12th INTERNATIONAL CONFERENCE

BIOMATERIALS AND NANOBIOMATERIALS:

Recent Advances Safety-Toxicology and Ecology Issues

Including Russian-Hellenic Workshop and School of Young Scientists

ABSTRACT BOOK

Public Health and Toxicology

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.

Full Journal Title: Public Health and Toxicology

Abbreviated Title: Public Health Toxicol

ISSN (electronic): 2732-8929

Publisher: European Publishing (www.europeanpublishing.eu)

Publisher Address: Science and Technological Park of Crete, Greece

Peer Review: Double Blind

Publication Frequency: Quarterly

Publication Medium: Electronic Only

Publication website: www.publichealthtoxicology.com

First Year Published: 2021

This abstract book includes the abstracts revised and accepted by the Bionanotox Scientific Committee. This abstract book has been produced electronically and all English articles are also available on-line as a special issue at www.publichealthtoxicology.com

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NEUROSOME

Biotransformation of phthalates by basidiomycete fungi from different ecophysiological groups

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Phthalate esters (PEs) – the esters of 1,2-benzenedicarboxylic (phthalic) acid – are the most commonly used plasticizers worldwide. Currently, there is an ever-growing concern about persistence, ubiquity and toxicity of PEs. Structurally resembling estrogen, PEs can potentially act as endocrine disrupting chemicals adversely affecting development and reproduction. A promising approach of PEs degradation is their biotranformation by fungal enzymes. The aim of this work is to study the biodegradation of diethyl phthalate (DEP) and dibutyl phthalate (DBP) by white rot fungi from different taxonomic and ecophysiological groups.

The selected fungi were: *Trametes hirsuta* – the primary colonizer on lignum; *Steccherinum ochraceum* and *Peniophora lycii* – the secondary colonizers on lignum; *Crucibulum leave* – the stramentum colonizer; and *Agrocybe praecox* – the humus colonizer. The overall oxidase activity and growth inhibition were assessed on malt agar plates containing 0.5, 1.0, and 1.5 $g*L^{-1}$ of DEP/DBP.

For biotransformation of phthalates fungal biomass was grown by a submerged method on a glucose-peptone medium. When the maximum biomass was accumulated, the mycelium was transferred to a medium of the same composition with peptone replaced by $NaNO_3$ and 1 $g*L^{-1}$ of phthalates. The samples were taken on days 3, 6, 10, and 14, and PEs were extracted with dichloromethane. The content of DEP and DBP in the extracts was estimated by a thin layer chromatography.

The decrease in the fungal growth rate on an agar medium containing phthalates has been shown. The growth inhibition by DEP was higher than by DBP. Only *T. hirsuta* and *P. lycii* were able to grow on a medium with a 1.5 $g*L^{-1}$ concentration of DEP. An increase in oxidase activity was shown during the growth on the DEP-containing medium for *T. hirsuta* and *P. lycii,* and on a DBP-containing medium – for *C. leave,* compared with control media without phthalates. *T. hirsuta, P. lycii* and *C. leave* completely degraded DBP within 10 days. The rate of DEP destruction by *T. hirsuta* and *P. lycii* was significantly lower than that of DBP. This correlated with the growth rate of these fungi on the solid agar media containing corresponding phthalates.

Thus, it was shown that white rot fungi are capable of degradation of phthalates. The effectiveness of biodegradation most probably depends on the enzymatic system of a particular species. The results of this work can be used for development of a phthalate biodegradation technology.

Funding
This study was financially supported by the Russian Science Foundation (grant no. 21-14-00306).
Public Health Toxicol 2021;1(Supplement):A1
DOI: 10.18332/pht/142008

Field study to assess the efficacy of slow-release formulations of the tribenuron-methyl herbicide in spring wheat

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The efficacy of slow-release formulations of tribenuron-methyl (TBM) was compared with the efficacy of TBM as the active ingredient of the Mortira commercial formulation. The embedding of TBM into degradable matrix enabled long-duration functioning of this unstable herbicide in soil. The TBM formulations controlled the weeds considerably more effectively, with the biological efficacy of 95.7%, than the commercial formulation (52.2%). TBM was more effective against *Amaranthus* species, which were killed at an earlier stage, than against weeds of the *Chenopodium album* and the *Galium aparine*. Wheat yield was the highest in the case of the deposited herbicide $33.6*10^2$ kg/ga and $32.5*10^2$ kg/ga in the case of spraying crops with Mortira. The highest quality parameters (grain hectoliter mass, gluten and protein contents) were obtained in the group of plants treated with the embedded herbicide. The application of embedded TBM in field trials confirmed the high efficiency of the experimental formulation.

Acknowledgements
This study was financially supported by Project "Agropreparations of the new generation: a strategy of construction and realization" (Agreement No 075-15-2021-626) in accordance with Resolution No 220 of the Government of the Russian Federation of April 9, 2010, "On measures designed to attract leading scientists to the Russian institutions of higher learning".
Public Health Toxicol 2021;1(Supplement):A2
DOI: 10.18332/pht/142036

Promising vehicules for intraocular drug delivery

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Local drug intake as eye drops is preferable for the treatment of the pathological processes within the eye. However, topical drug application is much less effective for the treatment of diseases, which include inner structures of the eye, due to the poor transport of the drug into the eye. And more, when one needs to deliver a protein molecule into the eye compartments, it especially represents a major challenge due to the limited ocular penetration. The work presents two different perspective ways for drug delivery into the eye. One implies biocompatible and nontoxic calcium phosphate (CaP) particles as vehicles for the low molecular weight substance, enalaprilat, an inhibitor of angiotensin-converting enzyme. The second one uses multilayer polyion complex nanoparticles of superoxide dismutase, SOD1, (Nano-SOD1), to treat inflammatory eye diseases. The drawbacks and prospects for both approaches are discussed.

Funding
Supported in part by Lomonosov MSU Reg. Theme AAAA-A21-121011290089-4, equipment used was purchased by the MSU Program of Development.

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Public Health Toxicol 2021;1(Supplement):A3
DOI: 10.18332/pht/142038

Immunochromatographic determination of macrolides in breast milk from medicated mothers to control safe breastfeeding

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Antibody against common carbohydrate moieties of macrolide antibiotics were generated in result of rabbit immunization with BSA-clarithromycin (CLA). For fabrication of immunochromatographic strip test, blue latex particles were labeled with antibody; GEL-CLA and anti-rabbit IgG were spotted on nitrocellulose membrane in test and control zones for the rapid simultaneous group determination of six macrolide antibiotics. Through careful optimization of parameters, namely the type of nitrocellulose membrane, the carrier for the coating antigen, its loading onto the strip (the number of nanodrops per spot, the number of spots per line and the number of lines), the loading of antibodies on the latex bead and the volume of the latex bead and stabilizing solutions, the color intensity was improved 100 times. CLA, roxithromycin, erythromycin, dirithromycin, azithromycin, and oleandomycin in buffer were visually detected at 1-1000 ng/mL level. The limits of instrumental detection were respectively 0.12, 0.15, 1.4, 2.1, 2.4, and 3.3 ng/mL. The composition of breast milk changes significantly both during a breastfeeding and during lactation period: protein levels decrease slightly, while carbohydrates increase, the fat content almost doubles. Therefore, for the unification of sample composition and avoiding matrix effect, a special 20-min procedure with methanol/hexane extraction was developed. Thus, the developed rapid on-site diagnostic assay format is suitable for monitoring of antibiotic content in breast milk during medication with any of six mentioned macrolides to ensure safe breastfeeding of infants.

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Public Health Toxicol 2021;1(Supplement):A4
DOI: 10.18332/pht/142039

Development of a new osteoplastic material based on bioactive ceramics and recombinant biologically active factors

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A wide list of clinical cases with bone injuries, as well as the absence of a universal osteoplastic material, leads to an active search for new materials with high biocompatibility, timely resorption, and the presence of osteoinductive properties. The regenerative potential of the material is associated with the presence in its composition of biologically active components - recombinant proteins – inducers of osteogenesis (BMP-2) and angiogenesis (erythropoietin, EPO). Materials based on bioactive silicate ceramics, for example, diopside ($CaMgSi_2O_6$), which provides a prolonged biological effect due to the slow release of immobilized proteins, can act as promising carriers. Such materials can be used both independently and in composites containing other biocompatible components (demineralized bone matrix, DBM; hyaluronic acid, HA).

We have developed a new composite osteoplastic material based on BMP-2 and EPO, immobilized on diopside particles, introduced into a scaffold based on DBM with the addition of HA. The immobilization capacity and kinetics of BMP-2 release from the diopside were studied under various conditions.

The study of biocompatibility and osteoinductive properties of the developed materials was carried out in vivo on a model of ectopic osteogenesis in mice (40 outbred male ICR (CD-1) mice of 38-47 days old). Experimental animals were divided into 5 groups, 8 animals per group: Group 1 - DBM + HA; Group 2 - DBM + HA + diopside; Group 3 - DBM + HA + diopside + BMP -2; Group 4 - DBM + HA + diopside + EPO; Group 5 - DBM + HA + diopside + BMP-2 + EPO. The implants were 1 x 1 x 4 mm DBM blocks inserted into the gluteal muscle of the hind paws of the animal (two implants per animal). Euthanasia, tomography and histomorphometry were performed 12 weeks after the operation. According to the results of tomography and histological analysis all implanted biocomposites demonstrated their high biocompatibility. The addition of rhBMP-2 to the scaffolds resulted in significant osteogenic effect and formation of large mineralization areas. The addition of erythropoietin led to an increase in the quality of the newly formed bone tissue, as well as, in some cases, to an increase in the amount of mineralized bone.

Public Health Toxicol 2021;1(Supplement):A5
DOI: 10.18332/pht/142040

Synergy of antibacterial effect of copper and silver nanoparticles with different functionalizations

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Introduction

Due to the aging of the population, the number of patients with chronic diseases like diabetes mellitus 2 type, atherosclerosis, venous insufficiency is becoming more severe. All these factors of poor blood supply, as well as a bedridden regimen in inpatient treatment facilities, lead to more chronic infected non-healing ulcers and pressure sores. Chronic infectious ulcers (CIU) lead to high health costs, as well as patient suffering. An especially big problem is the resistance of bacteria to antibiotics. As an alternative to traditional antibiotics, nanoparticles of various metals can be used. Recent studies have shown that there is a synergistic antibacterial effect between silver and some transition metals, such as copper, but synergic effect between metal nanoparticles with different functional groups is not described yet.

Material and Methods

In this project we studied antibacterial effect of silver and copper nanoparticles with different functional groups applying determination of minimal bactericidal concentration. Bacteria *Escherichia coli* K-12 and pathogenic *Pseudomonas aeruginosa* PAO1, *Staphylococcus aureus* ATCC 25923 and multiresistant ESBL *Escherichia coli* were used as models. Toxicity of the same nanoparticles and their combinations was also compared using Resazurin test with murine fibroblasts Balb/c T3T.

Results

We showed that silver and copper nanoparticles were synergistic in killing all tested bacteria. The antibacterial effect of the mixture of nanoparticles was up to 5 times higher than the sum of antibacterial effects of components separately. Especially high synergy with silver was observed

in combination with copper nanoparticles with positively charged functional coating. The difference in synergy between negatively and positively charged nanoparticles was especially relevant for Gram-positive bacteria. Unfortunately, the synergy was not specific for bacterial cells, but also killed mammalian cells *in vitro*. That can be a problem in developing safe antibacterials for practical use. Hopefully, future investigations of synergy mechanisms can help to develop new safe and effective antibacterial nanocomposites.

Public Health Toxicol 2021;1(Supplement):A6
DOI: 10.18332/pht/142325

Biomaterials based on nanosized forms of polyconjugated systems

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On the basis of polyconjugated systems, biomaterials have been developed that have hemocompatibility and are capable of releasing pharmacologically active substances. The prospects for the use of oxidative polymerization and copolymerization of aniline, pyrrole, dopamine and functional derivatives of aniline in the synthesis of electrically conductive polymers for biomedical purposes are considered. It has been shown that carrying out oxidative polymerization of aromatic amines, pyrroles and dopamine makes it possible to obtain nanosized polymer systems suitable for selective sorption of biologically active substances. As an alternative approach, it is proposed to carry out oxidative polymerization on the surface of inorganic carriers, which opens up new prospects for the creation of sorbents for lymphosorption, hemosorption and purification of biological fluids. The regularities underlying the production of hybrid composite materials are revealed and the prospects for their use for the creation of systems that imitate muscles are outlined. The prospects for the development of dopamine-containing complexing polymers for binding and controlled release of iron ions, which can be used in the treatment of anemia, are outlined. The materials under consideration have high mechanical strength, significant electrical conductivity and adsorption capacity.

Funding
The work was supported by Mendeleev University of Chemical Technology of Russia. Project Number K-2020-001.

Public Health Toxicol 2021;1(Supplement):A7
DOI: 10.18332/pht/142042

Properties of degradable polyhydroxyalkanoates (PHAs) synthesized by a new strain of *cupriavidus necator* ibp-21 on various c-substrates

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The bacterial strain isolated from soil was identified as *Cupriavidus necator* IBP/SFU-1 and investigated as a PHA producer. The strain was found to be able to grow and synthesize PHAs under autotrophic conditions; it also showed a broad organotrophic potential towards different carbon sources: sugars, glycerol, fatty acids, and plant oils. The highest cell concentrations (7-8 g/L) and PHA contents were produced from oleic acid (78%) and fructose, glucose, and palm oil (over 80%). The type of the carbon source influenced PHA chemical composition and properties: when grown on oleic acid, the strain synthesized the P(3HB-co-3HV) copolymer; on plant oils – the P (3HB-co-3HV-co-3HHx) terpolymer, and on the other substrates – the P(3HB) homopolymer. The type of the carbon source influenced molecular-weight properties of PHAs: P(3HB) synthesized under autotrophic growth conditions, from CO_2, had the highest number-average (290±15 kDa) and weight-average (850±25 kDa) molecular weights and the lowest polydispersity (2.9±0.2); polymers synthesized from organic carbon sources showed increased polydispersity and reduced molecular weight. Carbon source was not found to affect the degree of crystallinity and thermal properties of the PHAs. The type of the carbon source determined not only PHA composition and molecular weight but also surface microstructure and porosity of polymer films.

Acknowledgements
This work was supported by the was financially supported by Project "Agropreparations of the new generation: a strategy of construction and realization" (Agreement No 075-15-2021-626) in accordance with Resolution No 220 of the Government of the Russian Federation of April 9, 2010, "On measures designed to attract leading scientists to the Russian institutions of higher learning".

Public Health Toxicol 2021;1(Supplement):A8
DOI: 10.18332/pht/142044

Biomimetics of proteins: Artificial polymers made of α-amino acids

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Proteins are one of the most appropriate biomaterials for a variety of biomedical applications including resorbable surgical and pharmaceutical devices owing to their innate affinity to tissues, enzymatic biodegradability with releasing "the bricks of life" - α-amino acids (α-AAs) which could be assimilated by the organism promoting in that way tissue regeneration. However, the proteins have some serious shortcomings among which the most important is *immunogenicity* that is attributed to their molecular architecture.

More promising are α-AA based synthetic biodegradable (AABB) polymers having a new macromolecular architecture less recognizable by the immune system of the living organism. The presentation deals with an overview of the main families of the α-AA based synthetic polymers such as poly(amino acid) s, pseudo-poly(amino acid)s, polydepsipeptides, and provides somewhat more expanded information about the fourth class - so-called pseudo-proteins[1-4] which represent relatively new and broad family of the biodegradable polymers qualified for numerous biomedical applications[5-9], including nano-vehicles for targeted drug delivery[10]. A new class of pseudo-proteins composed of non-proteinogenic amino acids were obtained as well[11,12]. These new polymers are expected to have as wide range of biological activities.

Acknowledgments
This work was supported in part by the International Science and Technology Center [Grant # A-2289], and Shota Rustaveli NSF of Georgia [Grant # FR17-102].

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Public Health Toxicol 2021;1(Supplement):A9
DOI: 10.18332/pht/142047

The study on the experimental models *in vitro* toxic properties of nanomaterials in Belarus

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There is no doubt that nanomaterials require special control, regulation and legislation, but the main problem is that there is no legislative base for nanomaterials in the Republic of Belarus.

The aim of our work was to develop methodological approaches for screening safety assessment of nanomaterials on cell cultures.

The main damaging properties of nanomaterials are based on their ability to penetrate and accumulate inside the cell, disrupting the functioning of the internal systems, causing oxidative stress, as well as, when penetrating the cell nucleus, the ability to induce mutations. Thus, for preliminary screening testing of the safety of nanomaterials, it is most appropriate to use a sequential testing scheme based on alternative methods using cell cultures of different origin. On the basis of the main routes of entry of nano-sized particles into the body, cell cultures of similar specifications (A549, CaCo2, skin-muscle embryonic fibroblasts) were selected for research. The developed testing scheme includes a number of methods for determining the general toxic effect, mutagenicity, the ability to assess cell membrane damage and the method of assessing cell membrane damage. method for studying the induction of reactive oxygen species using fluorescein diacetate staining, cytogenetic analysis under a microscope and cytofluorimetric methods).

The study allowed us to develop a specific algorithm for screening assessment of nanomaterials. But the issues of proper sample preparation, as well as a comparative assessment of the number of tested nanoparticles, remained unresolved. We cannot adequately compare the concentrations of large molecular particles (for example, carbon nanotubes, which practically do not penetrate cell membranes, including due to the formation of agglomerates) and low molecular size (for example, silver nanoparticles). Thus, the question of the quantitative determination of nanoparticles for hygienic rationing nanoparticles in products and in the air of the working zone is still open.

No less important is the creation of a scientifically sound mechanism for assessing the safety of nanomaterials, a scientific base and a broad scientific platform for the exchange of experience.

Public Health Toxicol 2021;1(Supplement):A10
DOI: 10.18332/pht/142056

Mechanochemical approach to create biomaterials based on modified polysaccharides

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The possibilities of solvent-free extrusion for modifying polymers are of current interest, and mechanochemical technologies in general were named among the top ten technologies in chemistry at the IUPAC World Chemistry Congress 2019. The main benefits of the method are the absence of toxic additives during the synthesis and low temperature of the processes. From this point of view, this approach to obtaining polysaccharide-based biomaterials is very promising and leads to development of technologies of a new ecological level.

A number of compositions were developed by combining natural (chitosan, proteins) and bioresorbable synthetic polymers (polyesters) at shear deformation. Amphiphilic chitosan derivatives and copolymers were obtained through reactive solvent-free co-extrusion. The structure and properties of the products were studied; the regularities of the solvent-free processes were revealed. Generally, our studies have shown that, for the synthesis of chitosan derivatives and based on them composites, mechanical activation of solid reactive mixtures is preferable since it substantially reduces the consumption of reagents, the process duration (up to

several minutes), and the process temperature in contrast to a similar process in a melt or an organic solvent. In addition, fractions of graft copolymers of natural and synthetic components are formed during mechanochemical treatment, increasing the compatibility of the parent polymers in the compositions. A number of novel biodegradable polymeric materials have been obtained using innovative technologies for the processing of natural polymers. Their safety for solving biomedical problems ensures that there is no need for catalysts and process initiators at all stages of synthesis and processing. Laser-induced approaches to the formation of macroporous chitosan-based hydrogels with a well-defined architecture using laser stereolithography, a simple and fast technique of three-dimensional prototyping requiring no expensive equipment, are proposed. Multicomponent copolymer systems based on chitosan, collagen and oligo/polyesters which form ultrafine solutions in organic solvents have been developed. The products were analyzed using DLS, FTIR-spectroscopy and DSC.

Formulations for the electrospinning of nonwoven fibrous mats, containing at least 40% of natural components, have been proposed. Casting solution characteristics, namely viscosity, surface tension, and electroconductivity as well as electrospinning parameters were studied and optimized to obtain defect-free mats with an average fiber diameter of 7 μm. Morphology, chemical structure of surface layer, mechanical properties in dry and wet states and cytocompatibility were analyzed to confirm an appropriate functionality of the electrospun fibrous mats as scaffolds for tissue engineering.

Funding

This research was funded by RFBR, grant 18-29-17050.

Public Health Toxicol 2021;1(Supplement):A11
DOI: 10.18332/pht/142057

Development of approaches to the creation of an epitopic vaccine for preventing COVID-19

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Creation of epitope vaccines - vaccines, the active components of which are small protein epitopes of an infectious agent - is one of the most promising modern approaches in immunoprophylaxis. These vaccines do not have the disadvantages typical of live vaccines (reversal of pathogenic properties, residual virulence, incomplete inactivation, etc.). They are distinguished by a high degree of standardization, have a weak reactogenicity, with their help it is possible to avoid both the development of autoimmune processes during immunization and the formation of non-protective antibodies and antibodies that contribute to the development of antibody-dependent intensification of infection. Six epitopes (144-153, 337-346, 414-425, 452-494, 470-491, 496-507) were selected from the sequence of the Spike protein of the SARS-CoV-2, which implement protein-protein interactions in complexes with neutralizing antibodies and ACE2 (angiotensin converting enzyme 2). All epitopes, except for one with the alpha-helical conformation (337-346), have a loop-like conformation with close N- and C-terminus. To fix the conformation of the selected epitopes, an approach using protein (epitope) scaffolds was used. As an epitope scaffold, a homologue of the Rop protein form *Escherichia coli* was used, the structure of which contains a "helix - turn - helix" motif. Loop-shaped epitopes were inserted directly into the turn, and the alpha-helical epitope was inserted using flexible glycine-containing spacers. In the case of two epitopes, 452-494 and 470-491, the conformation was additionally fixed by a disulfide bond formed between the cysteine residues present in the epitopes. The presence of a disulfide bond was proved by mass spectrometry. For the purpose of multimerization, either an aldolase from *Thermotoga maritima*, which forms a trimer in solution, or an α-helical trimerizer of the SARS-CoV-2 Spike protein was attached to the epitopes inserted into the Rop-like protein. All obtained proteins (10 variants) showed a high level of immunogenicity after three parenteral administrations to mice with an interval of 2 weeks. Sera of mice immunized with fusion proteins containing epitopes with disulfide bonds (452-494, 470-491) interacted in a high titer with both the inactivated SARS-CoV-2 virus and the RBD (Receptor Binding Domain) domain of the Spike protein. Also, the interaction of sera with RBD was demonstrated in the case of the protein with the epitope 414-425. A study was carried out on laboratory animals (mice) of activation of cellular immunity, detected by the level of cytokine synthesis by splenocytes of immunized mice. The most pronounced increase of cytokines' level was observed in the case of a response to proteins, including epitopes with disulfide bonds (452-494, 470-491). The presented approach can be used in the future to create new epitope vaccines for the prevention of viral infections.

Funding

This work was supported by the Russian Foundation for Basic Research (Grant Number: 20 04 60373\20; "Development of approaches for design of a new type of preventive vaccine against COVID 19 based on the use of recombinant SARS CoV 2 antigens as a part of chimeric protein nanoparticles").

Public Health Toxicol 2021;1(Supplement):A12
DOI: 10.18332/pht/142058

Nanoformuation of 2,4-dinitrophenyl lipophylic derivatives as a promising tool for delivery to liver

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Type 2 diabetes mellitus (T2DM) and non-alcoholic fatty liver disease (NAFLD) are the pressing problems of the modern medicine. Nowadays there are no drugs approved for clinical use for the treatment of NAFLD and T2DM. One of the promising molecules that can be effective in the treatment of NAFLD and T2DM is 2,4-dinitrophenol, which acts as a proton ionophore, transferring protons into the mitochondrial matrix with the release of heat. But it has side effects (increase of metabolic rate, hyperthermia). Thus, the search for an effective formulation of this molecule for targeted delivery to the liver is of a great importance.

The purpose of this work is the development of a controlled-release nanoformulation of 2,4-dinitrophenol derivatives for intravenous administration. In fulfilment of the target, we used 2,4-dinitrophenol esters with various substituent lengths loaded into liposomes of various lipid composition and polymeric

micelles based on poly-(L-lactide-co-)glycolide (PLGA).
An effective 3-fold increase in the encapsulation of 2,4-dinitrophenol esters into liposomes in comparison with free molecule was observed. We investigated the dependence of the sustained release of 2,4-dinitrophenol from liposomes on the lipid composition and the LogP of the ester. It has been proven that the optimal ester chain length should be close to palmitic acid, and the lipid membrane should be composed of phospholipids with a certain phase transition point, depending on the desired release rate. We also demonstrated the possibility of 2,4-dinitrophenol esters to encapsulate in nanoparticles of PLGA block copolymer. In this case, the effectiveness of the molecule loading is increased up to 10 times compared to liposomes and loaded molecules released from the nanocontainer more slowly. Using ATP luciferin-luciferase assay efficacy of each derivative in comparison to free 2,4-dinitrophenol was shown. Also, the in vitro test of the formulations showed prolonged inhibiting ATP synthesis due to sustained release of active molecules.
The results open up the perspectives for future application of liposomes and polymer micelles of 2,4-dinitrophenol derivatives as a proton ionophores, whose task is to transfer protons to the mitochondria with the heat dissipation, that leads to accelerated fats oxidation and, as a result, harm-free medical treatment of T2DM and NAFLD.
Funding
This work was supported by a grant from the Russian Science Foundation № 20-73-00333.
Public Health Toxicol 2021;1(Supplement):A13
DOI: 10.18332/pht/142069

Encapsulation of antitumor copper coordination compounds into liposomes

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Medation is an antitumor drug classified as a copper complex compound of organic ligand containing 2-alkylthioimidasolone. According to the results of pre-clinical trials, the drug is recommended for Triple-Negative Breast Cancer treatment. Nevertheless, a significant limitation for in vivo use of this complex is its hydrophobic properties and, as a result, a low level of bioavailability. One of the ways to increase bioavailability of hydrophobic substances is their encapsulation into drug delivery systems. Liposomes are the most studied and well-described systems for biological substances delivery. Hydrophobic properties of our coordination compound do not allow us to use typical ways of encapsulation. Thus, the main aim of our work is to investigate for an 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 dipalmitoylphosphatidylcholine (DPPC), cholesterol (Chol) and PEG-distearoylphosphoethanolamine (DSPE-PEG(2000)) in the ratio DPPC:Cholesterol:DSPE-PEG (2000) 55:40:5. The presence of complex in liposomes has been proven by absorption spectra, quantitative complex determination was performed by HPLC. This approach allowed us to obtain colloidal stable suspension, loading capacity in molar complex to lipid ratio was up to 1,22%.
For obtained suspension we have estimated storage stability in 0,9% NaCl solution at 4°C, loading capacity was preserved up to one month. Liposomal suspension can be concentrated up to required values, the properties are retained in conditions close to blood flow (DMEM media, 37°C). We found that copper complex is included into lipid bilayer, localization has been studied under various conditions. Cytotoxicity and biocompatibility of liposomal formulation was estimated by MTT-test on MCF-7 cell line. The obtained liposomal formulation showed greater cytotoxicity in comparison with pure drug, which confirms the prospects for practical application.
The developed approach was used for encapsulation of copper coordination compounds similar to mediation, loading capacity values were of the same order as for the model complex.
Funding
This work was supported by RSF grant 19-74-10059 and MSU Program of Development.
Public Health Toxicol 2021;1(Supplement):A14
DOI: 10.18332/pht/142071

Analysis of the molecular interaction of asialoglycoprotein receptor with its ligands using surface plasmon resonance spectroscopy

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Liver cancer takes fifth place in the rating of most common types among men and the eighth among women worldwide. The most common type of liver cancer is hepatocellular carcinoma (HCC) - more than 75% of liver cancer is HCC[1]. Now, to treat this type of cancer oncologists are using cytotoxic drugs which also affect healthy tissues.
The main goal of the work was to study targeted delivery to the liver. So the focus was to find the most promising target cells and drug molecules. In this work, the asialoglycoprotein receptor was chosen as the delivery site, which is the best option for targeted delivery of drugs to the liver due to its location on the surface of hepatocytes. As targeting molecules native ligands of ASGPR were used. We chose modified triterpenic and bile acids as medical molecules. In their unmodified state they show anticancer and anti-inflammatory properties. Studies were held to obtain ligands' dissociation constants to determine which of them have the potential to be used in therapy. Spectroscopy of surface plasmon resonance was used to study the parameters of interaction of ASGPR with various ligands.
Multivalent ligands were demonstrating a cooperative effect. Dissociation constant decreases with increasing amount of N-acetylgalactosamine (GalNAc) residues: K_D = 19,6 ± 9,8 nM(mono-), K_D = 1,3 ± 1,1 nM(bi-), K_D = 0,7 ± 0,2 nM(three-). The length of the linker between the skeleton of the molecule and the residue of GalNAc had an impact on the obtained dissociation constant. This is presumably due to an increase in the mobility of GalNAc: K_D = 0,8 ± 0,1 nM for long linker and K_D = 19,5 ± 9,8 nM for the long one. The effect of the presence of hydroxyl groups in the skeleton of bile acid on binding to the receptor also had an impact on the obtained dissociation constant. We assume that this is due to a change in the hydrophobicity of the ligand. Steric factors on nonspecific binding of triterpenic acids to the hydrophobic site of ASGPR influenced obtained data. This can mean that some form of non-specific binding is taking place when ASGPR reacts with ligands.
Few of studied ligands with the obtained values of the dissociation constant less than 1 nM can be considered as leading compounds for the further development of a platform for targeted drug delivery to hepatocytes.
Funding
This study was supported in part by the Russian

Science Foundation grant 20-63-46029, State Topic AAAA-A21-121011290089-4 and MSU Program of Development.

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Public Health Toxicol 2021;1(Supplement):A15
DOI: 10.18332/pht/142076

Study of the influence of the surface of biopolymer chitosan fibrous and film materials on the morphofunctional state of cells

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Optical research methods have been widely used in pharmacology, medicine, and biotechnology to characterize new materials and objects In recent years. Modulation super-resolution interferometry is the most widespread and effectively used tool for studying surface properties in tissue engineering for the restoration of damaged tissues of a living organism[1]. The growth and controlled development of mesenchymal stem cells are carried out using matrices and scaffolds that promote cell proliferation.

This work aims to study the structure and proliferative properties of biodegradable polymer matrices and fibrous materials with anti-inflammatory activity created based on chitosan cross-linked by jenipin in combination with polypeptides secreted by mesenchymal stem cells[2]. The optimal conditions and methods for obtaining biopolymer matrices based on films of chitosan aminopolysaccharide crosslinked in the process of their production and fibrous materials have been determined[3]. In this work, we performed a non-invasive assessment of the results obtained (surface topography, microrelief character, heterogeneity of the polymer structure) and biological properties (biocompatibility, adhesiveness, immunogenicity, thrombogenicity, etc.) by interference microscopy using QPI technology. The effect of the obtained biopolymer composites on the viability, morphology, and functional state of cellular material (platelets, neutrophils, lymphocytes, MSCs) was studied. The morphodensitometric parameters of intact living cells were evaluated before and after contact with biopolymer composites.

Funding

This work was supported by Russian Foundation for Basic Research (project no. 18-29-17059).

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Public Health Toxicol 2021;1(Supplement):A16
DOI: 10.18332/pht/142077

The possibility of using gels based on polyvinylpyrrolidone in orthopedics and traumatology

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Endoprosthetics of joints is referred to as progressive and constantly developing methods of surgical treatment of patients with lesions of the musculoskeletal system of any genesis. However, the trend towards to increase of the number of endoprosthetics is inevitably accompanied by increasing in the number of patients with periprosthetic infection.

Prevention of periprosthetic infection consists not only in observing the rules of asepsis and antiseptics during the operation, but also in the selection of rational antibiotic therapy, which will ensure complete eradication of pathogenic bacteria and prevent the formation of biofilm on the surface of endoprosthesis, thereby preventing an acute postoperative periprosthetic inflammatory process that occurs for the first 3 months after surgery. The solution to the problem, from our point of view, can be achieved via using of polymer antimicrobial gels that provide a prolonged release of antimicrobial drugs in the area of periprosthetic inflammation.

To develop this kind of medical devices, a number of studies are required, including studies of local tissue reactions at the cellular level, studies of parenchymal organs and blood tests in order to determine the response of surrounding tissues to implantation and study of general toxic effects. We studied gels based on polyvinylpyrrolidone (PVP) with different viscosities and with addition of antibiotics. The test samples were implanted into the femoral muscle group of experimental animals.

In all studied tissue samples, after the use of different types of gels, differing in viscosity, the presence of bacterial microflora was not detected.

The inflammatory reaction in the form of infiltration by lymphoid cells was noticed only near the injected gels and did not spread into deeper layers of tissues.

The manifested tissue reaction is associated with mechanical stress, depending on the size and viscosity of the samples.

The detected diffusion of PVP-based gels into adjacent tissues and the formation of capsules from connective tissue is associated with the onset of its biodegradation.

The developed gels based on PVP, regardless of the manufacturing method, are inert in relation to the surrounding tissues, do not have a toxic effect on soft tissues and the body as a whole. The revealed insignificant inflammatory reaction in the surrounding tissues indicates an adequate local tissue reaction to the injection of foreign substance and the corresponding mechanical action during the period of exposure of the gels at the injection site.

Despite the different physicochemical properties of the gels, no critical local and general pathologies were identified, which suggests gels inertness in relation to the surrounding tissues and the absence of toxicity.

Public Health Toxicol 2021;1(Supplement):A17
DOI: 10.18332/pht/142078

Design and antimicrobial profiling of silver-chitosan nanocomposites for biomedical applications

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Health-care-associated infections and the development of antimicrobial resistance are one of the most serious public health problems worldwide. Bacterial infections are often difficult to treat because the bacteria can adapt rapidly to conventional antibiotics (ABs), which in turn has made their choice limited and expensive. Nowadays, nanotechnology holds great promise for the design of new antimicrobials, reducing the use of existing ABs, development of AB-resistant "superbugs" and the spread of pathogenic microbes in healthcare facilities.

One of the biomedically promising biopolymers is chitosan, which has gained interest in the design of wound dressings and implants, mainly due to its biocompatibility, biodegradability, antimicrobial and immune-modulating properties. Crosslinking of chitosan with biocidal metal-based nanoparticles, such as Ag or Cu can be a promising approach to create novel nano-antimicrobials that have both antimicrobial and immune-modulating effects.

The aim of the study was to (i) synthesize *Ag-chitosan-nanocomposites* (CS-AgNPs) and (ii) evaluate their antimicrobial potency and mode of action against the medically important bacteria Gram (-) *Escherichia coli* and *Pseudomonas aeruginosa*, and Gram (+) Staphylococcus aureus. In parallel, $AgNO_3$ and chitosan were analyzed as ionic and coating/stabilizer control. CS-AgNPs were synthesized by reduction of $AgNO_3$ with trisodium citrate in the presence of chitosan (Sigma, low molecular weight). Three types of CS-AgNPs with different silver to chitosan weight ratios (1:0.3, 1:1 and 1:3) were synthesized. The antimicrobial potency of CS-AgNPs was addressed by determining their minimum biocidal concentration (MBC) in deionized water to minimize the effect of silver ions speciation on its bioavailability and toxicity. Flow-cytometry and laser-scanning confocal microscopy (LSCM) was used to assess nanocomposite cell interactions.

We showed that the studied CS-AgNPs were effective antimicrobials against *E. coli, P. aeruginosa*, and *S. aureus*: 24-h MBC values ranged from 0.07 – 0.19 mg Ag/L, 0.31 – 0.63, and 0.50 – 0.69 mg Ag/L, respectively, and the antibacterial potency depends on the chitosan content. Flow-cytometry and CLSM study revealed the CS-AgNPs attachment onto the surface of bacteria.

Funding

This work was supported by the Estonian Research Council project PRG749, and ERDF projects NAMUR+ and TK-134.

Public Health Toxicol 2021;1(Supplement):A18
DOI: 10.18332/pht/142080

Evaluation of biological effects of nanomaterials: Cost-efficient tool box and corona challenges

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Nanomaterials and nanotechnologies allow breakthroughs practically in all fields of development, from car industry to medicine. As the progress cannot be achieved at any price, i.e. not taking into account the potential risks to human and environment, safety evaluation must be performed in parallel to the development of novel (nano)materials.

The authors of this presentation have long-time experience in eco-safety evaluation of different types of nanomaterials (NMs) using a combined approach based on thorough physico-chemical characterization of NMs coupled to the bioassays with crustaceans, protozoa, algae, plants and microbes. This approach has been successfully used on metallic NMs[1-3,5], e.g., antimicrobial NMs[3,4], rare-earth-elements and their oxides[5] and more recently also micro- and nanoplastics[6,7]. Last but not least, our pioneering studies incorporating the assays based on metal-sensing recombinant bacteria[4] have shown the leading role of shed metal ions in toxic effect of metal-based nanomaterials[8].

The COVID-19 outbreak created an unexpected challenge also to ecotoxicologists: firstly, the urgent need for novel antiviral materials has led to the rapidly increasing interest in use of e.g., copper and silver in different surface coatings, textiles and face masks etc. Indeed, silver, copper and zinc are efficient antimicrobials but have hazardous effects to aquatic species, especially to algae and crustaceans – important representatives of aquatic food chain[3]. Secondly, the need for protection of humans against Coronavirus has led to warning increase of use of single-use plastics (masks, gowns, gloves) being contradictory to the pre-COVID strategy envisaging cutting down the single-use plastics.

Thus, the NM safety research is very challenging but indispensable allowing to (i) connect the materials physico-chemical properties with their biological properties; (ii) discover the types of materials that can be safely applied, or, if not so, (iii) modified to be more safe (safe-by-design) or (iv) used as biocides and/or antimicrobials/antivirals (toxic-by-design)[3]. The current COVID-outbreak has proven the need to address all of these aspects.

Funding

This work was supported by ERDF TK-134 project, Estonian Reseach Council projects PRG749, PSG311, PUT1015 and MOBJD509.

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Public Health Toxicol 2021;1(Supplement):A19
DOI: 10.18332/pht/142081

Three main directions of magnetic field impact on biomaterials and bio-objects: Its efficiency and safety for biomedicine

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Exposure to magnetic field (MF) is one of the oldest instruments in biomedical practice along with the use of chemical, thermal and mechanochemical tools or approaches. However, in comparison to the latter ones, it has much more limited physical background so that its impact is much less predictable and reliable. As a result, its applications are scarce currently. This paper briefly discusses three main directions of the MF application in biomedicine in the past, nowadays and in the future.

The first one is the exposure to steady or low frequency (f<300 Hz) non-heating MF. It is the oldest but still the most controversial and the least reliable approach. There is no universally accepted theory or even mechanisms of its impact on biological objects. The second one is the use of radio frequency (f ~ 10-100 MHz) MF for heating biologic materials or objects due to eddy currents or dielectric losses. The influence of the MF itself in this approach appears to be negligible or insignificant.

And the third approach employs magnetic nanoparticles (MNP) introduced into the object beforehand or existing there naturally as a mediators and enhancers of MF action on biomolecular structures. Depending upon MF characteristics, heating (at f = 100-800 kHz) or magnetomechanical activation (at f < 1 kHz) within the region of interest can be implemented. In the latter case, the heating is negligible and the impact is due to the nanomechanical activation of biomolecular structures localized within the region close in size to MNP, i.e. within several tens of nanometers. Such nanomechanical activation can affect target cell functioning or induce its apoptosis.

Comparison of requirements and outcomes of these 3 approaches is presented, and advantages, drawbacks, uncertainties and risks of each of these approaches are analyzed.

Funding

Funded by RFBR grant 18-29-09154, supported also in part by Lomonosov MSU (Reg. Theme AAAA-A21-121011290089-4). The experimental work has been carried out in part at the Center of Collective Use of Derzhavin Tambov State University.

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Public Health Toxicol 2021;1(Supplement):A20
DOI: 10.18332/pht/142084

Surprises from diagnostic kit antibodies: Reinvestigation their carbohydrate specificity using synthetic oligosaccharides

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In recent decades the significant spread of fungal diseases has become a serious global problem. The mortality rate from fungal infections in the world is comparable to that of tuberculosis or malaria and already amounts several million cases per year. Due to the high mortality rate and the absence of specific clinical manifestations, the most important requirement for the successful treatment of mycoses is their early diagnosis. However, the enzyme immunoassay systems currently present on the market have certain drawbacks.

Information about the fine carbohydrate specificity of diagnostic antibodies is the basis for understanding the origin of false-positive and false-negative results and for improving the existing diagnostic tools. The development of effective methods for stereospecific synthesis of oligosaccharides allows us to create thematic libraries of ligands corresponding to the main polysaccharides of the cell wall of pathogenic fungi. These compounds and conjugates thereof are a powerful tool for the generation, selection and analysis of monoclonal antibodies. This approach allowed us to review the carbohydrate specificity of a number of known monoclonal antibodies and obtain new antibodies that recognize a given epitope, which is necessary for the development of new a generation of diagnostic tools.

Funding

This work was supported by the Russian Science Foundation (grant 19–73–30017).

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Public Health Toxicol 2021;1(Supplement):A21
DOI: 10.18332/pht/142086

Trail dr5-b-conjugated polymeric nanoparticles for targeted delivery in tumors

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In current study, polymeric nanoparticles based on poly-N-vinylpyrrolidone amphiphilic derivatives with hydrophilic polymer fragment and one anchor n-alkyl hydrophobic group were obtained, which were covalently conjugated to

the antitumor cytokine TRAIL DR5-B. Such system possesses a therapeutic perspective due to the selective induction of apoptosis in tumor cells by specific binding of TRAIL DR5-B to the DR5 receptor, increased stability and apoptotic signal amplification caused by higher local concentration of cytokine. In this work, we also investigated the effect of the particles composition on the size and sorption capacity, as well as antitumor activity in 3D in vitro models.

A pattern was revealed for an increase in the sorption capacity of particles with an increase in the content of the maleimide-modified polymer in the composition of the particles. It was shown that the particle size depends on the length of the polymer molecules, and the combination of macromolecules with different molecular weight of the polymeric fragment in the composition of the particles affects the steric accessibility of maleimide groups for TRAIL DR5-B/V114C protein molecules, and, thereby, on their sorption capacity.

The cytotoxicity of TRAIL DR5-B-conjugated polymeric nanoparticles with the maximum sorption capacity was tested in 3D models based on multicellular tumor spheroids of various origins in vitro. TRAIL DR5-B-conjugated polymer particles demonstrated enhanced cytotoxicity compared to free TRAIL DR5-B in 3D MCF-7 breast adenocarcinoma tumor spheroids model. At the same time, the absence of cytotoxicity to normal mesenchymal stem cells of the FRSN line was shown. As a result, a completely novel type of polymeric nanoparticles for targeted delivery of the receptor-selective cytokine TRAIL DR5-B in tumors was obtained. After optimization of the polymer composition, size, and sorption capacity, TRAIL DR5-B-conjugated polymeric nanoparticles based on poly-N-vinylpyrrolidone amphiphilic derivatives can be investigated in xenograft preclinical models in vivo with promising clinical application for the treatment of a wide range of tumors.

Funding

The work of Kuskov A.N. was supported by D. Mendeleev University of Chemical Technology of Russia (Project Number K-2020-018).

Public Health Toxicol 2021;1(Supplement):A22
DOI: 10.18332/pht/142087

Silver-containing hydrogel based on polyvinyl alcohol modified with nanoscale cyclotriphosphazene

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The development of a new generation of wound coverings, such as those based on silver-containing polyvinyl alcohol hydrogels, is required to combat microbial infections. Such hydrogels are a reticulated polymer system that is biocompatible with the tissues of living organisms and allows imitation of biological tissues. This is due to the water-retention capacity, film-forming properties of the gels, vapor permeability, and the ability to absorb a degree of wound exudate.

Silver nanoparticles are known for their antiseptic, anti-inflammatory, and multi-level antimicrobial effects. However, silver nanoparticles are highly cytotoxic and also prone to aggregation, which can lead to a loss of antimicrobial properties.

To solve the problem of silver particle aggregation in this work, functional substances were used that can be evenly distributed in the matrix of polyvinyl alcohol. For this purpose, nanosized p-β-carboxyethenylphenoxy-p-formylphenoxycyclotriphosphazene was used as a crosslinking agent. This compound is nontoxic, which makes its application safe for wound healing. Due to the fact that phosphazene has carboxyl and aldehyde functional groups at the same time in its composition, it is able to act both as a crosslinking agent and as a carrier of silver ions.

Based on the developed system, wound coverings were obtained using different amounts of crosslinking agent. It was found that as the content of the crosslinking agent decreases, the degree of water absorption of the material increases. At the same time, wound coverings with crosslinking agent concentration of 4,5 wt.% have the highest water absorption. After silver introduction into the hydrogel, its X-ray fluorescence elemental analysis was carried out. The silver content was 0.87 wt.%, which is sufficient to exhibit antiseptic properties.

Funding

The work was supported by Mendeleev University of Chemical Technology of Russia. Project Number 2020-014.

Public Health Toxicol 2021;1(Supplement):A23
DOI: 10.18332/pht/142088

Biodegradable 2D and 3D scaffolds based on chitosan for regenerative medicine

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Chitosan (Chit) is a promising material, as it possesses the properties necessary for tissue engineering structures. Chit is a biodegradable, biocompatible natural polymer. Based on Chit scaffolds with specified physicochemical properties can be obtained, for example, by modifying chitosan by amino groups[1]. Improvement of mechanical properties, as well as regulation of the rate of biodegradation and hydrophilic-hydrophobic balance, can be achieved by copolymerization of chitosan with other monomers / oligomers. Polylactic acid (PLA) meets the requirements of tissue engineering for biomaterials due to its biocompatibility and the possibility of obtaining scaffolds with the required morphology on its basis. PLA is one of the few synthetic polymers used clinically. However, the hydrophobic nature of PLA and the lack of functional groups limit cell adhesion, and slow biodegradation limits the use of PLA scaffolds. The creation of copolymers based on chitosan and oligolactides will allow combining the advantages and leveling the disadvantages of each component. The purpose of this work is to obtain polymer matrices in the form of films (2D) and microporous hydrogels (3D) based on chitosan, as well as its graft copolymers with oligolactides, the study of their structure, physicochemical properties, as well as the growth and differentiation of cells on / in them in an in vitro model. For the formation of films and hydrogels, we used copolymers of chitosan, (MW 60 kDa, SD 0.9), with oligo (L, L- and / L, D-lactides) with MW 5 kDa, (Chit-L, L and Chit-L, D, respectively), which were obtained by the method of solid-phase synthesis[2]. Films (2D) were obtained from copolymer solutions by pouring, and 3D macroporous hydrogels - by lyophilization of these solutions. The structure of the hydrogels was studied by confocal laser microscopy and represented a system of interconnected micropores with an average size of 120-140 μm. The cytotoxicity of the obtained scaffolds was tested after incubation with the culture medium for 24 h by examining the toxicity of the obtained extracts, using the MTT test. It was shown that the surface of the modified scaffolds ensured adhesion of mouse fibroblast L929 cells (confocal microscopy), as well as their growth and proliferation during long-term cultivation in hydrogels (MTT test). During the

cultivation of human mesenchymal stromal cells (MSC) isolated from adipose tissue, it was found that hydrogels provide the conditions necessary for cell proliferation, and copolymer films promote their differentiation in the osteo direction. MSC differentiation was assessed by the activity of alkaline phosphatase.

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

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Public Health Toxicol 2021;1(Supplement):A24
DOI: 10.18332/pht/142090

Development and evaluation of 3-hydroxyquinoline-4-carboxylic acid derivatives for ASGPR-targeted drug delivery

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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.

Discovery of new ligands is an important goal in drug delivery. Quinoline derivatives has shown to be potent inhibitors of another C-type lectin (P-selectin)[1,2,3], marching to the clinical studies for the treatment of athero-thrombotic vascular events. Similar structures were obtained in the previously reported[4] rational in silico screening for ASGPR ligands, albeit the measured affinity was relatively low. In this work we try to optimize the affinity of quinoline derivative structure. After *in silico* screening sixteen 3-hydroxyquinoline-4-carboxylic acid-derivatives were selected and synthesized. Due to the presence of hydroxyl at position 3, all structures had a solubility of at least 0.5 mM. The affinities to ASGPR has been assessed using surface plasmon resonance (SPR) spectroscopy technique. All structures exhibited strong binding affinity with K_D values in the nanomolar range (0.1 - 30 nM), that considerably exceed the affinities of native ASGPR ligands – N-acetylgalactosamine and galactose.

As all of the obtained compounds turned out to be potent ASGPR ligands one may consider quinoline-4-carboxylic acids to be a practically useful monovalent ligand as a cheaper, more available and more efficient GalNAc substituent as a vector for targeted drug delivery in hepatocytes.

Funding

This study was supported in part by the Russian Science Foundation grant 20-63-46029, State Topic AAAA-A21-121011290089-4 and MSU Program of Development.

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Public Health Toxicol 2021;1(Supplement):A25
DOI: 10.18332/pht/142092

Novel synthetic bone substitute materials based on dyaldehyde dextran

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Currently, the most urgent problem around the world is the treatment of bone defects resulting from injuries and diseases. An ideal material for bone tissue replacement should have the following properties: biocompatibility, optimal rate of biodegradation, bioinertness, the ability to release physiologically active substances and long-term protection against bacterial attack.

The aim of the work was to create fully synthetic biodegradable composites with polymodal drug release.

The basis for the implant was obtained by periodate oxidation of dextran according to the Malaprade reaction. After that, openly porous samples were obtained by preparing a conjugate of collagen with dialdehyde dextran, then it was mixed with water and various additives, freeze-dried and tableted. The pore size was determined by the polymer:water ratio and monitored by atomic force microscopy and microphotography. The rate of *in vitro* biodegradation was determined by the rate of release of L-hydroxyproline, since collagen contains a large amount of this amino acid. The study of osteoinductive properties in vivo was carried out according to the following method: a tablet of a given composition was placed in the tibia. Untreated demineralized bone matrix was placed in the other bone as a reference. Osteoinductive properties were monitored using tomography.

The results of atomic force microscopy and micrographs show that the resulting composite tablet mainly has pores with a diameter of 0.8-1 microns, which is optimal for the germination of capillaries and successful regeneration of bone tissue.

Based on *in vitro* experiments, it was found that the biodegradation of pure collagen under the action of collagenase takes 5 days. The use of dialdehyde dextran as a biodegradation inhibitor makes it possible to reduce the rate of enzymatic hydrolysis by three times.

Tomographic observations demonstrate that the material

partially biodegraded, and callus began to form, which indicates the initial stage of bone fusion. The demineralized bone matrix was completely resorbed.

Thus, completely synthetic biodegradable, bone-replacing polymer composites based on dialdehyde dextran capable of releasing physiologically active substances at different times have been developed.

Funding

The work was supported by Ministry of Education and Science of the Russian Federation as part of the state assignment for the FSSM-2020-0004 project.

Public Health Toxicol 2021;1(Supplement):A26
DOI: 10.18332/pht/142093

Study of diagrams of the phase state of the PVA-water system in a wide range of temperatures and compositions

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Polyvinyl alcohol is a synthetic polymer that is widely used in industry for commercial, medical, and food applications. Crosslinked PVA can be used as high porosity hydrogels due to the network structure of crosslinked polymer chains that induces molecular diffusion[1]. Small molecules (eg peptides, proteins) can diffuse in and out of the matrix, while larger molecules (eg plasmid DNA) often enter the pores and become trapped within the mesh. This property makes it possible to use crosslinked PVA as porous hydrogel filter systems.

In this regard, we are faced with the task of obtaining composite porous filtration systems that allow multiple contact of the air mass with the biocidal surface formed in these systems intended for disinfection.

At this stage, a diagram of the phase state of the PVA - water system was constructed in a wide range of temperatures and compositions, from which it can be seen that the phase equilibrium in the PVA - water system is characterized by a complex amorphous-crystalline equilibrium.

It has been established that for the formation of porous hydrophilic sorbents with a predominantly binding and stable pore system under conditions of prolonged operation in humid environments, it is necessary - (1) to carry out the process of structure formation in the PVA region, solutions in water corresponding to the location of the water liquidus line on the phase state diagram; (2) a network of chemical bonds should be created in the dispersed phase of the sorbent, which can then be used to regulate and stabilize the hydrophilic properties of the sorbent during its operation and regeneration.

Funding

This work was supported by Russian Foundation to Basic Research 18-29-18039.

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Public Health Toxicol 2021;1(Supplement):A27
DOI: 10.18332/pht/142094

Nanoscale pharmacy carriers based on surface-active derivatives of ethyl-2-cyanoacrylate

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Alkyl-2-cyanoacrylates are widely used in medicine due to the following features: biodegradability, low toxicity, and the ability to quickly polymerize in a neutral aqueous medium without the use of initiators. This work is devoted to the synthesis of surface active derivatives of ethyl 2-cyanoacrylate and various long-chain primary alcohols, as well as drug-containing nanoparticles based on them. The work was carried out in several stages. First, adduct monomers were formed from various primary fatty alcohols and purified ethyl-2-cyanoacrylate by acid catalysis in an inert atmosphere. Recrystallized 2-cyanoacrylic acid, that was previously obtained from pure ethyl-2-cyanoacrylate by the mechanism of thermal β-decay of carboxyl compounds, was used as a catalyst, due to its high acidity, non-toxicity and good solubility in cyanoacrylates and their derivatives. A series of empty model particles, as well as particles containing the anti-tuberculosis drug rifampicin, were obtained in a neutral aqueous medium from surface-active adduct monomers based on linear primary alcohols by the mechanism of emulsion polymerization with partially cross-linked polymer chains formation. The particle size was investigated by the method of dynamic light scattering. It was assessed that size of the particles depends on the used primary alcohol molecular weight. For the filled particles, the average hydrodynamic diameter did not exceed 350 nm. The content of the physiologically active substance in the filled particles was determined by UV spectrophotometry. It was 90% of the initially loaded preparation. The formation of adduct monomers and the structure of nanoparticles obtained from them were confirmed by the combined method of 1H NMR and mass spectroscopy. Thus, a technique for obtaining nanoscale carriers of drugs that have prospects in the treatment of such serious diseases as tuberculosis was developed.

Funding

The work was supported by Ministry of Education and Science of the Russian Federation as part of the state assignment for the FSSM-2020-0004 project.

Public Health Toxicol 2021;1(Supplement):A28
DOI: 10.18332/pht/142095

Resorption of highly porous pleural implants based on polylactide and polycaprolactone during intrapleural implantation in an experiment

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Introduction

The functional purpose of a bioresorbable pleural implant is to provide a reversible collapsosurgical effect on the area of lung tissue in which the tuberculous cavity of destruction (cavity, fibrous cavity) is located, by preserving the volume and shape of the implant for a given time, followed by bioresorption and restoration of the volume and function of the affected lung.

Objective

To evaluate changes in the volume and shape of highly porous implants based on polylactide (PLA) and polycaprolactone (PCL) during intrapleural implantation in an experiment.

Material and methods of research

The objects of research are porous disks with a diameter of 20 mm and a height of 10 mm of two compositions. Sample No. 1 consists of a mixture of PLA/PCL with a polymer ratio of 3:1 (3 wt.%). Sample No. 2 is made of PLA/PCL with a polymer ratio of 1:1 (1.7 wt.%). Laboratory samples of pleural implants were obtained by cryoliophilization. The porosity ranges from 97.0 to 98.3%, and the Young's modulus is from 100 to 1800 kPa.

Implantation was performed in 5 rabbits simultaneously in both pleural cavities under endotracheal anesthesia. The duration of implantation was 7-21-54-76-78 weeks. The consistency, dynamics of changes in the volume and shape of the implants were evaluated.

Results

There is a stable preservation of the volume and shape of both samples for 21 weeks. After 54 weeks, the volume of samples No. 1 and No. 2 decreases only by 10 and 20%, which is not a clinically significant decrease in volume. A significant decrease in volumes is observed in the fourth and fifth periods of implantation, for samples No. 1 and No. 2 – 90% and 96%, respectively. The loss of volume occurs mainly due to a decrease in the thickness of the implant. At all implantation periods, the soft-elastic consistency of both samples is preserved.

Conclusions

The clinically significant preservation of the volume and shape of the samples until the third implantation period (54 weeks) indicates the prospects of both studied laboratory samples in clinical use. Considering that the terms of bioresorption of pleural implants in living organisms correlate with the level of metabolism, it can be assumed that in clinical conditions the studied highly porous pleural implants will provide a collapsosurgical effect in the target time from 1 to 3 years or more.

Public Health Toxicol 2021;1(Supplement):A29
DOI: 10.18332/pht/142096

Biodegradable macroporous polyvinyl alcohol hydrogel in the treatment of patients with perineal wound suppuration after pelvic evisceration surgery

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One of the main methods of treatment of locally advanced tumors of the small pelvis and soft tissues of the perineum is surgery, which consists of removing the tumor affecting the small pelvic organs, external genitalia, and rectum. For tumors of the distal parts of the rectum, abdominopelvic extirpation of the rectum was proposed by Miles in 1908, and somewhat later, in 1948, the American surgeon A. Brunschwig first published the results of pelvic extirpation. Modern medicine increasingly uses methods of surgical interventions using various materials or products capable of replacing or restoring the function of an organ or tissue damaged as a result of a pathological process or trauma. Depending on the purpose of its application, it can exist permanently in the body or be replaced by its own tissues. When used in the body, the implant must meet a number of requirements: not to emit harmful substances; to withstand sterilization conditions by standard methods; to be made of available materials using a fairly simple technology.

When using polymers as implants, their properties such as biological inertness, biocompatibility, biodegradation, biodegradation, and biosubstitution are taken into account. We bring to your attention the results of the first experience of using a biodegradable material based on a macroporous polyvinyl alcohol hydrogel combined with betadine in the treatment of patients with perineal wound suppuration after (EPO) Exenteration of the Pelvic Organs Surgery. This method of treatment was used in the complex treatment of 5 patients, operated on for locally advanced forms of pelvic cancer, 3 women of 56 to 71 years old. In two cases anterior, in two cases posterior and in one case total pelvic evisceration was performed. Four patients underwent laparotomy and one patient underwent laparoscopy, and the perineal wound suppuration was observed 3-7 days after the surgery. In all patients during the first two weeks local treatment was carried out according to the same scheme - daily washing of the wound with antiseptic solutions, drainage and wound tamponization with the use of Levomycol and Betadin ointments. Due to the low efficiency of local treatment of perineal wounds, a polyvinyl alcohol-based biodegradable implant was used. The scheme of material application was as follows: during the first week, the implant was placed for three days followed by replacement with sterile material cut to the size of the cavity; subsequently, replacement was usually performed on the seventh day. The average treatment period was 21±3 days. The patient was then discharged with the implant installed permanently.

Thus, the proposed method of using the biodegradable material based on cross-linked PVA combined with povidone-iodine (betadine) in the treatment of patients with perineal wound suppuration after (EPO) Exenteration of the Pelvic Organs Surgery can become an effective method in the complex of therapeutic measures.

Public Health Toxicol 2021;1(Supplement):A30
DOI: 10.18332/pht/142098

Polymer photo-curing dental composition with prolonged antimicrobial action

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Dental fluoride compositions are of significant interest for the treatment of caries. This is due to the fact that the fluoride ion, released from the composition, is able to inhibit the enzymes of cariogenic bacteria, as well as reduce the demineralization of enamel by forming a protective layer on its surface. However, various fluoride-containing additives can be released from restorative materials and entered the human body orally or by resorption in oral mucosa, which can cause intoxication of the surrounding tissues and the body as a whole.

In this work, a fluorine-containing photo-curable dental composition based on bisphenol A-glycidyl methacrylate, triethylene glycol dimethacrylate and a fluorine-containing modifier, representing fluorocyclotriphosphazene, also containing 4-allyl-2-methoxyphenoxy groups, was obtained. The presence of fluorine in the modifier provides an antimicrobial effect, and due to the allylic groups, phosphazene is embedded in the polymer matrix during copolymerization with acrylates, so it doesn't wash out of the composite and doesn't penetrate into the human body.

During the study of the properties of the modified compositions, it was found that they have a prolonged antimicrobial effect in relation to the oral microflora. In addition, mechanical properties such as microhardness, compressive and flexural strength are not deteriorated when modifying the compositions with phosphazene. The resulting composition is proposed to be used as a restorative dental material, in

particular, for the manufacture of photo-curing fillings.

Funding

The work was supported by Mendeleev University of Chemical Technology of Russia. Project Number 2020-014.

Public Health Toxicol 2021;1(Supplement):A31
DOI: 10.18332/pht/142130

Glycosylation of gossypol as a method to reduce its toxicity

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Gossypol is a naphthaldehyde with various types of physiological activity: antiviral, interferon-inducing, antitumor, antioxidant, etc. But along with this, gossypol is highly toxic. This work is devoted to the development of a method for chemical modification of gossypol to eliminate its toxicity while preserving its biological properties.

A method was developed for the synthesis of gossypol-containing compounds by glycosylation. The properties of the products formed as a result of the acid hydrolysis process, similar to the reaction in the acidic environment of the stomach, have been studied.

Model substances containing gossypol were obtained by the Böeseken reaction in an alkaline medium. Briefly, a solution of gossypol in an alkaline medium was added to the glucose dissolved in a borate buffer. Then, the solutions were mixed and reacted at room temperature under argon flow for 48 hours. Then the mixture was washed to remove unreacted reagents and freeze-dryed. Acid hydrolysis of model glycosides was carried out at 37°C for 4 hours in a buffer solution containing hydrochloric acid (pH=2). The resulting water-insoluble apogossypol was separated by filtration and dried in air. The obtained compounds were investigated by 13C NMR and MALDI-TOF methods.

As a result of the condensation reaction of naphthaldehyde with glucose in a borate buffer, addition occurs at the C1 hydroxyl group of anomeric carbon atom of glucose. Addition to the C2, C3, C4 atoms does not occur. This is due to the orientational effect of the Böeseken complex used to ensure the regioselectivity of the reaction. During acid hydrolysis of model compounds, which are di-, tri-, and tetraglycosides in the form of complexes with boric acid, apogossypol and its oxidation products are formed. These substances are much less toxic due to the absence of aldehyde groups that cause cytotoxicity.

Thus, methods of synthesis were developed and model compounds of gossypol were characterized, which gives the right to further study the physiologically active compounds of gossypol from a biological point of view.

Public Health Toxicol 2021;1(Supplement):A32
DOI: 10.18332/pht/142131

Properties of degradable polyhydroxyalkanoates with different monomer compositions

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To synthesize and investigate polyhydroxyalkanoates (PHAs) with different monomer composition and percentages and polymer films prepared from them. Various PHAs: homopolymer poly-3-hydroxybutyrate P(3HB) and 2-, 3-, and 4-component copolymers comprising various combinations of 3-hydroxybutyrate (3HB), 3-hydroxyvalerate (3HV), 4-hydroxybutyrate (4HB), and 3-hydroxyhexanoate (3HHx) monomers were synthesized under specialized conditions. Relationships were found between the monomer composition of PHAs and their molecular-weight and thermal properties and degree of crystallinity. All copolymers had decreased weight average molecula rweights, M_w (to 390–600 kDa), and increased values of polydispersity (3.2–4.6) compared to the P(3HB). PHA copolymers showed different thermal behavior: an insignificant decrease in T_{melt} and the presence of the second peak in the melting region and changes in parameters of crystallization and glass transition. At the same time, they retained thermostability, and the difference between T_{melt} and T_{degr} was at least 100–120 °C. Incorporation of 4HB, 3HV, and 3HHx monomer units into the 3-hydroxybutyrate chain caused changes in the amorphous to crystalline ratio and decreased the degree of crystallinity (C_x) to 20–40%. According to the degree to which the monomers reduced crystallinity, they were ranked as follows: 4HB – 3HHx – 3HV. A unique set of films was produced; their surface properties and physical/mechanical properties were studied as dependent on PHA composition; monomers other than 3-hydroxybutyrate were found to enhance hydrophilicity, surface development, and elasticity of polymer films.

Funding

The part of the reported study on polymer synthesis and examination was funded by RFBR and KKRF Grant No. 19-43-240012 "Biological and physical principles of production of new generation biomaterials". The work on production and investigation of polymer films was carried out as part of the State Assignment of the Ministry of Education and Science of the Russian Federation No. FSRZ-2020-0006.

Public Health Toxicol 2021;1(Supplement):A33
DOI: 10.18332/pht/142133

Novel porous matrices based on modified polyvinyl alcohol for air filtration

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The use of polymeric cryogels based on PVA for the creation of composite filter systems for the disinfection of the gaseous medium in confined spaces is of great interest in connection with the information that has appeared on the low efficiency of the Potok device used at the International Space Station for air purification[1].

It is known that carrying out the process under cryostructuring conditions makes it possible to obtain PVA cryogels with directed pores, which makes it possible to use the samples as filtering systems.

To date, a number of methods are known for obtaining physically and chemically crosslinked PVA gels. However, the gels obtained by the methods described in the literature have relatively low porosity. One of the ways to overcome this problem is the original approach proposed by us earlier, which consists in crosslinking in water-frozen systems of acrylic derivatives of PVA[2]. This method makes it possible to obtain a product with a porosity of 85-86% from polymer solutions with a concentration of ~ 4%.

However, to obtain matrices with even higher porosity, a new combined approach was developed in this work. At the first stage, the PVA solution is frozen with an initiator, followed by freeze drying, as a result of which a three-dimensional porous structure is formed. In the second stage, crosslinking occurs under controlled heating.

As a result, it was possible to obtain crosslinked systems even with a polymer concentration in the solution of only 1%.

At the next stage, the equilibrium swelling of the obtained samples was assessed. It has been established that with prolonged heat exposure, a decrease in equilibrium swelling is observed. This fact can be associated both with a change in the hydrophilicity of the crosslinked polymer chains due to the oxidation of hydroxyl groups under the influence of an excess of initiator, and due to a change in the porosity of the system.

Funding

This work was supported by Russian Foundation to Basic Research 18-29-18039.

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Public Health Toxicol 2021;1(Supplement):A34
DOI: 10.18332/pht/142134

Polyelectrolyte multilayer capsules modified with antitumor cytokine DR5-B and loaded with doxorubicin for targeted drug delivery to tumor cells

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The development of targeted drug delivery systems is a challenge in biomedicine. Polyelectrolyte multilayer capsules (PMC) are promising due to their biocompatibility and biodegradability as well as drug encapsulation efficacy[1]. Cytokine TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) could be used for targeted anticancer therapy due to cell apoptosis induction via death receptors, in particular DR4 and DR5. However, a short half-life and rather low apoptosis-inducing capacity obstruct TRAIL use in clinic. Recently, a DR5-specific TRAIL mutant variant, namely DR5-B with enhanced pro-apoptotic activity has been developed[2]. On the other hand, conjugation of TRAIL or its mutants to various nanocarriers is of great importance, since it allows to increase TRAIL stability, half-life and apoptosis-inducing activity. Moreover, loading TRAIL-containing nanocarriers with antitumor drugs could provide an enhanced targeted drug delivery.

The aim of the study was to obtain biodegradable polyelectrolyte capsules, modified by conjugation with DR5-B and loaded with doxorubicin (DOX) and to evaluate their cytotoxicity in both 2D and 3D *in vitro* models. For this purpose, two types of PMC which differed in mean diameter, namely 500±110 nm and 300±60 (after thermally induced compression) were obtained from poly-L-arginine and dextran sulphate using layer-by-layer technique. The PMC physical-chemical properties (mean diameter, ζ-potential, storage stability, DOX encapsulation efficiency) were studied.

The PMC accumulation and localization in human breast adenocarcinoma MCF-7 cells and human colorectal carcinoma HCT-116 cells in both 2D (monolayer culture) and 3D (tumor spheroids) in vitro models were studied by confocal microscopy and flow cytometry. Thermally compressed PMC with mean diameter of 300 nm were found to accumulate in cells faster than those with mean size of 500 nm. Nevertheless, both types of PMC-DR5-B accumulated in cells faster than free DR5-B. Cytotoxicity of PMC-DR5-B and loaded with DOX was studied by MTT-test for HCT-116 and MCF-7 cells, as well as in human fibroblasts Bj-5ta (normal cells) in both 2D and 3D in vitro models. PMC-DR5-B (300 nm) were shown to be more cytotoxic than PMC-DR5-B (500 nm) in both 2D and 3D models. PMC-DR5-B (300 nm) loaded with DOX were non-toxic to normal Bj-5ta cells.

Thus, PMC-DR5-B could be assumed as promising targeted drug delivery system to cancer cells.

Funding

The work was supported by Russian Science Foundation (grant No. 21-14-00224) and the grant of the President of the Russian Federation (МК-1109.2021.1.3).

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Public Health Toxicol 2021;1(Supplement):A35
DOI: 10.18332/pht/142136

Micellar nanosystems based on functionalized amphiphilic copolymers of N-vinylpyrrolidone for creating theranostic platforms

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Polymeric drug delivery systems attract increased attention due to their ability to ensure accurate delivery of active substances exact to the site of action as well as reduce off-target cytotoxicity, which is extremely important during the chemotherapy of cancer. Amphiphilic copolymers of N-vinylpyrrolidone containing a terminal hydrophobic moiety are capable of forming micellar structures with hydrophobic core and hydrophilic corona in an aqueous medium, where a hydrophobic drug can be encapsulated in the micelles core. In this work, the PVP copolymers containing one terminal thiooctadecyl group were functionalized with acrylic acid. The presence of free carboxylic acid groups in the hydrophilic micellar shell provides ample opportunities for modifying the surface of micelles to create theranostic platforms.

Copolymers of acrylic acid and N-vinylpyrrolidone containing only one terminal hydrophobic group were obtained in the molecular weight range from 3.5 to 11 kDa. The synthesis was carried out in dioxane solution for 3 hours at a temperature of 70°C in the presence of dinitrile azobisisobutyric acid as radical polymerization initiator and octadecyl mercaptan ($C_{18}H_{37}SH$) as chain transfer agent.

The structure of the copolymers was confirmed by ^{13}C and ^{1}H NMR spectroscopy and MALDI-TOF mass spectrometry. Nanoaggregates of copolymers were obtained by ultrasonic dispersion. The average hydrodynamic diameter of the particles and the particle size distribution (polydispersity index, PDI) were determined by dynamic laser light scattering (DLS). The determination of the surface charge of the particles (zeta potential) was carried out by microelectrophoresis on

the Zetasizer Nano ZS analyzer (Malvern Instruments, Great Britain). The nanoparticles had sizes ranging from 30 to 200 nm and a weakly negative surface charge from -21 to -14 mV. The obtained micelles can be functionalized with gadolinium as a contrast agent for MRI. Furthermore, the presence of free groups of carboxylic acids allows us to covalently attach fragments aimed at cancer cells to the polymer (FALGEA peptide).

Thus, the unique structure of the obtained nanoscale micelles can ensure the delivery of an active substance encapsulated in the micelle core simultaneously with a selectivity agent and/or an imaging agent immobilized on the micellar corona, which open up broad prospects for the diagnosis and therapy of cancer diseases.

Public Health Toxicol 2021;1(Supplement):A36
DOI: 10.18332/pht/142137

Gel coatings of bone implants based on modified polysaccharides with immobilized antibiotics for use in regenerative medicine

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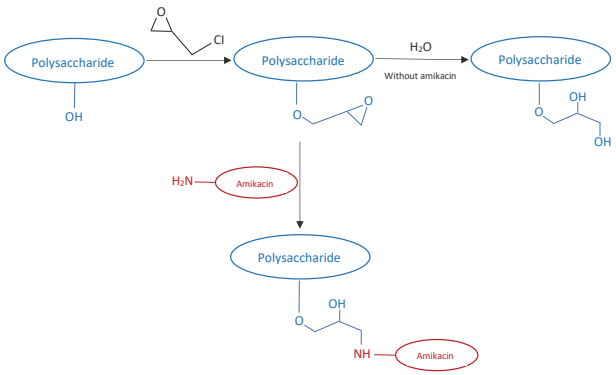
A variety of biomaterials are widely used in modern regenerative medicine. Particularly in traumatology, products made from chemically stabilized bone matrices are used. Throughout the entire life of the implant, long-term protection against bacterial infection is required. Unlike living bone, a bioprosthesis is not able to resist bacterial attack on its own. This study was carried out within the framework of the general concept of creating physiologically active biocomposite materials and devoted to the creation of materials necessary for replacement of damaged bone tissue capable of locally releasing antibiotics into the bloodstream. A method has been developed for treating biological tissue (bovine bone) used as the basis for a physiologically active biocomposite. It contains a polymeric active bioabsorbable polysaccharide gel cross-linked with epichlorohydrin and bis-epoxides under alkaline conditions. Commonly used clinical blood substitutes hydroxyethyl starch and dextran as well as hydroxyethyl cellulose was used as a polysaccharide base.

Comparative tests of the bacteriostatic effect of the gel layer were carried out using the method of inhibition of the growth of the culture of Staphylococcus aureus P209 on agar medium. When the bioprosthesis comes into contact with the staphylococcus lawn a zone of growth inhibition is observed in a day around the sample, under the samples and on their surface. Thus, the release of amikacin occurs locally under the influence of a bacterial attack and stops with the destruction and removal of bacteria.



The dynamics of antibiotic release was studied. It has been shown that in the absence of bacterial attack, the dextran-based gel does not decompose or release an antibiotic into the bloodstream. Under the influence of the dextranase enzyme released by staphylococcus, the gel decomposes with local antibiotic release, which provides local protection against bacterial attack. The effectiveness of the protection was confirmed in the in vitro experiments.

Funding
The work was supported by Ministry of Education and Science of the Russian Federation as part of the state assignment for the FSSM-2020-0004 project.
Public Health Toxicol 2021;1(Supplement):A37
DOI: 10.18332/pht/142143

Mapping the mechanical properties of wood annual growth rings using nanoindentation as an additional quantitative method of dendrochronology

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Analysis of the morphology and sizes of annual growth rings of wood by means of technical vision gives a lot of information about the climatic, ecological and other conditions of plant growth. However, optical methods do not allow assessing the physical, in particular micro- and macro-mechanical properties of wood in each year of growth and, moreover, within each growing season.

The paper proposes a quantitative method for assessing Young's modulus and wood hardness with a resolution from units of nm to tens of microns by the method of automated mapping of these characteristics using an atomic force microscope operating in a contact mode or a nanoindentometer. The latter is specially designed for continuous recording of the immersion depth of the diamond probe and the penetration resistance force, which, after processing the loading diagrams, makes it possible to determine up to 5 independent local characteristics of the material on a nano- or microscale. Modern nanoindentometers allow in automatic mode to make several thousand individual measurements at programmed points and then build a map of local mechanical properties.

The analysis of such maps makes it possible to determine with high accuracy the position and thickness of annual growth rings and layers of early and late wood, the course of changes in mechanical properties within each annual ring, and to link these dependencies with climatic and ecological growing conditions.

Examples of such analysis of maps of mechanical properties on cross sections of pine (Pinus sylvestris L.), linden (Tilia cordata Mill.), Oak (Quercus robur L.) and birch (Betula pendula Roth) are given.

Funding
This research was funded by the Russian Science Foundation, grant number 21-14-00233.
Public Health Toxicol 2021;1(Supplement):A38
DOI: 10.18332/pht/142150

Recultivation of oil-contaminated soils with humic acids and their polymer forms

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To restore oil-contaminated soil in this work, humic acids in

monomeric and polymeric forms were used in the process of sorption decontamination and purification of oil-contaminated soils. The choice of these reagents is due to high reactivity of humic acids in relation to petroleum hydrocarbons, which is associated with presence of a hydrophobic aromatic framework in their structure. This made it possible consider humic substances as natural detoxicants capable of reducing ecological load of oil pollution on environment.

The studies were carried out on soil sampled from bottom of open evaporation ponds of LLP "Atyrau Oil Refinery" on herbaceous plants from legume family - alfalfa, melilot, sainfoin. The initial oil content in the studied soil -17.38%, the pH of water extract of soil -7.62, electrical conductivity -2735Sm/m, the salt content -1680mg/kg.

Among leguminous grasses, alfalfa occupies leading place, being the main source of vegetable protein. Due to its productive longevity and ability to assimilate atmospheric nitrogen in grass mixtures, it increases their yield. Melilot saturates the soil with oxygen and nitrogen, improves the structure of the soil. Sainfoin is a soil conditioner, used as a green fertilizer, which gives high yields even on poor washed-out soils of the slopes of hollow, ravines.

The soil was treated with 0.1% and 0.5% solutions of humic acids, then samples were stirred with an electric stirrer until uniform and dried in air at room temperature for 24 hours. The seeds were also treated with a 0.0025% solution of polymeric form of humic acids.

The study of the phytotoxicity of soils was carried out in laboratory conditions; the sowing of herbaceous plants was carried out in plastic cups. Sowing depth of seeds - 1 cm.

In the experiments, phenological observations were carried out in each variant. Research have shown that seedlings were not observed in soils treated with 0.1% and 0.5% solutions of monomeric form of humic acids. However, with additional treatment of seeds with a 0.0025% solution of the polymeric form of humic acids, the first shoots appeared on the 7th day from the moment of sowing. The largest number of sprouts was observed for sainfoin seeds.

Analyzing the data obtained, it is not difficult to notice that the polymeric form of the growth stimulator turned out to be a factor affecting the growth of the assimilation surface, and, as a result, an increase in seed germination.

Funding

This work was financially supported by grant funding for scientific research from the Committee of Science of the Ministry of Education and Science of Republic of Kazakhstan for 2021-2023 №AP09260008.

Public Health Toxicol 2021;1(Supplement):A39
DOI: 10.18332/pht/142152

Oil-contaminated waste water treatment

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Wastewater from industrial production, as well as from enterprises for the extraction and processing of oil, containing organic substances, polluting groundwater and rivers through the soil is one of the main environmental problems.

Effective technologies of wastewater treatment from these enterprises and bringing them to production standards for further use is one of the most important tasks of environmental protection.

In this work, to solve the noted problem, a purification method has been developed, including the addition of a coagulant based on an aluminum-containing component, followed by thickening and filtration of the precipitate. Rau-85 aluminum alloy was used as the aluminum-containing component. The aluminum alloy is used in the form of a powder that is stable in air. Alloy composition (wt%): 85 aluminum, 5 indium, 5 gallium and 5 tin. The method takes place in one stage, it is environmentally friendly, since it does not require large amounts of coagulant (10-50 g/t). When the alloy is added to oily waste water, it actively interacts with water with the formation of a precipitate of metal hydroxides that sorb polluting oil molecules. The formation of hydroxides of aluminum and other metals included in the alloy occurs with the release of hydrogen and heat. The release of hydrogen creates an additional effect of gas flotation. Higher efficiency of separation of oil impurities is achieved by increasing the efficiency of collision and adhesion of oil droplets, which are smaller in size of gas bubbles. The presence of coagulants promotes coalescence of the droplets, which increases the probability of collision and adhesion with gas bubbles. Hydrogen evolution turbulence results in better collision and trapping of bubbles and oil droplets. Treatment of oily wastewater is carried out within 30 minutes; the formed precipitate is filtered. The oily wastewater treatment process is carried out on standard equipment at room temperature.

Studies on oily, low-mineralized wastewater from enterprises producing bitumen (oil content from 4.01-17.79 mg/l, salt content 500 mg/l) showed that for wastewater with a high oil content - 110 mg/l with the use of a coagulant in a wide in the pH range from 5.5 to 10, the oil removal efficiency was 98.6% (residual oil content 1.6 - 3 mg/l). For wastewater with an oil content of 4.0-17.8mg/l, the oil removal efficiency was 92.7%. The reagent effectively coagulates oil at pH 7, temperature 25°C and flow rate 10g/t. Apparently, with an increase in the concentration of oil in water, the probability of collision of colloids increases, which possibly leads to a greater removal of oil.

Public Health Toxicol 2021;1(Supplement):A40
DOI: 10.18332/pht/142153

Investigation of the indicative properties of Daucus carota extract in a complex system based on polysaccharides

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Currently, research is being actively conducted to find natural dyes and indicators, since they are safer to use for humans and renewable. Anthocyanins, curcumin, chlorophyll, carotenoids, brasiline, and tannins are often used as natural pH indicators Natural indicators are widely used in films, xerogels and coatings based on polysaccharides of various origins for pH control in food and medical products. Polysaccharides are able to absorb vapors or moisture, and the indicator, immobilized in the polysaccharide matrix, reacts to changes in pH in situ, acquiring the appropriate color. The use of acid-base indicators in the diagnosis of the condition of wounds can provide information about the healing stage. Using the properties of polysaccharides to absorb moisture, a formula of a polyethylene oxide-based ointment containing a mixture of anionic polysaccharides of sodium alginate and kappa-carrageenan (1:3) was proposed, in which a dry extract of Daucus carota (1%) was introduced. The behavior of anthocyanins from Daucus carota extract in the matrix of hydrophilic ointment based on marine polysaccharides was studied in model media with different pH (pH 5,0, 6,0, 7,0, 8,5, 10,0). Anthocyanins are sensitive to changes in pH: in an acidic environment they show various shades of red, in a neutral environment shades of purple, in an alkaline environment from blue to yellow-green. In the experiments, the time of contact with the model solution and the ratio of the model

solution and the indicator ointment were changed (10:1 and 20:1). The color change occurred quite quickly, but at a ratio of the model solution and the ointment of 10:1 due to the limited amount of the model liquid, there was an incomplete swelling of the sorbing complex in the ointment, which in some cases contributed to an uneven color distribution. The indicator ability of the ointment when applied to wounds will depend on the amount of exudate released and the applied ointment. The condition of the wound can be determined both by the express method (after 10 minutes) when applying the ointment to the wound with a thin layer, and when changing the dressing after 6, 12 or 24 hours.

Public Health Toxicol 2021;1(Supplement):A41
DOI: 10.18332/pht/142242

Amphiphilic carboxyl containing copolymers of n-vinylpyrrolidone

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Amphiphilic copolymers of N-vinylpyrrolidone and acrylic acid were prepared by radical polymerization of comonomers with initiator azodiisobutironitrile in the presence of octadecylmercaptan.

By analyzing the trajectory of nanoparticles, the ability of the synthesized polymers to form stable nanosized particles (60-170 nm) in an aqueous medium has been revealed.

The conditions have been identified that make it possible to obtain products with the highest yield at various contents of acrylic acid units in the copolymers. The dependences of the number average molecular weight and the yield of copolymers on the concentration of octadecyl mercaptan and the ratio of monomers was determined. The resulting copolymers can be used as drug carriers.

Funding
The work was supported by Ministry of Education and Science of the Russian Federation as part of the state assignment for the FSSM-2020-0004 project.
Public Health Toxicol 2021;1(Supplement):A42
DOI: 10.18332/pht/142244

Laser processing of polymer films fabricated from PHAs differing in their monomer composition

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The study reports results of using a CO2-laser in continuous wave and quasi-pulsed modes to treat films prepared by solvent casting technique from four types of polyhydroxyalkanoates (PHAs), namely poly-3-hydroxybutyrate and three copolymers of 3-hydroxybutyrate: with 4-hydroxybutyrate, 3-hydroxyvalerate, and 3-hydroxyhexanoate (each second monomer constituting about 30 mol.%). The PHAs differed in their thermal and molecular weight properties and degree of crystallinity. Pristine films differed in porosity, hydrophilicity, and roughness parameters. The two modes of laser treatment altered these parameters and biocompatibility in diverse ways. Treatment in either mode resulted in different modifications of the films, depending on their composition and irradiation mode. Roughness parameters were changed by the treatment in both modes. Continuous wave line-by-line irradiation caused formation of sintered grooves on the film surface, which exhibited some change in water contact angle (76–80°) and reduced roughness parameters (to 40–45 mN/m) for most films. Treatment in the quasi-pulsed raster mode resulted in the formation of pits with no pronounced sintered regions on the film surface, a more considerably decreased water contact angle (to 67–76°), and increased roughness of most specimens. Colorimetric assay for assessing cell metabolic activity (MTT) in NIH 3T3 mouse fibroblast culture showed that the number of fibroblasts on the films treated in the continuous wave mode was somewhat lower; treatment in quasi-pulsed radiation mode caused an increase in the number of viable cells, depending on PHA composition. This is an important result, offering an opportunity of targeted surface modification of PHA products aimed at preventing or facilitating cell attachment.

Public Health Toxicol 2021;1(Supplement):A43
DOI: 10.18332/pht/142245

Detection of antibiotic streptomycin by fluorescence polarization immunoassay

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Streptomycin (STR) is the first discovered antibiotic in the group of aminoglycosides, which was the first effective against tuberculosis and plague. It is formed during the vital activity of radiant fungi Streptomyces globisporus streptomycini or other related microorganisms. STR is still widely used in medicine and animal husbandry and therefore STR can be detected in food and the environment. Trough serum concentrations of streptomycin greater than 3 ug/mL are more likely to be associated with toxic effects; the streptomycin data sheet recommends that dosage should be reduced if 24 h serum concentrations exceed 3 ug/mL and those in patients over 60 years the trough serum concentration should not exceed 1 ug/mL. Regulatory authorities have established maximum residue limits (MRLs) for STP in food. In the European Union (EU), the MRL for STP in bovine and ovine milk is 200 μg/kg (0.2 ng/mL). Until now, there had been little testing in these areas for drugs in water. In general wasterwater could be contaminated up to 50 ug/mL and the water samples with higher than 0.1 ug/mL concentration of antibiotics will be not recommended to drink.

The aim of this work was determination of streptomycin in wastewater and river samples by Fluorescence Polarization Immuno Assay (FPIA) method. The FPIA method was chosen for streptomycin determination in water, because it is rapid, selective, homogeneous, well reproducible and quite sensitive. The FPIA method for the determination of streptomycin was optimized for different antibodies and tracers, the optimal concentrations of working solutions of immunoreagents were selected. The analytical characteristics of the optimized FPIA method were determined: the detection limit for streptomycin was 0.03 μg/ml. Linear range of determined concentrations: 0.1 - 3.4 ug/ml. The obtained FPIA technique was successfully applied to the determination of streptomycin in 30 river and wastewater samples from the Moscow region and in 4 of which streptomycin was detected in the concentration higher than 0.1 μg/ml.

Funding
This research has been supported by the Interdisciplinary Scientific and Educational School of Moscow University, “Future Planet and Global Environmental Change”.
Public Health Toxicol 2021;1(Supplement):A44
DOI: 10.18332/pht/142246

Synthesis of complex dressings containing various therapeutic agents based on modified cellulose and their properties studying

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In wound therapy, it is customary to adhere to the T.I.M.E. rule. That stands for tissue debridement, inflammation/infection, moisture imbalance, epithelial edge advancement. In this regard, it is necessary to create a dressing with a complex effect.

Biopolymers, especially polysaccharides, are known to be used in the treatment of purulent wounds due to their biocompatibility, biodegradability, and low toxicity. To that end, dialdehyde cellulose (DAC) was chosen as a matrix for the immobilization of subsequent therapeutic agents in our research work.

The most important property of wound healing dressings is their ability to biodegrade without the formation of toxic decay products. Consequently, experiments to study the kinetics of dressings' destruction were carried out.

Importantly, the process of transition of low-molecular-weight products formed by periodate oxidation of cellulose into a solution was mentioned by many authors. In particular, there is an opinion that during the high-temperature destruction of DAC, formaldehyde is formed.

The data obtained by HPLC methods on the composition of the products of hydrolytic degradation are difficult to identify and do not give an unambiguous answer as to what exactly are the products of hydrolytic degradation of the support.

The analysis data obtained utilizing capillary electrophoresis by the method of additions showed that the peak of the formaldehyde yield is outside the region of retention of degradation products. During titration of the studied enzyme preparations with the destruction products of the studied polysaccharide carriers, monomer units and formaldehyde, it was shown that low molecular weight aldehydes (for example, formaldehyde) reduce the activity of proteases in concentration dependence by binding, apparently, to the amino groups of the active center of the enzyme. It was shown that the products of hydrolytic destruction of various test carriers do not affect the proteolytic activity of the studied enzymes, regardless of the exposure time, similar to glucose solutions, or have an activating effect.

The results of the experimental toxicological studies of the samples of the used cellulose materials allow us to conclude that the studied samples do not have toxic, allergic effects, as well as mutagenic activity.

Public Health Toxicol 2021;1(Supplement):A45
DOI: 10.18332/pht/142258

Study of the toxicological properties of microfertilizers

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Aim

Microfertilizers based on nanomaterials differ from traditional ones and require more detailed studies of their toxicological properties. The aim of the investigation was to study the toxicological properties and safety of the micronutrients in the experiment.

Objects of the research

Microfertilizer of various brands based on bio-polymer-stabilized colloidal solutions of nanoparticles of microelements (Co, Mn, Cu, Fe, Zn, Cr, Se, Mo, Ag in various combinations and concentrations).

Methods of investigation

Toxicological, physiological, hematological, biochemical and statistical.

Results

A study of acute toxicity with a single intragastric administration for all brands of microfertilizer revealed a complete absence of symptoms of intoxication and death of animals in the limiting dose (15 000 mg / kg b.w.). Studies of cumulative properties (the effect of toxicity during accumulation) were performed with repeated intragastric administration of microfertilizer (within 30 days) at a dose of 1,500 mg / kg b.w. Symptoms of intoxication and death are not revealed. To verify immunotoxicity and sensitizing effect, intradermal administration of microfertilizer doses above 5,000 mg / kg b.w. was performed. The test showed complete absence of edematous proliferative reaction. When tested on seeds of radish, cucumber and oats, the complete absence of phytotoxicity of microfertilizer is shown. On the contrary, the effect of stimulation of the process of germination of test seeds and an increase in the length of rootlets of seedlings was revealed. In the study of reproductive toxicity microfertilizer was daily administered per os to a group of pregnant female rats. There were no clinical signs of poisoning of the mother organism. Miscarriages and premature births were not noted. When examining the effect on the reproductive function of males and females, microfertilizer was intragastric administered to a group of males and females 7 weeks before mating. Then the females were introduced during pregnancy and feeding. It has been established that microfertilizer does not affect the reproductive function of males and females.

Conclusion

It is established that microfertilizer of all brands is low-toxic, is not eco- and phyto- toxic, is not cause long-term consequences when used (no mutagenicity, genotoxicity, effects on reproductive function).

Public Health Toxicol 2021;1(Supplement):A46
DOI: 10.18332/pht/142259

Vaterite particles: Influence of their size and shape on drug loading and release

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The latest advances in nanomedicine are aimed at targeted delivery of drugs to tumor foci after intravenous administration with the possibility of avoiding excessive accumulation of toxic drugs in normal tissue. The slow transport of nanoscale drugs in the tumor after their extravasation from capillaries significantly impedes their delivery to cancer cells. In the absence of convection in the tumor, the transport of nanomedical drugs is determined solely by diffusion, which turns out to be much slower than the diffusion of low-molecular-weight chemotherapeutic drugs. One approach to solving this problem is to develop and apply high-capacity containers with good biodegradability. The choice of the optimal size and shape of submicron particles, as well as the targeted modification of their surface, will significantly increase their adhesion to the vascular and capillary endothelium of primary and metastatic tumors, which are typically characterized by a reduced blood flow. Vaterite $CaCO_3$ particles may be suitable candidates, but the optimization of their loading efficiency is required.

The aim of the study was to obtain $CaCO_3$ submicron particles with the size less than 500nm of various shapes (spherical, ellipsoidal and lamellar) and maximum content of crystallite vaterite phase. Colloidal particles of

calcium carbonate were obtained by mass crystallization in aqueous solutions of salts containing calcium ions and carbonate ions. In particular, crystallization of spherical and ellipsoidal vaterite particles was carried out in a mixture of water/glycerin, water/ethylene glycol, water/gelatin[1]. Samples have been characterized using scanning electron microscopy, X-ray powder diffraction, and porosity measurements. The loading efficiency of photodynamic and chemotherapy drugs (photosens and doxorubicin) have been studied depending on the size, shape and porosity of the particles. For the encapsulation of therapeutic drugs, a number of techniques have been used: adsorption on pre-synthesized porous particles, coprecipitation during the formation of particles, and a new method - adsorption from solution during the freezing of the solvent. To evaluate the effect of the physicochemical characteristics and the rate of dissolution of particles on the release profile of the incorporated substances, the particles were incubated in a model medium at a pH from 4 to 8. The stability of the obtained nanostructured containers and the processes of their recrystallization and dissolution under various external conditions (pH, proteins) have been studied.

Funding

This work was performed using the equipment of the Shared Research Center FSRC "Crystallography and Photonics" RAS and was supported by the Russian Ministry of Education and Science" in part of particles characterization. This research was supported by the Russian Science Foundation (Project No. 21-74-10058) in the part of drug loading and release studies.

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Public Health Toxicol 2021;1(Supplement):A47
DOI: 10.18332/pht/142260

Development of environmentally and toxicologically safe waterless hair cleansers

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In recent years, the safety requirements of the components included in personal care have become stricter in relation to the environment and consumers. Reducing the water and carbon footprint is possible through the development of solid washable cosmetics. The absence of an aqueous phase also minimizes the risk of microbial contamination of the finished product, which simplifies its storage and transportation.

When developing solid flushable cleansers, it is important to choose a mixture of surfactants that could ensure the hardness of the product, sufficient foaming and optimal pH. In this work, Sodium Cocoyl Isethionate (SCI) and Sodium Lauroyl Glutamate (SLG) were studied in a mixture with Coco Glucoside (CG). These soft surfactants are characterized by excellent biodegradability, low tendency to skin irritation, and are also produced from bio-renewable sources, which makes them an environmentally friendly alternative to other synthetic surfactants.

By measuring the foaming capacity of 5% solutions of binary mixtures using the modified Ross-Miles method, it was obtained that the height of the foam column for a mixture with SCI is 177 mm, with SLG is 253 mm, and the foam stability is 84%, which indicates that both compositions provide the necessary foaming and can be used for the development of solid flushable cleansers.

The hydrogen index of the studied compositions was 5.89 for SCI and 4.88 for SLG. Both samples have a hydrogen index close to the pH of the scalp and hair (pH = 4.5-5.5), which means that the product will not damage the hair cuticle when used.

On the basis of these components, compositions of solid detergents were compiled and an in vivo study was conducted, which showed a good washing ability of the product.

Thus, it was shown that mixtures of surfactants: SCI/CG, SLG/CG, provide the necessary physical and chemical characteristics of the washing composition and can be used for the manufacture of safe and environmentally friendly solid cleansers.

Public Health Toxicol 2021;1(Supplement):A48
DOI: 10.18332/pht/142261

Magnetic small-scale robots: Principles, applications and challenges

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Last two decades has seen a growth of the research on untethered mobile small-scale robots. These motile devices display the ability to travel through fluids by transforming the energy generated by an external power source into mechanical motion. As a result, these devices are being recognized as promising platforms to break through the drawbacks of nanoparticle drug delivery systems. Among the family of small-scale devices, magnetic micro- and nanorobots, which refer to those devices wirelessly controlled by external magnetic fields, are arguably the most appealing systems for biomedical applications. Magnetic fields display biocompatibility characteristics in a wide range of conditions, and they can penetrate body tissues with minimal interaction. Additionally, magnetic fields can be generated in several forms (rotating, oscillating, gradients), enabling a rich collection of motion mechanisms, including several that mimic those of cells and microorganisms. In the present talk, we will introduce magnetic small-scale robots, their actuation principles, designs and constituent materials. Next, we will discuss about existing and potential applications in the biomedical area. Finally, we will conclude with remaining challenges for their translation into clinical applications, with a special focus in the area of intravascular drug delivery.

Acknowledgements

The authors acknowledge support from the European Union's Horizon 2020 research and innovation programme under grant agreement No 952152, project ANGIE (MAgnetically steerable wireless Nanodevices for the tarGeted delivery of therapeutIc agents in any vascular rEgion of the body).

Public Health Toxicol 2021;1(Supplement):A49
DOI: 10.18332/pht/142290

Soft-hard magnetically driven microrobotic devices

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Magnetic micro- and nanorobots are promising candidates for the delivery of therapeutic agents in difficult-to-reach locations of the human body. These devices can swim through fluids by means of external magnetic fields. Depending on the specific design, these devices can exhibit a plethora of motion patterns as a function of the applied magnetic field. Many of these devices can also mimic the motion strategies of small organisms and cells. The vast majority of magnetic micro – and nanorobots are constructed either with magnetic hard blocks, or soft magnetic polymer nanocomposites. The first type usually comprises materials with poor biocompatibility characteristics (except for fully iron devices and few iron alloys), limited motion versatility and mechanical features far from those of biological tissues. The second, while being more versatile and adaptable in terms of motion and shape, and more similar to tissues in terms of their mechanical properties, are limited in terms of magnetic force. To overcome these limitations, devices that marry the excellent magnetic performance of magnetic hard components with the advantageous properties of soft polymeric materials for biomedicine should be developed. In this talk, we will showcase a strategy to produce microrobotic devices that comprise interlocked metallic and polymeric parts. The devices are constructed by means of template-assisted 3D printing by combining electrodeposition and mold-casting. We will demonstrate the richness of these devices in terms of motion. Finally, we will mention the potential applications of these machines for intravascular applications.

Acknowledgements
The authors acknowledge support from the European Union's Horizon 2020 research and innovation programme under grant agreement No 952152, project ANGIE (MAgnetically steerable wireless Nanodevices for the tarGeted delivery of therapeutIc agents in any vascular rEgion of the body).
Public Health Toxicol 2021;1(Supplement):A50
DOI: 10.18332/pht/142296

Marrying inorganics and biologics: Opportunities and challenges

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Stroke is the second leading cause of death worldwide. The gold standard for the emergency treatment of ischemic stroke is recombinant tissue plasminogen activator (tPA). However, its use is associated with serious side effects e.g. internal bleeding, which drives the effort for the development of targeted tPA delivery platforms. Some of the most promising active delivery strategies encompass the use of microdevices based on inorganic materials. Despite the promise of achieving synergistic effects, the consequences of the interactions between complex biological molecules and inorganic materials need careful investigation. Among them is the unspecific adsorption of tPA to inorganic surfaces. Co-incubation of nanoparticles and tPA lead to a reduction in enzyme recovery as investigated by SDS gel electrophoresis. Interestingly application of a fluorescence activity assay showed that at least part of the adsorbed enzyme retained its catalytic activity of 58% +/-3%. These findings have two-fold implications for future development of tPA associated microdevices. On one side their interactions could be monitored and optimized by surface treatments to achieve minimal adsorption and inactivation. On the other side adsorption of the active molecule could be employed for site-specific delivery with release kinetics determined by physiochemical interactions.

Acknowledgements
The authors acknowledge support from the European Union's Horizon 2020 research and innovation programme under grant agreement No 952152, project ANGIE (MAgnetically steerable wireless Nanodevices for the tarGeted delivery of therapeutIc agents in any vascular rEgion of the body).
Public Health Toxicol 2021;1(Supplement):A51
DOI: 10.18332/pht/142300

Microfluidic technologies for chemistry, materials science and biotechnology

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Self-assembly has long being used to control covalent and non-covalent interactions where molecular design has been the major driving force to achieve a desired outcome. Like in nature, a full control over self-assembly processes could lead to rationalized structure-property correlations, a long-time sought in chemistry, materials science and biotechnology. However, the pathways followed and the mechanisms underlying the formation of supramolecular aggregates are still largely unknown and unresolved. Additionally, the effective integration of supramolecular matter into small-scale robots for controlled drug delivery applications is yet in its infancy. Accordingly, it is highly required to find new technologies that can allow to overcome all these challenges. In this contribution, I will present how microfluidic devices can be used to uncover pathway complexity as well as to asses drug delivery applications with small-scale robots. Specifically, I will show that microfluidic technologies provide an unprecedented kinetic control over self-assembly processes; for example, enabling the isolation of well-defined kinetically trapped states as well as unprecedented metastable intermediates. Moreover, I will show that microfluidic conditions can also be used as a phantom environment to test drug delivery applications of rationally designed small-scale swimmers.

Acknowledgements
The author acknowledges support from the European Union's Horizon 2020 research and innovation programme under grant agreement No 952152, project ANGIE (MAgnetically steerable wireless Nanodevices for the tarGeted delivery of therapeutIc agents in any vascular rEgion of the body).
Public Health Toxicol 2021;1(Supplement):A52
DOI: 10.18332/pht/142301

In flow controlled synthesis of blood clots

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Nanoscience is one of the most developed fields in today's world. Its applications cover examples in natural, physical, pharmacological, and chemical sciences, with a high impact on human advancement in the present and the future. The application of nanotechnologies includes several advantages, for

example, the decrease of manufacturing costs, the development of new technologies based on new properties, and the mitigation of side effects on human and environmental health.

A stroke is a medical condition where poor blood flow causes the cell's death because of a cerebrovascular or cardiovascular blockage. Strokes have been reported as one of the primary causes of death in 2019, with a 22% increase since the beginning of this century. This condition was responsible for 8.9 million deaths in 2019, with an expected increase, consequence of its relationship with the SARS-COV-2 (COVID19) virus[1]. Its high impact in human health has pushed scientists to develop and improve technologies for the study and treatment of strokes, as well as the relationship between stroke and viral diseases[2-4]. Current treatments for the disease have not overcome the medical limitations, where the administration times, drug efficiency, complex medical interventions, and secondary effects (such as haemorrhage) are liable for causing deaths among the treated people[5]. In this context, nanoscience concepts such as Lab-On-A-Chip technologies, controlled drug carrier transport, and micro-particle synthesis and manipulation can serve as a tool to overcome current challenges in stroke therapy. In this contribution, we will show how microfluidic technologies can have a great impact on this field enabling a controlled localization of clots as well as the study of their dissolution.

Acknowledgements

The authors acknowledge support from the European Union's Horizon 2020 research and innovation programme under grant agreement No 952152, project ANGIE (MAgnetically steerable wireless Nanodevices for the tarGeted delivery of therapeutIc agents in any vascular rEgion of the body).

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Public Health Toxicol 2021;1(Supplement):A53
DOI: 10.18332/pht/142302

Evolution, gaps, and trends in the origins of innovation ecosystems

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The "innovation ecosystem" is an umbrella term used to account for the common efforts of different stakeholders to achieve innovation. Suppliers provide key parts and technologies that are complemented by products and services provided by a variety of other actors, while customers establish demand and capabilities. In this process of joint value creation, companies gain a competitive advantage by appreciating the overall value of the products and services delivered to customers. Themes including the cooperation between actors, the creation and acquisition of value by organizations, and ecosystem leadership have received increasing interest from both practitioners and scholars. Nevertheless, many knowledge gaps remain and there is urgent need to increase our understanding regarding the formation of innovation ecosystems. Although the genesis of innovation ecosystems has received limited attention to date, it has significant impact not only for research and practice, but also for policies aiming to promote the economic welfare of sectors, regions, and countries. It is crucial to understand the process of innovation ecosystem formation, because this period of ecosystem evolution is the most fragile. Therefore, external provision of the necessary conditions, resources, and activities during this period will have the highest impact. We are conducting a systematic review to understand the origins of innovation ecosystems, as well as the evolution, gaps, and trends associated with this process. Following PRISMA guidelines, we are searching the ISI Web of Science from database inception to Sept 1, 2021, for relevant studies. No restrictions on language or study design have been applied. Risk of bias, data extraction, and sensitivity analysis are being performed by two independent investigators. This talk presents the results from this work in an effort to increase our understanding regarding the formation of innovation ecosystems.

Acknowledgements

The authors acknowledge support from the European Union's Horizon 2020 research and innovation programme under grant agreement No 952152, project ANGIE (MAgnetically steerable wireless Nanodevices for the tarGeted delivery of therapeutIc agents in any vascular rEgion of the body).

Public Health Toxicol 2021;1(Supplement):A54
DOI: 10.18332/pht/142303

In vivo co-exposure monitoring to Pb and Mn from conception until adulthood

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One of the most important concerns for public health related to toxic elements is exposure to Lead (Pb). By now there is no evidence that Pb has any benefit or an essential role for humans. On the contrary, several studies have demonstrated that there is no safe threshold of exposure, especially for children. On the other hand, Manganese (Mn) is an essential element for humans. It works as a cofactor for a variety of enzymes, therefore, is a crucial nutrient.

Our experiment was organized to increase the understanding, by exposure of mice in vivo, of the behavior for co-exposure to Pb and Mn. Shortly, four groups of mother were divided into: control, Pb exposure, Mn exposure, and Pb + Mn exposure groups respectively. The exposure via was done through drinking water and Pb solution had a 100 ppm concentration and Mn solution had 2 g/L concentration. Exposure begins 5 weeks before the delivery.

Then the offspring were grouped according to the mother exposure group, and the sub-grouped until they reach the ages of 0 –3 (0-3 PND), 14 –18 (18 PND), and 60 (60 PND) post-natal days respectively.

During this period the groups 18 PND and 60 PND continue the exposure via lactation. The exposure continues, until the sacrifice, for the through lactation until the 21 days after born. When every group reach the need its age, they were sacrificed and the blood samples, among others, were taken for analyse the element concentrations.

The metal concentration analysis for the blood samples was performed using ICP-MS. Briefly, the samples were digested using ultrapure HNO_3 at 80 °C, from it the analytical solution was prepared. As quality control were used CCL® and SERONORM® Level 1 and 2. The full procedure is reported elsewhere.

The principal statistic for the concentrations are presented in the Tables 1 and 2.

Here the control groups were expected to show the lower concentration, base line, for each mice age group. The lower values for Pb and Mn correspond to the age PND (60) with an average of 1.56 ± 2.35 ppb and 11.42 ± 3.65 ppb for Pb and Mn respectably.

On the other hand, the higher values for Pb correspond to "Pb exposure" group at the age of 18 PND. This due probably to the constant exposure of the subjects from the maternal womb until the end of their life. The value found for Pb corresponds to 341.16 ± 107.16 ppb. Nevertheless, for the Mn concentrations, the higher values were presented for the "Pb + Mn coex" at the age of 0-3 PND, with an average value of 262.66 ± 429.80 ppb. A comparison between the values got to Pb "Pb exposure" and "Pb + Mn coex" show that the presence of Mn have inhibitory effect in the absorption of Pb for the subject. Meanwhile the behavior for the Mn seems to work at contrary. When the groups "Mn + exposure" and "Pb + M coex" are compared the amount of Mn seems to increase when Pb is present. This can reveal a tide relationship between the metabolism involving these metals.

Due to the exposure (single and co-exposure) is finished 20 days after the delivery, the age group of 60 PDN, seems to return to base values equal as the control group. In this way is possible to hypothesize that for exposure time of 20 days, a period of 40 days is enough to eliminated from the blood the excess of Pb and Mn. For a better understanding of the metabolism pathways of the storage organs, a set of samples from liver, bones, and brain were collected from the subjects and will be analysed to continue the study.

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Public Health Toxicol 2021;1(Supplement):A55
DOI: 10.18332/pht/142304

Targeted analysis of organic contaminants, exposure assessment and vulnerability of populations to hazardous compounds

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The exposure of people to chemicals has been of increasing interest over the past decades covering a large number of chemicals including synthetic industrial chemicals and production by-products, such as phthalates, DINCH, parabens, bisphenols, triclosan, and persistent organic pollutants (POPs).Results from the DEMOCOPHES study as well as the first national HBM study (HBM I) suggest that Slovenian women, children, men, and lactating women are exposed to numerous contaminants simultaneously. We found associations between phthalate metabolite concentrations and plastic as well as personal care products[1], whereas exposure to POPs seems to be associated with the residential environments (old building materials, local contamination events) and the consumption of animal products[2]. Additionally, we evaluated the risk resulting from exposure to phthalates, parabens, bisphenols, and triclosan using the hazard quotient[3]. The resulting values for men and lactating women are 0.76 and 0.74, respectively, suggesting no risk at the point. However, this approach neglects individual susceptibilities

Table 1: Pb concentrations in ppb (µg/L)

Offspring´s age	*Exposure group*											
	1 Control			*2 Pb exposure*			*3 Mn exposure*			*4 Pb + Mn coex*		
	n	*x*	*sd*	*n*	*x*	*sd*	*n*	*x*	*sd*	*n*	*x*	*sd*
0–3 PND	11	2.68	2.11	7	280.81	149.15	17	2.86	4.14	30	178.98	69.55
18 PND	18	2.80	1.41	17	341.16	107.99	17	3.49	1.04	54	249.88	71.00
60 PND	18	1.56	2.35	18	9.33	3.09	20	1.12	0.58	42	3.70	2.05

Table 2: Mn concentrations in ppb (µg/L)

Offspring´s age	*Exposure group*											
	1 Control			*2 Pb exposure*			*3 Mn exposure*			*4 Pb + Mn coex*		
	n	*x*	*sd*	*n*	*x*	*sd*	*n*	*x*	*sd*	*n*	*x*	*sd*
0–3 PND	11	37.10	55.36	7	80.26	136.19	17	134.61	119.28	30	262.66	429.80
18 PND	18	11.77	2.05	17	15.57	5.24	17	63.52	33.52	54	87.07	43.31
60 PND	18	11.42	3.65	18	9.33	3.09	20	10.85	3.92	42	10.1	3.24

based on genetic predispositions. We investigated the influence of selected cytochrome P450 enzymes on the biotransformation of di-(2-ethylhexyl) phthalate DEHP4 and identified three single nucleotide polymorphisms (SNPs) in CYP2C9 (rs1799853, CYP2C9*2; rs1057910, CYP2C9*3) and CYP2C19 (rs12248560, CYP2C19*17) that influence the biotransformation. The results suggest that carriers of CYP2C9*2 and *3 have significantly reduced excretion of secondary metabolites (men only) and lower ratios among metabolites. Carriers of both variant alleles had the most pronounced negative effect on DEHP biotransformation. Carriers of CYP2C19*17 had significantly higher excretion of secondary metabolites, however no effect on the was observed on the ratios. We therefore propose the named SNPs as biomarkers of susceptibility or resilience towards DEHP. Currently, we are developing an offline sample preparation method for the analysis of phthalate and DINCH metabolites using liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) for the application in HBM studies. In this method, we carefully selected - among others - metabolites of high molecular weight (> 7 c atoms) compounds to evaluate the influence of the proposed biomarkers of susceptibility on other compounds structurally similar to DEHP.

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Public Health Toxicol 2021;1(Supplement):A56
DOI: 10.18332/pht/142305

Clarity of health risk assessment elements in human biomonitoring and risk assessment studies published between 2016 and 2021

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Human biomonitoring (HBM) is an area that has been developing rapidly with continuously growing number of scientific publications, especially in the last ten years. HBM has been emphasized multiple times as one of the important new approaches for the assessment of health risks. Nevertheless, the actual value of HBM for the purpose of health risk assessment (HRA) and particularly for informing decision-making remains to be clarified.

The goal of our study was to evaluate the inclusion and clarity of different HRA elements in the recent examples of HRA. The study was a part of research efforts aiming to evaluate the characteristics and issues affecting the success of HRA and risk analysis' informing of public health decisions (i.e. risk-informed decision-making). As a collaboration organized within the NEUROSOME Innovative Training Network project funded by the Horizon 2020 Research and Innovation Marie Skłodowska-Curie programme, it aimed to consolidate the understanding of selected HRA topics among the early stage researchers involved. The appraisal involved ten of the fourteen early stage researches working in the NEUROSOME project. Thirty-six scientific papers dealing with HBM and HRA published between 2016 and 2021 were identified by pre-defined search criteria. Their appraisal was performed with a help of a simple appraisal tool, which focused on the clarity of ten important HRA elements, identified in a prior study by a group of established risk and decision analysis professionals: the assessment context of HRA, dose/exposure—response relationship, exposure setting, exposure sources, exposure duration, exposed population, magnitude of risk, uncertainty of HRA results, options for mitigating/avoiding exposure, and transparency and clarity of the assessment process.

We observed inconsistencies in the understanding of fundamental HRA and risk analysis principles in the publications dealing with HBM and risk assessment. None of the HRA elements has been identified and understood as clear in all the appraised publications. Risk assessment practice should be based on solid, clear and consistent theoretical foundations regardless of the areas of its application.

Public Health Toxicol 2021;1(Supplement):A57
DOI: 10.18332/pht/142306

Advancing exposure assessment through environmental monitoring, human biomonitoring and use of personal sensors

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In recent years, a vast number of epidemiological evidences for the effects of a wide range of environmental contaminants on child health outcomes were revealed. Common chemical exposures include: outdoor air pollutants (carbon monoxide (CO), nitrogen dioxide (NO_2), ozone (O_3), particulate matter (PM – including PM 2.5 and PM 10), polycyclic aromatic hydrocarbons (PAHs), and sulfur dioxide (SO_2); toxic heavy metals (lead, mercury, cadmium, arsenic), organochlorine compounds (PCBs, DDT/DDE, HCB, dioxins), perfluoroalkyl substances, polybrominated diphenyl ethers, pesticides, phthalates, and bisphenols. Exposure to these chemicals is widespread globally, through air, water, soil, dust and food contamination, and many consumer goods including plastics and cosmetics. Pollutant exposure may disrupt normal development and thus result in adverse cognitive outcomes with global intelligence quotient as most studied outcome, and behavioral disorders including autism and autism spectrum disorder and attention deficit hyperactivity disorder.

This research aims at combining the information from environmental contamination data with HBM data and personal sensors data aiming at calculation of robust human exposure model. This was achieved by collecting biological samples from 550 participants residing in Thessaloniki, Greece; data from 120 participants using personal silicone samplers from Thessaloniki, Athens, Ljubljana, Madrid, Basel and Milan. Identification of exposure levels for phthalates was carried with both biological samples and personal samplers, PAH's exposure levels were identified with silicone wristbands. Thus, the use of individual HBM data, accompanied by ancillary information will shed light on the mechanistic link between exposure dynamics and observed HBM data.

Public Health Toxicol 2021;1(Supplement):A58
DOI: 10.18332/pht/142307

Developmental exposure to Pb and Mn in mice: Longitudinal studies

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Developmental exposure to metals may have serious consequences for mental health[1,2]. Longitudinal in vivo studies can improve our ability to identify mechanisms of metal neurotoxicity and consistency of long term effects. In our first study, we developmentally exposed CD1 outbred mice to Pb, in the second one to a mixture of Pb and Mn. We mimicked the real-life exposure scenario with low, human-related levels of the two metals administered in drinking water to female mice throughout pre-conception, gestation and lactation periods. Behavioural tests were subsequently conducted in the offspring (to evaluate metal effects on neonatal, juvenile and adult behavioural profiles). Metal levels were monitored in blood, brain and bone at different ages.

The first study revealed the effect of Pb on selected neonatal responses (reduced locomotor activity in the nest area during homing test on PND 11 and spatial learning and memory performances at adulthood. These behavioural alterations were observed in animals with blood lead levels (BBLs) below 5 μg/dL, the identified blood Pb reference value for children by the Center for Disease Control and Prevention (CDC)[3], thus confirming that that there is no safe level for Pb. Moreover, Pb monitoring in different tissue showed that brain Pb levels remain significantly higher than controls at later ages, when BLLs did not longer differ. Interestingly, adult Pb-exposed males appeared more vulnerable than females to detrimental Pb effects on spatial learning and memory, as previously reported[4]; brain Pb levels in males did not differ from females, suggesting a different Pb neurodevelopmental effect rather than higher accumulation in male brains.

In the second experiment, whereas most Pb effects of the previous study were confirmed, co-exposure of Pb and Mn did not show synergistic effect of the two metals; Mn-exposed males appeared selectively impaired in reactivity to social/olfactory cues, a result that certainly needs further investigation. As a whole, we found behavioural effects of developmental metal exposures, suggesting that Pb and Mn interfered with maturation of cognitive and social competencies.

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Public Health Toxicol 2021;1(Supplement):A59
DOI: 10.18332/pht/142308

ESR 12: Transcriptomics, metabolomics, and toxicity pathway analysis of combined exposure to neurotoxicants: transcriptomic statistical method development

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Integrated omics technologies have been instrumental in a host of applications including disease discovery. In ESR 12, the main objectives include the identification of the molecular signatures (using multi-omics analysis of transcriptomic and metabolic data) of co-exposure to neurotoxicants in the human biosamples. Multi-omics are a powerful tool in disease discovery that have undergone large advancements in recent years; available technologies allow for the generation of datasets with tens of thousands of outputs. This requires large computational power and advanced statistical analysis for datasets. While the in-house methods for metabolomics analysis are well-established, there is a need for a statistical pipeline for transcriptomic data analysis. Unfortunately, there is debate within the scientific community over the ideal methods to process raw data, analyze and identify differentially expressed genes (DEGs), and generate robust results while limiting Type I and Type II errors.

We developed two statistical method pipelines using Linear Models for Microarray Data (LIMMA; R software) and moderated t-test (Agilent GeneSpring™ software) for DEG discovery and functionality comparisons. R is an open-source software that allows extensive control of statistical pipelines and parameters and the ability to handle complex statistical designs, although it requires basic knowledge of programming languages. GeneSpring™ is a commercially available, user-friendly analysis software that is efficient for basic statistical analyses with the benefit of freely available technical support. However, Genespring™ is geared towards parametric data analysis and tends to be rigid in its pipeline capabilities.

We analyzed Agilent™ microarray data generated from three real datasets from experiments from within the lab group (One-Color, SurePrint Zebrafish Gene Expression v3 4 x 44k Microarray, design ID: 026437) that aim to detect the molecular mechanisms involved in metabolic disorders associated with environmental contaminants. 3-day post fertilization (dpf) zebrafish larvae (n = ~17 per replicate, 4 replicates) were exposed for 48 hours (sampled at 5-dpf) to two concentrations of the plasticizer bis(2-ethylhexyl) phthalate (DEHP; 25 nM and10 μM), positive control (amiodarone; 1 μM), and a carrier control [dimethyl sulfoxide (DMSO); 0.1%]. Samples were processed following manufacturer protocols and features were extracted using Agilent Feature Extraction™ software. Raw data was exported and analyzed using the above methods. Preliminary results suggest that LIMMA is more conservative than the moderated t-test and generates fewer DEGs. Overall, this work is instrumental in future efforts to generate statistically robust and reliable computational models and systems biology for the prediction of a host of metabolic diseases.

Public Health Toxicol 2021;1(Supplement):A60
DOI: 10.18332/pht/142309

Genome-wide profiling and identification of Single Nucleotide Polymorphisms (SNPs) relevant to susceptibility to neurodevelopmental disorders

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Objective
The research is based on the study of the interplay between genetic variability and co-exposure to chemicals for extended period of time and how this contributes to the development or exacerbation of central nervous system disorders during child development. Identification of single nucleotide polymorphisms (SNPs) may help predict an individual's susceptibility to environmental pollutants, and the risk of onset of neurodevelopmental disorders. Genome-wide association studies (GWASs) have identified a large number of genetic variants, significantly associated with a wide range of complex traits. However, these variants typically have a small effect and correspond to a small fraction of truly associated variants, meaning that they have limited predictive power. Therefore, our approach to this constraint is the polygenic risk score (PRS) application which is an estimate of an individual's genetic liability to a trait or disease, calculated according to their genotype profile and relevant GWAS data.
Methods
Cord blood/tissue and saliva samples from the Italian (604 samples), Slovenian (249 samples) and Croatian (149 samples) PHIME cohorts were collected for DNA extraction. All samples (total 1002) were genotyped with Infinium Global Screening arrays by Illumina (Illumina Inc., San Diego, CA, USA). Genotype imputation was followed in order to estimate missing genotypes from the genotype reference panel. Reference data were obtained from a GWAS meta-analysis, in 269,867 individuals which identifies new genetic and functional links to intelligence. Both reference data and our PHIME target data underwent quality control using Genome studio, plink and R software, where SNPs and individuals were removed due to mismatching, duplicate and ambiguous SNPs or sample overlap and relatedness. Using a linear mixed model in the genome-wide complex trait analysis software, PRSice-2, PRS analysis was applied on the remaining samples (983 individuals), in order to explain the heritability of children cognitive scores at 18 months of age by evaluating the effects of all SNPs simultaneously.
Results
Cognitive scores of 983 children were normally distributed and the value range was from 70 to 145. A multiple linear regression model was applied to find the relationship between children cognitive scores and a variety of explanatory variables. Statistically significant associations were found between cognitive scores and PRS scores (p=0.000212), maternal age at delivery (p=0.000640) and child fresh and homogenized fish intake, p=0.016357 and p=0.07626, respectively. While, no association was found between cognitive scores and THg cord blood levels, maternal BMI, maternal alcohol consumption, child birthweight and birth length (p>0.05).
Conclusions
We found some evidence that the health outcome of our study is directly associated with the genotype profile and can be adversely influenced by the maternal age as well as, by child fish consumption.
Public Health Toxicol 2021;1(Supplement):A61
DOI: 10.18332/pht/142311

Neurodevelopmental effects of prenatal co-exposure to heavy metals and phthalates

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Evidence on neurodevelopmental effects of co-exposure to heavy metals and plasticisers is continuously growing, yet there is also a marked lack of mechanistic interpretation to date.
Phthalate and heavy metal (Pb and Hg) prenatal exposure was determined in mothers during the third trimester of pregnancy (prenatal exposure) and from their children at the 24th month of age (postnatal exposure). Urine untargeted metabolomics analysis was also carried out in both a Thermo Orbitrap LC/MS-MS and NMR. Psychomotor development was assessed in children at the age of 2 years using the Bayley Scale. Associations were investigated using the linkage disequilibrium method of the exposome-wide association study paradigm (EWAS), while pathway analysis was mapped with the Mass Profiler Pro (Agilent Technologies).
Based on our results, co-exposure to phthalates and metals is highly associated with the metabolism of citrate (Krebs cycle). Plasma untargeted metabolomics analysis using NMR led to the detection of pyruvate, 2-oxo-glutarate, and succinate, while urinary untargeted metabolomics using LC/MS/MS led to the detection of citrate, and (s) - malate. These compounds are main metabolites of the Krebs cycle. Perturbations of the TCA cycle may result in altered ATP levels, affecting in this way mitochondrial function. In addition, NMR plasma untargeted metabolomics resulted in the detection of L-arginine, and Fumarate, while urinary untargeted metabolomics using LC/MS/MS led to the detection of L-aspartate. These three compounds (L-aspartate, L-arginine, and Fumarate) are the main compounds of the urea cycle.
Regarding the links between the urea cycle and the Krebs cycle, the generation of aspartate from fumarate is well known. Also, the flux of acetyl-CoA through the TCA cycle can indirectly affect the urea cycle by altering the levels of N-acetyl glutamate.
Overall, although phthalates and metals affect mitochondrial respiration through different mechanisms (endocrine disruption and oxidative stress respectively), this synergistic effect is essential for the expression of neurodevelopmental defects.
Keywords
neurodevelopmental outcomes, exposome, phthalates, heavy metals, metabolomics
Public Health Toxicol 2021;1(Supplement):A62
DOI: 10.18332/pht/142312

Unveiling the effect of telomeres length in cancers development

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Cancer represents the most prominent medical, social, and financial burden regarding cause-specific Disability-Adjusted Life Years (DALYs) among all human pathologies. Given its multifactorial aetiology cancer has been related with a wide variety of risk factors such as exogenous, endogenous and individual, including genetic predisposition. Given that telomeres represent a crosslink connecting numerous inter and intra cellular pathways, great attention has been attracted to them regarding their potential role in cancer development. Telomeres are specific repetitive DNA sequences (5'-TTAGGG-3') located at the end of chromosomes which, together with sheltering proteins, facilitate the maintenance of chromosomes' stability and protect them

from degradation and damage. Many studies have indicated a correlation between telomere length status and cancer, but do not reach a consensus, suggesting that both long and short telomeres are associated with a high risk of cancer incidence. This review comprehensively examines different types of cancer, focusing on telomere length and cancer incidence association. When evaluating risk associations between cancer and telomere length, a disparity seems to be emerging. In some cases, shorter telomeres seem to be associated with higher risk of cancer, whilst in others longer ones. However, it is demonstrated that both short and long telomeres could promote carcinogenesis, suggesting that the association with cancer risk would vary among telomere length distribution and not be linear. Further studies are needed to detect specific associations and establish telomere length as a cancer-type specific risk marker.

Public Health Toxicol 2021;1(Supplement):A63
DOI: 10.18332/pht/142314

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