infection in Lithuania is among the highest in Europe for nearly 10 years [1,2]. Although the whole country is considered endemic, TBE is a focal infection [3]. In the majority of TBE foci detection studies ticks were primarily analyzed for TBE RNA presence. However, TBE viral RNA prevalence rate in endemic sites collected ticks is low. In addition, a large number of ticks has to be analyzed and tick collecting is time and effort consuming work with a risk of tick bite and tick-borne infection [4].

Small wild rodents are considered to be one of the most important amplifying reservoir virus hosts, therefore they may be suitable for TBE foci detection studies. To investigate wild rodent suitability for TBE foci confirmation, we have trapped small wild rodents (n = 139) in various locations (n = 19) in Lithuania where human TBE cases has been previously confirmed. In addition, to increase the chances of viral RNA detection, we have inoculated murine neuroblastoma (Neuro-2a) cells with each rodent's brain and internal organ mix suspensions. TBE viral RNA was detected in 74.8% (CI 95% 66.7-81.1) of the rodent's brain and/or internal organ mix suspensions from all suspected foci tested, and the prevalence rate increased significantly ($p < 0.0001$) after sample cultivation in Neuro-2a cell culture, reaching 96.4% (CI 95% 91.8-98). Furthermore, average viral load was measured in rodent (n = 30) suspension and respective isolate samples and viral load increased significantly ($p < 0.05$) after isolation in neuroblastoma cells (5.71 $\log_{10}$ copies/ml and 6.35 $\log_{10}$ copies/ml, respectively). Moreover, a strong correlation ($r = 0.8859$; $p = 0.0346$) was found between the TBE viral RNA prevalence rate in cell culture isolates of rodent sample suspensions, considered negative prior cultivation in neuroblastoma cells and the average monthly air temperature. In addition, vital virus was detected in 5 fetal samples after isolation in cell culture.

The present study shows that the majority of wild rodents trapped in TBE foci in Lithuania carry TBE virus and wild rodents can be suitable sentinels for foci confirmation, and possibly for foci detection. Moreover, the study demonstrates that rodent sample cultivation in murine neuroblastoma cell culture is a highly efficient method to increase viral load to detectable quantities.

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ASSESSING THE DIAGNOSTIC UTILITY OF COFILIN 1 AND COFILIN 2 INPATIENTS WITH ACUTE CORONARY SYNDROME

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Introduction: ACS is a form of coronary heart disease, which causes one-third of total mortality for people over 35 years. It is caused by decreased blood flow to a portion of the heart muscle that often occurs after plaque rupture and thrombus formation, leading to ischemia and infarction of that part of the heart. Cofilins are the protein involved in the actin dynamics by severing and dissociating actin monomers from filaments toincrease actin filament turnover.

Purpose: 1. To measure and compare serum cofilin 1 and cofilin 2 levels between patients with acute coronary syndrome and healthy participants. 2. To determine TIMI (Thrombolysis for Myocardial Infarction) and GRACE (Global Registry of Acute Coronary Events) risk scores in patients with acute coronary syndrome. 3. To study the associations between the serum cofilin 1 and cofilin 2 levels, clinical and biochemical parametersand risk scores in patients with acute coronary syndrome.

Methods: Forty-five patients with ACS as cases and forty-five healthy controls were enrolled in the study.Physical details and personal and family histories were recorded from all study participants. The blood sampleswere collected to analyse routine biochemistry investigations by random access analysers and serum CFL1, CFL2 and heart-type fatty acid-binding protein (HFABP) levels by enzyme-linked immunosorbent assay.

Analysis: Cases had low serum CFL11{0.574 (0.46-0.73)} Vs {0.577 (0.41- 0.88)} ng/mL, P = 0.939) which was not statistically significant. However, serum CFL2 {2.93 (1.95-3.32} Vs 4.35 (3.4-6.61)} pg /mL, P = < 0.001) levels were significantly low in cases compared to controls. A positive correlation of serum CFL2 levels was observed with history of DM, BMI, and serum HDL-C levels and a negative correlation with weight, height, systolic and diastolic blood pressure, pulse rate, serum CK-Total, CK-MB, troponin-T, total cholesterol, triglycerides and LDL-C levels. TIMI and GRACE risk scores for ACS patients showed a positive correlation with serum troponin-T levels and H-FABP levels in cases.

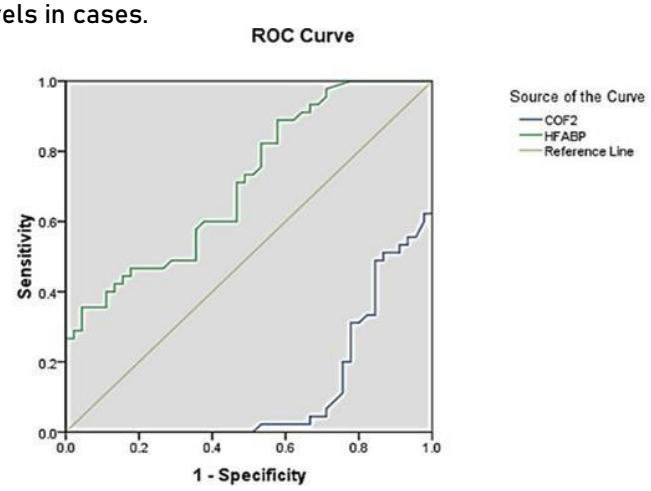

Figure 1. Reciever operating curves of serum cofilin (pg/mL) and HFABP (ng/mL) for prediction of ACS.

Conclusion: Serum CFL1 and CFL2 levels were not significant enough to be used for the diagnosis and prognosis in patients with ACS. can be tried for a more specific method and approach.

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