Public Health and Toxicology

13th INTERNATIONAL CONFERENCE

BIOMATERIALS AND NANOBIOMATERIALS:

Recent Advances Safety-Toxicology and Ecology Issues

VIRTUAL PRE-CONFERENCE

ABSTRACT BOOK

Aims and Scope

Public Health and Toxicology (ISSN: 2732-8929) is a double-blind peer-reviewed open access journal. Its primary focus is to assess the interaction between public health and toxicology, including how population data on disease incidence can suggest possible etiologies and how genetic and epigenetic factors can influence risk for adverse health effects. The journal also focuses on the application of how these concepts provide evidence relevant to the understanding and prevention of morbidity and mortality resulting from environmental exposures to toxic substances.

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Development of micellar approach for inhibition analysis of galactonolactone oxidase from *Trypanosoma cruzi*

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Trypanosoma cruzi is a parasite causing Chagas disease, which is nowadays treated with only two drugs: benznidazole and nifurtimox. However, these drugs are effective only in the early or acute infection phases and have side effects. It should be added that about 7 million people all over the world suffer from the disease and thus the development of new drugs against Chagas disease is a significant task. The action of new drugs may be based on their inhibitory effect on the biosynthesis of compounds vital for the parasite, for example vitamin C (antioxidant). Galactonolactone oxidase from *Trypanosoma cruzi* (TcGAL) is an enzyme which catalyzes the final stage of the biosynthesis of vitamin C, and so the inhibition of TcGAL may be a suitable way to suppress the growth of the parasite. However, the development of a technique for the analysis of activity and, hence, the search for an effective inhibitor of the enzyme has been impossible until now due to its membranotropic nature and, accordingly, the tendency to aggregate in aqueous solutions. Thus, the expression of the recombinant enzyme TcGAL from *Trypanosoma cruzi* in *E. coli* cells leads to the formation of an aggregated and inactive protein, the so-called inclusion bodies. Attempts to restore the catalytic function of TcGAL using various methods traditionally used for enzyme refolding have not been successful.

In this project, an innovative technology has been developed for obtaining genetically engineered TcGAL in a soluble and catalytically active form using a system of reverse micelles of surfactants.

The developed approach makes it possible to create a high-throughput screening system for the search for a selective TcGAL inhibitor, on the basis of which it is possible to conduct a targeted search for new drugs against diseases caused by trypanosomal infection.

We have developed a micellar approach for measuring TcGAL activity and successfully applied it for inhibition analysis of TcGAL. The main point of the approach is the use of a reverse micellar system as a membrane model. Membrane has many important functions (transition of ions, proteins, fusion and compartmentalization in membranes and so on) and micellar non-bilayer structures were found in mitochondria so the used model is reliable. The use of bis(2-ethylhexyl) sulfosuccinate (AOT) reverse micelles system allowed us to learn the influence of a number of compounds on TcGAL activity including lycorine (which is known to suppress the growth of *T. cruzi*), chalcones (they have lycorine-like structure) and derivates of allylbenzene and apiole (some of them also suppresses *T. cruzi* growth).

Inhibition analysis of TcGAL showed that the inhibitory effect of apiole, dillapiole, lycorine and chalcones is probably associated with a common structural fragment, benzodioxol. The effect of eugenol, estragole and tetramethoxyallylbenzene may be explained by a common structural fragment namely allylbenzene which is an inhibitor itself. In order to enhance the inhibitory effect, two inhibitors (tetramethoxyallylbenzene and dillapiole) were conjugated with triphenylphosphonium (TPP) cations. TPP provides the selective delivery of the desired compounds into the mitochondria driven by the inner negative transmembrane potential according to literature data, and so we observed a similar effect in the case of micellar reverse system too. The conjugates of tetramethoxyallylbenzene and dillapiole with TPP showed 100% inhibition at 100–200 μM, which was not obtained using the compounds without TTP fragment.

Thus, the approach to inhibition analysis of TcGAL has been developed using AOT reverse micelles system as a mitochondrial membrane model and a number of inhibitors of TcGAL were characterized. The dependence of the inhibition effect on the structure of the inhibitor was investigated and allylbenzene and benzodioxol were found to be necessary structural fragments for TcGAL inhibition.

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The effect of antifibrotic and antibacterial drugs on the physicochemical properties of liposomes: A spectral study

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Introduction

The task of biomedical chemistry is to fight against the new coronavirus infection and overcome severe complications, including pulmonary fibrosis and bacterial infections. Liposomes are a promising inhalation delivery system with a high affinity for lung tissues. However, the inclusion of active molecules in liposomes can significantly affect the physicochemical properties of the container, which must be taken into account when designing delivery systems. The aim of this work is to identify the main binding sites of moxifloxacin, levofloxacin, pirfenidone, nintendanib, and rifampicin with the lipid bilayer and to detect the effect of these drugs on the phase transition of liposomes of different composition.

Methods

Drugs were obtained from Sigma Aldrich; DPPC, cardiolipin, cholesterol - Avanti Polar Lipids. Sodium phosphate buffer tablets for solution preparation Pan-Eco.

Liposomes were prepared by thin layer hydration technique with lipid composition: DPPC, DPPC:CL 80:20, and DPPC:Cholesterol 90:10. The same procedure was used to prepare liposomal preparations, but a thin lipid film was dispersed with a buffer solution containing the active molecule. The resulting liposomes were purified from the non-incorporated drug by dialysis.

Spectroscopy studies were conducted with UV-Vis spectrometer AmerSharm UltraSpec2100pro and ATR-FTIR Bruker Tensor 27.

Results

Depending on the nature of the active molecule, it was possible to achieve an efficiency of passive loading into liposomes from 25 to 80%. The loading proceeded best for liposomes containing cardiolipin, while the addition of cholesterol reduced this parameter. It has been established that the lipophilicity of the drug and the presence of ionogenic groups in its structure play a decisive role in the loading efficiency. For fluoroquinolones, the key binding mechanism is electrostatic, while for antifibrotics and rifampicin, stacking interactions with cholesterol also play an important role, as evidenced by characteristic spectral changes in the infrared spectra.

Conclusions

The main binding sites of drugs are usually carbonyl and phosphate groups of lipids, and stacking interactions are also important. Depending on the lipophilicity of the drug, they can either accelerate the phase transition of liposomes (rifampicin, pirfenidone) or slow it down (levofloxacin).

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Development of molecular containers based on polyethylenimines, cyclodextrins, spermine and mannose for targeted delivery to alveolar macrophages of fluoroquinolone-based formulations combined with essential oils

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The aim of the work is to develop and characterize molecular systems based on cyclodextrins (CDs), polyethylenimines (PEIs) and mannose – carriers for targeted delivery to alveolar macrophages of drugs for the treatment of a wide range of diseases, including post COVID-19 pneumonia and tuberculosis. Biopharmaceutical properties of drugs loaded into molecular containers can be improved: increased solubility and bioavailability, slowing release in tissues, protection from destruction, concentration of drugs in the target organ and tissues, due to the complexation of fluoroquinolones with cyclodextrin derivatives, as well as their copolymers.

Four groups of conjugates with different molecular architecture and physicochemical parameters were synthesized: 1) mesh conjugates of cyclodextrins with a large hydrodynamic size 350–700 nm and a zeta potential of 3–5 mV; 2) linear polyethyleneimines of 35 kDa with grafted CDs – 55 nm, +5–10 mV; 3) star-shaped cyclodextrin conjugate with grafted mannose clusters on spermines – 150 nm, -6 mV; and 4) star-shaped cyclodextrin conjugate with grafted branched light (2–10 kDa) chains of PEI – 70 and 370 nm, +4 mV. The variety of conjugate structures will make it possible in the future to select the best drug carriers for specific therapy tasks.

The 'targeting' of macrophage mannose receptors was confirmed by the high affinity binding of ligands with the model lectin concanavalin A due to the presence of mannose clusters on spermines or PEI in conjugates: by changes in the intensity and position of peaks of Amide I and II in the FTIR spectra, by quenching and polarization of tryptophan fluorescence in the protein $K_D \approx 10^{-6}$–10^{-7} M, and the affinity of the natural trimannoside ligand (10^{-5} M).

The inclusion of antibacterial levofloxacin and moxifloxacin molecules in the CD cavity and interaction with the conjugate polymer chains leads to a decrease in the intensity of 'aromatic' peaks in the infrared spectra and a shift in the maxima/minima of ellipticity in the near-UV region in the circular dichroism spectra. Conjugates with high affinity bind from 10–15 to 70 drug molecules with constants of 10^3–10^5 M. To reduce the dose of the toxic main component, double complexes of inclusion of levofloxacin (Lev) and the synergist eugenol were obtained, which showed a significant increase antibacterial activity: the minimum inhibitory growth of *E. coli* (NCIB 12210) levofloxacin concentration was reduced from 0.1–0.15 to 0.02–0.03 μg/mL due to the adjuvant effect.

The adjuvant apiol significantly enhances and accelerates the antibacterial effect of Lev in the star-shaped conjugate HPCD-spermine-Man in experiments on liquid media, therefore, the MIC of the antibiotic can potentially be reduced. Levofloxacin in conjugates acts more effectively than the free form. Moreover, the effect of prolonged action is observed (the decline of curves against the plateau in the case of Lev).

The advantage of the dosage forms in the composition of conjugates is a prolonged release from 1 hour, but more than 6–12 h (up to several days), depending on the architecture of the conjugate – to reduce the dosage of the toxic agent, reduce the frequency of administration, and increase the friendliness of therapy.

The conducted research has demonstrated a significant therapeutic potential of targeted delivery systems of combined drugs for the treatment of respiratory diseases.

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Preparation methods and biocatalytic properties of covalent conjugates of rhodospirillum rubrum l-asparaginase

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Bacterial L-asparaginases have been successfully used for more than 50 years in the treatment of various types of leukemia, in particular for acute lymphoblastic leukemia (ALL). However, their use is associated with a number of side effects such as allergic reactions, blood clotting, and liver and nervous system disorders. Various methods of covalent modification of enzymes are used to improve the pharmaceutical characteristics of L-asparaginase preparations. For example, PEGylated *E. coli* L-asparaginase has a longer circulation time in the bloodstream and less immunogenicity. In our laboratory, the effect of different copolymers of chitosan, polyethylene glycol (PEG) and polyethyleneimine (PEI) on the properties of L-asparaginases from various bacterial sources is being studied. Special attention was paid to L-asparaginase of *Rhodospirillum rubrum* (RrA), which has a small amino acid sequence and high antitumor activity compared with commercial preparations.

We studied previously obtained covalent conjugates of L-asparaginase. The conjugates were obtained by modification of amino- and carboxyl groups of the protein. The first method was used to obtain RrA conjugates with chitosan-PEG (30 PEG chains) and chitosan-glycol (72 kDa). Carboxyl groups were modified using PEI (2 kDa), PEI-PEG (branched, 5 kDa), and polyamines (spermine, spermidine). Purification was performed on Amicon centrifugal filters. RrA preparations were characterized by infrared (IR) and CD spectrometry. The IR spectra of the native enzyme and the conjugates contain characteristic protein peaks of amide I (1600–1700 cm^{-1}) and amide II (1500–1600 cm^{-1}). The spectra of RrA-chitosan-PEG and RrA-chitosan-glycol conjugates contain a peak at 1080 cm^{-1} corresponding to vibrations of C-O-C bonds in the PEG chains and C-O-C, C-N and C-C bonds in the pyranose cycle of chitosan. The presence of these peaks confirms the modification of the protein by the mentioned copolymers. For the RrA-PEI-PEG conjugate one can also note a peak at 1100 cm^{-1} related to vibrations of the PEG chains and C-N bonds of PEI. The peak at 1465 cm^{-1} is responsible for vibrations of the N-H bonds as well as the scissor CH_2 bonds. In the circular dichroism spectra of both the native enzyme and its modified forms, there is a distinct peak at 207–210 nm corresponding to alpha helices. To compare the specific catalytic activity of the conjugates, enzyme concentrations were determined from the ellipticity calibration dependence at wavelengths of 207 and 220 nm. The hydrolysis activity of 20 mM

L-asparagine native RrA and its conjugates was determined by CD spectroscopy. The greatest increase in activity compared with the native enzyme (46 IU/mg) was observed for the RrA conjugate with chitosan-PEG (64 IU/mg). Cytotoxic activity was determined for the native enzyme and conjugates on cells of chronic myeloid leukemia (K-562) and T-cell acute lymphoblastic leukemia (Jurkat). RrA-chitosan-PEG and RrA-chitosan-glycol at a concentration of 10 IU/mL approximately equally reduced the survival of K-562 cells to 10–12% of the control, while the native enzyme only to 56%. On Jurkat cells, RrA-chitosan-glycol was more toxic at concentrations of 1–10 IU/mL than RrA-chitosan-PEG and native enzyme. Thus, the cell type and copolymer structure influence the toxicity of the L-asparaginase RrA conjugate. In addition, the cytotoxicity of the drug depends not only on its catalytic activity but also on its zeta potential.

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Fluorescence polarization immunoassay for microcystin-lr

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Phycotoxins produced by several algae, microalgae, and cyanobacteria, are highly toxic contaminants of environmental water and sea foods. They cause necessity of efficient techniques for their simple and rapid detection. In the study, fluorescence polarization immunoassay (FPIA) was developed for microcystin-LR (MC-LR), one of the most widespread phycotoxins. Several fluorescent derivatives of MC-LR were synthesized and compared for this purpose. The reached detection limit for MC-LR was 7.5 ng/mL, and the working range of determined concentrations was 50–500 ng/mL. The developed FPIA provides rapid (10–30 min, including sample preparation) and sensitive testing. The developed portable detectors of fluorescence polarization allow testing environmental objects directly at the sampling points and making decisions about their contamination and safety rapidly.

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Development of fluorescence polarization immunoassay to detect dibutylphthalate (dbp) and its active metabolite monobutylphthalate (mbp) in environmental samples

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Dibutylphthalate (DBP) is widely used in cosmetics, printing inks, and pharmaceutical coatings as a plasticizer to impart softness, strength, flexibility, and elasticity to plastic products. DBP is one of the most widely studied phthalates and it has been found that when ingested, DBP and its active metabolite monobutylphthalate (MBP) can cause harm to the hormonal system, liver and lungs. Therefore, the development of a simple, fast and cost-effective method for the immunoassay of phthalates is urgently needed. Promising methods for the determination of phthalates for quality control of food products and environmental objects are immune methods. The method of fluorescence polarization immunoassay (FPIA) makes it possible to determine low molecular weight analytes in a homogeneous medium without separation with high specificity and sensitivity, has greater accuracy, stability of reagents, as well as the speed and simplicity of analysis. The aim of this work is to obtain immunoreagents and develop a rapid method for the determination of DBP and its metabolite MBP in water, using the FPIA method. For the development of FPIA phthalates, we used rabbit polyclonal antibodies obtained against the conjugate of BSA with an amino derivative of DBP by the diazo method (Ab-DBP) or against the conjugate of cationized BSA with MBP by the carbodiimide method (Ab-MBP). Fluorescein-labeled tracers were synthesized from the amino derivative DBP or MBP, activated by NHS at the carboxyl group, with various fluorescein-containing labels (FITC, DTAF, EDF, AMF, GAF). The resulting immunoreagents: antibodies and tracers were studied for binding kinetics. It was shown that antibodies to MBP (Ab-MBP) after 3 and 4 cycles of immunization bind well to the MBP-GAF tracer, but the time to reach equilibrium is about 90 min. Calibration dependences were constructed, FPIA for MBP determination was optimized, and the analytical characteristics of the method were determined (detection limit 5 μg/mL, linear range 10–100 μg/mL). Cross-reactivity studies have shown that Ab-MBPs are highly specific to MBPs only. When developing FPIA to determine DBP, the time to establish equilibrium in the system was only 15 min, the matched pair of Ab-DBP immunoreagents and the DBP-FITC tracer showed a limit of detection on the calibration curve of 3 μg/mL and a linear range of 4–13 μg/mL. Total time for FPIA performance is 1 min. Using the developed FPIA method, water samples taken from the Moscow River and from a water reservoir in the Moscow region were tested for the possibility of determining phthalates.

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Magnetic nanoparticles in combination with a non-heating low-frequency alternating magnetic field can increase the lysis of *Escherichia coli* cells under the action of bacteriophage lys394 endolysin

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Magnetic nanoparticles (MNPs) are increasingly attracting the attention of scientists. Over the past few years, magnetic nanoparticles have become known as an important class

of nanomaterials finding their application in many fields of science. The greatest interest of scientists is attracted by the use of MNPs in the field of biomedicine: hyperthermia, targeted drug delivery, tissue engineering, theranostics, magnetic resonance imaging, stimulation of cell growth, etc. Of particular interest is the magnetic properties of nanoparticles, which make it possible to manipulate a biological object using an external magnetic field.

In this work, we developed a method for the synthesis of dopamine-functionalized rod-shaped MNPs based on iron oxide. The synthesized nanoparticles were used in combination with a low-frequency alternating magnetic field to study the lysis of *E. coli* cells under the action of Lys 394, for which the outer membrane of the cell wall is normally impermeable. We studied the lysis of the cell suspension with particles in the presence and absence of an external field under the action of the enzyme (Lys394 endolysin). It was shown that MNPs in the absence of a magnetic field do not affect the cell lysis. In the work, particles with a length of 40 to 70 nm and a width of 20 to 40 nm were used. For each particle size, different magnetic field parameters were tested. The highest efficiency of the cell lysis was achieved with particles 56±13 nm long and 13±3 nm wide, and with a magnetic field intensity of 68.5 mT. According to the experimental data, cell lysis under the action of endolysin in the presence of MNPs and a low-frequency alternating magnetic field increases almost 2-fold. The possibility of manipulating a biological membrane with MNPs under the action of a magnetic field was additionally demonstrated by two independent experiments: the use of a hydrophobic Nile red (NR) dye and an assessment of the yield of the periplasmic protein β-lactamase. In one of them, disordering leads to the release of more than 80% of the periplasmic enzyme β-lactamase, and in the other, to a significant change in the fluorescence of the hydrophobic dye NR.

Thus, we have shown that it is possible in principle to remotely enhance the efficiency of the enzyme in the lysis of the bacterial cell wall as a result of the disordering of its structure under the action of rod-like MNPs driven into oscillatory motion by an external non-heating low frequency magnetic field. The study demonstrates the prospects for using an external low-frequency magnetic field to enhance the action of bacteriophage antibacterial endolysins against gram-negative pathogens.

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Tunable synthetic polymeric scavengers for toxic xenobiotics

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Toxins present danger to human health. This work is focusing on ochratoxin A (OTA). It exhibits mutagenic, carcinogenic, neurotoxic properties which are harmful to human health. OTA is produced by some species of *Aspergillus* and *Penicillium* and exposure can occur by consumption of contaminated food products such as grain, pork, coffee and other food products.

The selective binding of toxic compounds finds its potential application both in sensor construction for analytical purposes and in treating patients recently exposed to harmful substances. Chemically designed macromolecules may demonstrate enhanced affinity for target compounds. Main goal of this work was to synthesize polymeric scavenger, that would be able to bind OTA. A polymer bearing azide groups was synthesized and was further introduced into Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) with different alkynes. The ability of alkyne-modified polymers to bind OTA was estimated using plasmon resonance spectroscopy technique. OTA was immobilized on the gold surface of the chip, and equilibrium dissociation constant (KD) was measured.

For the proof of the concept, the side groups of the block copolymer PEG_{114}-$pGlu_{10}$ were modified using 3-azido-1-propylamine via EDC/NHS reaction. The product was further introduced in CuAAC with first alkyne 1-ethyl-4-ethynylbenzene. The resulted polymer exhibits moderate affinity to OTA (K_D = 0.5 ± 0.1 mM). HPMA-based polymer was synthesized for future use as parent compound. Six different methods of N-(2-hydroxypropyl)methacrylamide (HPMA) synthesis were used to obtain the higher yield [76%, 1H-NMR (300MHz, D_2O) δ (ppm): 1.05 (d, 3H, CH-CH_3), 1.79 (s, 3H, CH_3-C), 3.10 (m, 1H, CH_2-NH), 3.85 (m, 2H, CH-CH_3), 5.31 (s, 1H, CH_2=C), and 5.56 (s, 1H, CH_2=C)].

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Nanoparticles based on polyferylic and polygentisic acids as new carriers of anticancer drugs

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Nanoparticles (NPs) as a tool of delivering drugs to tumor cells are considered as one of the most important options for enhancing the effectiveness of tumor treatment. Recently, the successful use of natural lignin polymers for the formation of biodegradable NPs has been shown. But the lack of certainty of the chemical structure and the limited possibility of making modifications to the chemical composition limits their use in pharmacology. We proposed to synthesize *de novo* polymers from phenolic monomers to be able to control polymerization and form lignin-like NPs. This makes it possible to improve such characteristics of nanosystems such as charge, stability, efficiency of penetration into human cells, and the possibility of use for targeted antitumor therapy.

This work is devoted to obtaining stable NPs based on lignin-like polymers synthesized enzymatically and chemically, and evaluating their physicochemical properties, as well as studying their penetration and cytotoxicity *in vitro* in tumor cell lines.

It was shown that out of the 12 studied phenolic monomers, NPs from polyferulic (pFA) and polygentisic (pGA) acids, which were synthesized using the enzyme laccase, have optimal physicochemical properties (diameter 100–300 nm, PDI lower 0.2, zeta-potential lower -30mV). The resulting NPs are stable in protein solutions and can carry hydrophobic low molecular weight compounds (Doxorubicin, Dox). When studying the internalization of NPs by tumor cell lines, a more

active uptake of pFA NPs was shown. For both types of breast cancer (MDA-MB-231 and MCF-7), as well as for normal human foreskin fibroblasts (Bj-5ta), no toxicity of the empty NPs was found even at a concentration of 100 mg/mL. Loaded pFA NPs and pGA NPs have similar toxicity values to free Dox in MDA-MB-231. MCF-7 is resistant to the samples with pFA NPs, and at high concentrations of loaded Dox, NPs have a more pronounced toxic efficacy. Using 3D spheroids based on MCF-7, we have shown that, with two methods of samples incubation (with removal after 15 min and without removal, complete incubation for 48 h), NPs penetrate more efficiently and cause apoptosis.

A comparative analysis of two types of polymer NPs showed the possibility of using pFA NPs to develop new approaches to antitumor therapy, such as complex therapy using mixtures of NPs carrying different pharmacological compounds and targeted drug delivery exclusively to tumor cells.

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Matrices from chitosan/oligolactide copolymers with osteoinductive properties for regenerative medicine

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Chitosan (Ch) is a deacylated form of chitin, which is widely distributed in nature. As is well known, positively charged chitosan-based matrices can support cell adhesion and proliferation. However, chitosan has rather low mechanical strength and degradation rate. To overcome these limitations, various copolymers of chitosan obtained using Ch amino groups could be proposed. Thus, chitosan-based composite matrices can be obtained by copolymerization of Ch with some other monomers/oligomers without additional cross-linking agents, which allows to reduce matrix cytotoxicity. Polylactic acid (PLA) approved by FDA for clinical use, is a promising synthetic polymer for grafting chitosan. Polylactic acid meets the requirements for biomaterials due to its biocompatibility and ease of matrix formation with the desired morphology. However, rather low negative charge of the PLA surface limits cell adhesion. The combination of the properties of Ch and PLA may be of great interest: 1) to adjust the hydrophilic-hydrophobic balance of chitosan-based matrices and their mechanical properties; and 2) to enhance cell adhesion to PLA-based matrices. Mesenchymal stromal stem cells (MSC) are known to possess tri-lineage differentiation ability, including osteogenesis. By varying the properties of the matrix, one can affect this process. For example, by adopting the matrix properties it is possible to enhance the osteoinductive potential of MSC.

The aim of this study was to obtain polymer matrices in the form of films (2D) and macroporous hydrogels (3D) based on chitosan, as well as its graft copolymers with oligolactides: to study their structure and some physicochemical properties; and to evaluate the growth and differentiation of MSC on/in them in an *in vitro* model. For the formation of 2D films and 3D hydrogels, we used copolymers of Ch, (MW 80 kDa, DA 0.1), with oligo (L, L- and / L, D-lactides) with MW 5 kDa, (Chit-L, L and Chit-L, D, respectively). The copolymers were obtained by the method of solid-phase synthesis[1]. The structure of the hydrogels studied by confocal laser microscopy represented a system of interconnected macropores with an average size of 120–140 μm. The obtained matrixes were tested by examining the toxicity of the obtained extracts using the MTT-test after previous matrix incubation with culture medium for 24 h. The surface of the copolymers matrixes was found to ensure rather good adhesion of MSC isolated from human adipose tissue (confocal microscopy), while cell growth and proliferation at long-term cultivation for 7 days in the matrices was studied by MTT-test. Chit-L,D matrices (hydrogels) were shown to improve the proliferation of MSC, while copolymer films contributed to the cell differentiation in the osteo direction. MSC differentiation was assessed by estimating alkaline phosphatase activity (light microscopy) and by qRT- PCR which confirmed the expression of ALPL, RUNX2, SPP1 gene markers of osteogenesis on day 7 and 14 after induction.

Thus, hydrogels based on copolymers of chitosan with oligolactides are promising biomaterials for regenerative medicine.

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Fibroin cryostructures cross-linked with genipin for tissue engineering: Preparation and in vitro evaluation

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Biodegradable biopolymers are widely used to create matrices for tissue engineering. Protein-based hydrogel materials are known to well mimic the natural cell environment, and thus provide optimal conditions for tissue regeneration. Matrices from regenerated fibroin (Fb) are water-soluble. To prevent solubility of Fb-based matrices and to improve their mechanical properties chemical cross-linking is used. However, it may not be effective enough because of rather low content of primary amino groups in the Fb structure. Several possible conformational states of Fb are known, namely water-soluble α-helices and insoluble β-folded structures. Creating conditions for the α → β conformational transition at the hydrogel formation from regenerated Fb allows to influence the polymer solubility.

The aim of the study was to prepare Fb-based cryostructures cross-linked with genipin and to study their structure, some physicochemical properties, as well as to evaluate their biocompatibility in an *in vitro* model.

To obtain porous cryostructures (matrices), Fb solutions (20 and 30 wt.%,) were incubated at -10°C for 30 min and freeze-dried for 18 h. The obtained porous matrices were cross-linked by incubation in a genipin solution (10 wt.%) in ethanol for 24 h. Using confocal laser microscopy, the matrices were shown to provide an open-pore system. The mean pore sizes were 149 ± 7 μm and 355 ± 14 μm for the Fb-20 (20 wt.%) and Fb-30 (30 wt.%) cryostructures, respectively. The absence of solubility was ensured both by Fb transformation into the β-conformational state and by chemical cross-linking

with genipin in an alcohol medium. Cytotoxicity of fibroin cryostructures was studied by extract test using mouse fibroblasts L929 as model cells. The number of viable cells was determined by MTT-test. Some decrease in cell viability was observed at their incubation in undiluted extracts from the matrices. However, after incubation of the cells with the extracts no changes were found in cell morphology. After diluting the extracts, cell viability increased. To study cell behavior in the matrix, L929 cells and immortalized human mesenchymal stem cells (hTERT-MSC) were seeded in matrices and cultured for 7 days. Cell distribution and morphology in the cryostructures were analyzed by confocal microscopy after 7 days of cultivation. Both hTERT-MSC and L929 cells formed a dense cell monolayer on Fb-20 and Fb-30 cryostructures.

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Enzymatic copolymerization of aniline and 3-aminobenzoic acid in a deep eutectic solvent

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In recent years, a new class of 'green' solvents named deep eutectic solvents (DESs) has appeared[1]. DESs are obtained by simple thermal mixing of two compounds, which results in the formation of a eutectic solution with a melting point lower than those of the individual components. Due to the properties of DESs such as high thermal stability, low toxicity, biodegradability, conductivity, and others, DESs or their mixtures with a buffer solution can be used in various fields of chemistry, including biocatalysis. However, there are only a few studies describing the use of oxidoreductases for the synthesis in DES-buffer mixtures.

Laccase (*p*-benzenediol:oxygen oxidoreductase, EC 1.10.3.2) belongs to the 'blue' oxidases; it catalyzes the oxidation of various organic compounds with molecular oxygen. This enzyme attracts much attention as a catalyst for fine organic synthesis. One of the promising laccase substrates is aniline, the oxidation of which results in the formation of polyaniline, the most important compound among conducting polymers. Polyaniline and its functionalized derivatives possess antimicrobial properties and could be used to create film coatings, protecting various surfaces from bacterial contamination[2].

In this work, DES betaine-glycerol (molar ratio 1:1) was used as a co-solvent for efficient laccase-catalyzed template copolymerization of aniline and 3-aminobenzoic acid. The fungal laccase *Trametes hirsuta* retained ~50% activity in a DES/buffer mixture (60/40 vol.%) after 120 h incubation. Enzymatic reactions conducted in DES/buffer mixtures met the requirements of sustainable chemistry. The synthesized copolymer was characterized by UV-Vis and FTIR spectroscopy, atomic force microscopy, and cyclic voltammetry.

The copolymer strongly inhibited the *Staphylcocus aureus* and *Escherichia coli* growth. The minimum growth inhibitory concentration (MIC, the lowest concentration of a compound that inhibits bacterial growth for 24 hours) was determined by the standard serial two-fold dilution method in LB nutrient medium. It was found that the MIC values of copolymer against gram-positive *S. aureus* and gram-negative *E. coli* bacteria were 0.125 and 1.0 mg/mL, respectively.

Thus, it has been shown that betaine-based deep eutectic solvents are promising media for the polymerization of various laccase substrates, and functionalized polyanilines synthesized enzymatically can be used to create antimicrobial coatings.

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Development of nanocontainers for the delivery of 2,4-dinitrophenol derivatives to the liver in the treatment of non-alcoholic fatty liver disease and type 2 diabetes

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Type 2 diabetes mellitus (DM-2) and non-alcoholic fatty liver disease (NAFLD) are two of the most urgent problems of medicine nowadays. According to the World Health Organization forecasts, by 2030 these diseases will take the 7th place among all causes of death. Clinics use a number of drugs aimed at treating DM-2, but there is no drug treatment for NAFLD.

Thus, the development of a drug for the prevention and treatment of NAFLD and CD-2 is relevant. One of the promising molecules that can be effective and safe in the treatment of these diseases is 2,4-dinitrophenol, which changes the activity of mitochondria. The mechanism of action of 2,4-dinitrophenol is to transfer a proton across the mitochondrial membrane, dissipating the mitochondrial proton gradient and promoting thermal dissipation of energy resulting from the oxidation of the mitochondrial substrate, which leads to accelerated fat oxidation. Previously, 2,4-dinitrophenol was widely used as a weight loss agent, but was discontinued due to the occurrence of fatal hyperthermia. In order to remove the established side effects from its use, the task arose to develop an effective formulation of this molecule for targeted delivery to the liver.

The aim of this work is to develop nanocontainers of 2,4-dinitrophenol derivatives with controlled release in the liver for oral administration. To solve the problem, we used esters and alkyl derivatives of 2,4-dinitrophenol, as well as aromatic polycyclic nitroalcohols based on naphthol-1 and benzylphenol, loaded into nanoparticles based on polylactic-co-glycolic acid (PLGA).

Polymer micelles have a number of advantages such as high stability, simplicity of preparation and delayed prolonged release. The efficiency of encapsulation of 2,4-dinitrophenol derivatives increases up to 30 times compared to the free molecule, and slow release from the nanocontainer is observed. Using the analysis of ATP-luciferin-luciferase, the

effectiveness of derivatives was shown in comparison with free 2,4-dinitrophenol. In addition, *in vitro* testing of the formulations showed a prolonged inhibition of ATP synthesis due to the prolonged release of the active molecules.

The obtained results open up the prospects for further application of polymeric micelles of 2,4-dinitrophenol derivatives as a harm-free drug treatment for DM-2 and NAFLD.

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Stability study of copper antitumor coordination compound loaded into liposomes

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Organometallic complexes are one of the most promising types of anticancer drugs. Studies of the last few years show that copper coordination compounds are particularly promising as antitumor therapeutic agents. Many copper complexes are characterized by hydrophobicity and, as a consequence, low bioavailability, which significantly complicates their practical use. Such complexes include Medation, an antitumor drug, which is a coordination compound of copper based on 2-alkylthioimidazolone. According to the results of preclinical trials, the drug is recommended for triple-negative breast cancer treatment.

One of the ways to improve the parameters of solubility and bioavailability of hydrophobic active molecules is their encapsulation into nanocontainers for drug delivery. The most studied and used system for the delivery of biologically active substances are liposomes. The hydrophobicity of the copper complex does not allow the use of usual methods to hydrophilic substances for its loading into liposomes, therefore, the purpose of this work is to find the optimal method for encapsulation of copper coordination compound into liposomes.

We have developed an approach for copper complex encapsulation into liposomes, which is realized by injection of organic solvents mixture into copper salt solution. Liposomes were prepared from dipalmitoyl phosphatidylcholine (DPPC), cholesterol (Chol) and PEG-distearoyl phosphoethanolamine (DSPE-PEG(2000)) in the ratio DPPC:Chol:DSPE-PEG(2000) 55:40:5. Quantitative determination of the ligand and complex in liposomal suspension was carried out using HPLC. This new approach allowed us to obtain a suspension resistant to aggregation and sedimentation for up to two weeks, the loading efficiency was 1.22% (molar ratio of the complex to lipids). Ionic strength of copper solution and cholesterol content in lipid membrane affected the loading capacity. The developed technique has been successfully applied to obtain liposomal forms of similar copper coordination compounds based on 2-thioxoimidazolone, which indicates the possibility of using the technique for encapsulation of hydrophobic copper complexes in liposomes.

The stability of the resulting suspension was evaluated in NaCl solution at 4°C and in RPMI cell medium at 37°C. The suspension retained loading for several days in both environments, which indicates the advantage of the developed technique. It was shown by derivative spectroscopy that the complex in liposomes is stable when stored at a temperature of 4°C and dissociates within 24 hours at temperatures of 37°C and 45°C. Incubation at 37°C leads to the release of 10% of copper loaded into liposomes. Quenching constants for fluorescently labeled liposomes were calculated using spectrofluorimetry, which made it possible to analyze the behavior of the complex in the lipid bilayer. The cytotoxicity of the liposomal formulation was evaluated using an MTT test on MCF-7 cells – the test showed that the formulation has greater cytotoxicity due to increased bioavailability. In addition to antitumor activity, copper complexes have also demonstrated antibacterial properties, which can be used for joint therapy in the future.

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Synthesis and characterization of a bifunctional platform based on magnetite-gold nanoparticles for theranostics of cancer

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One of the most interesting objects from the point of view of application in biomedicine is hybrid structures based on magnetic nanoparticles (NPs) and NPs of noble metals (including gold), which make it possible to simultaneously introduce two types of ligands onto the surface of NPs for their further use for photodynamic cancer therapy (PDT) [a combination of a photosensitizer (PS) for therapy and a fluorophore (FP) for platform detection]. Synthesis of the system is a promising direction and is of interest to scientists. In connection with the above, the aim of this work was the synthesis and study of Fe_3O_4-Au NPs with the 'dumbbell' structure as a bifunctional platform for PDT of oncological diseases using fluorescent diagnostics.

As a result of the decomposition of iron pentacarbonyl in diphenyl ethers in the presence of hydrogen tetrachlorourates, hybrid magnetite-gold NPs with Fe_3O_4 size of 10.8 ± 1.5 nm and Au 4.4 ± 0.8 nm (according to transmission electron microscopy) stabilized with oleic acid were synthesized. According to the results of x-ray phase analysis, the synthesized nanoparticles have a 'spinel' type crystal structure with a lattice period of 0.8387 nm (an intermediate value between magnetite and maghemite). According to the results of measuring the magnetic properties, the NPs had a saturation magnetization of 62 Am2 · kg (Fe_3O_4) -1 and a coercive force of 13 Oe. Further, the NPs were modified with 3,4-dihydroxyphenylacetic acid (DOPAC) for further coating with a stabilizing polyethylene glycol (PEG) using the carbodiimide method.

For further grafting of chromophores, a series of PS based on bacteriochlorin and FP based on cyanine were studied for use as components of a bifunctional platform for PDT. Since two different colored substances (PS and FP) must be combined in one system, the synthesized Fe_3O_4-Au NPs with the 'dumbbell' structure (stabilized) were used as a 'link'. The FRET pair FP/PS (PS/Cy5) was selected and the optical properties were studied. Modification of NPs with DOPAC and PEG followed by activation by EDC/NHS (carbodiimide method) makes it possible to effectively attach a PS to the magnetic surface of NPs in a two-phase system (water–DMSO). In the future, it is planned to 'cross-link' the fluorophore (Cy5) onto the gold surface, study the optical properties of the final platform Fe_3O_4-

Au/PS/FP, and calculate the physicochemical parameters of the synthesized system to optimize the system and conduct therapy *in vitro* and *in vivo*.

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Development and evaluation of asgpr-targeted atorvastatin derivatives

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According to the World Health Organization, the number one cause of death is ischemic heart disease, responsible for 16% of the world's total deaths in 2019. Statins are effective therapeutic agents used to reduce cardiovascular disease by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-R). Its adverse effects on muscles are generally explained by poor bioavailability and systemic administration route. Targeted delivery of statins in liver may be one way to reduce undesired side effects.

The asialoglycoprotein receptor (ASGPR) is a C-type (Ca^{2+}-dependent) lectin which is highly expressed on the surface of hepatocytes. The primary physiological role of ASGPR is considered to be binding, internalization, and subsequent clearance from the circulation of galactose- and N-acetylgalactosamine-terminated glycoproteins. The location and the function make the receptor an ideal target for delivery of therapeutic agents to liver cells.

Herein, we report on synthesis and evaluation of the ASGPR-targeted atorvastatin conjugates. The conjugation of the selected carbohydrate fragments to atorvastatin generally produced a favorable effect on the hydrophilicity of the drug. Obtained conjugates demonstrated at least 4-fold higher molar solubility than that of atorvastatin. The molar solubility of the atorvastatin calcium salt was 0.11 ± 0.01 mM, while the molar solubilities of the obtained unprotected conjugates valued from 0.19 to 0.54 mM. The affinities to ASGPR have been assessed using surface plasmon resonance (SPR) spectroscopy technique. All of the synthesized conjugates decorated with N-acetylgalactosamine as a targeting moiety demonstrated greater affinity to ASGPR compared to unmodified atorvastatin. Compounds 2a and 2b exhibited subnanomolar binding to the receptor (K_D = 0.33 and 0.15 nM, respectively) which is 4 orders of magnitude lower than that measured for atorvastatin itself and the native ASGPR ligand N-acetylgalactosamine (K_D = 1.0 and 4.5 μM, respectively). The amide-based conjugates exhibited higher water solubility and stability for hydrolysis than ester-based atorvastatin conjugates. The release of atorvastatin after incubation of the conjugate in the presence of lysosomal protease was demonstrated. The results suggest that designed amine-based compounds are potential orally administrated liver-targeted prodrug of atorvastatin that can be used in clinical practice. The hepatic selectivity of synthesized conjugates is the subject of the further exploration.

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