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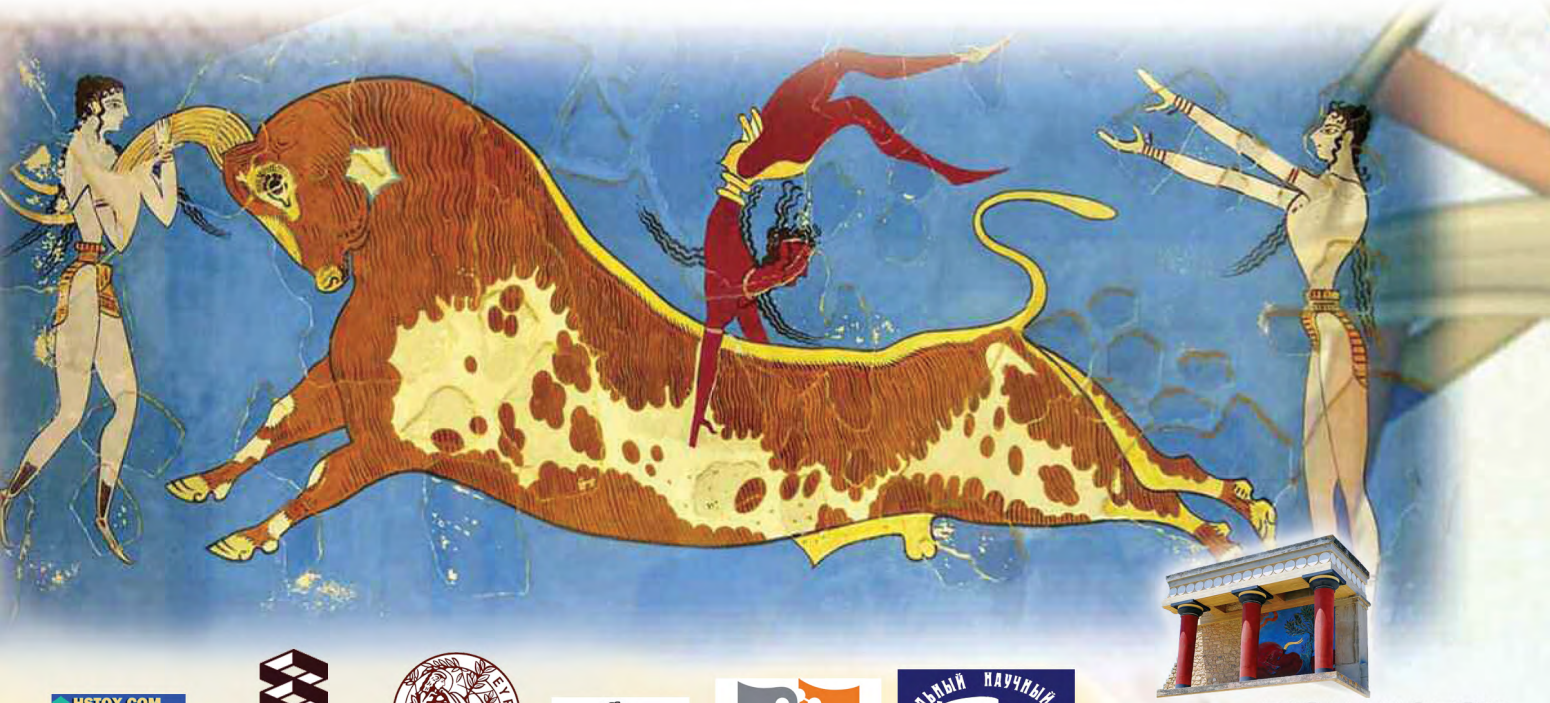

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AMF membrane destabilizing effect was confirmed by β-lactamase release from *E.coli* cell periplasm as well as significant decrease of the hydrophobic dye Nile Red intensity. Next, we were trying to determine the optimal magnetic properties of MNPs and their synthesis conditions. For this purpose, non-magnetic rod-like nanoparticles of akaganeite (β-FeOOH) were prepared by aqueous hydrolysis of ferric chloride in acid medium, followed by modification with dopamine and reduction with hydrazine hydrate in a microwave reactor. Magnetic properties and stability of MNPs can be altered significantly by varying dopamine and reducing agent concentrations enabling to have different ratios between magnetic and non-magnetic phases.

Experimental data of MNP optimal compositions for enzymatic lysis of *E.coli* cell wall are discussed.

The work was supported by RSF-14-13-00731П and RFBR 17-54-33027 ОНКО_а grants.

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STAPHYLOCOCCUS AUREUS LYTIC ENZYMES KINETICS STUDIES

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Staphylococcus aureus is one of the most dangerous bacterium for humans and animals. It is a cause of human organs and tissues infections. The treatment of staphylococcal infections is difficult because of 90% of its strains are resistant to antibiotics. Lytic enzymes of bacteriophages can be considered as promising alternative to conventional antibiotic therapy.

The stability (residual activity dependence on the incubation time) of the lysins of phages 8161, K, and chimeric enzyme K-L (LysK CHAP endopeptidase and amidase domains, and the entire mature lysostaphin protein) was investigated at the physiologically relevant conditions (37°C, human serum, enzyme concentration of 0,2-0,4 mg/mL). Activity of the enzymes was measured in a turbidity reduction assay from time-dependent turbidity changes in a suspension of S. *aureus* cells (A_{600} = 0,6, 37°C, C_{NaCl} 137 mM, C_{KCl} 2,7 mM, $C_{Na2HPO4}$ 10 mM, C_{KH2PO4} 1,76 mM, pH 7,4).

The stability of lysin of phage 8161 depends on the enzyme concentration. Increase of the enzyme concentration from 0.2 to 0.4 mg/mL leads to the enzyme stability decrease. It can be concluded that intermolecular interactions (aggregation) is likely the main reason of the enzyme inactivation. At high concentration of phage 8161 lysin (0,4 mg/mL) two linear parts are presented on the dependence of residual activity logarithm versus incubation time. Such dependence corresponds to two different inactivation stages.

The rate of chimeric enzyme K-L inactivation does not depend on its concentration and is described by the first order equation. Both inactivation constant and half-inactivation time were calculated (k ~ $3{,}33\times10^{-5}$ s^{-1} τ1/2 ~ 347 min).

The inactivation rate of phage K lysin depends on its concentration. Increase of the enzyme concentration from 0.2 to 0.4 mg/mL (inactivation curve with fracture) leads to the enzyme stability increase. Such dependences correspond to dissociative mechanism of the enzyme inactivation.

It can be concluded that 8161 lysin inactivation has aggregative mechanism, chimeric enzyme inactivation has denaturation mechanism and phage K lysin inactivation has dissociative mechanism.

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CATIONIC LIPOSOMES MODIFIED WITH FOLIC ACID FOR ACTIVE TARGETED ANTITUMOR DRUG DELIVERY

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Liposomes loaded with antitumor drugs are known to be delivered into cancer cells by so-called Enhanced Permeability and Retention (EPR) effect (passive delivery) as well as by ligand molecules which can bind only with specific cancer cell receptors (active delivery). Targeted delivery is a promising approach to anticancer treatment.

The aim of the study was to obtain doxorubicin(DOX)-loaded folate-associated cationic liposomes (FLPs) and to study their cytotoxicity *in vitro*. Cationic liposomes are supposed to have faster penetration into tumor cells compared to neutral and anionic ones due to their high positive membrane charge. The FLPs surface was modified with folic acid residues to provide their specific interaction with folate receptors. These receptors are known to be an attractive target for active drug delivery systems, since they are located mainly on the membrane of tumor cells.

To prepare FLPs, two types of lipid compositions were used as bases, namely cationic lipopeptide (1) and a mixture of polycationic amphiphile (2) with 1,2-Dioleoyl-sn-glycero-3- phosphoethanolamine (DOPE). Both bases were combined with two different folate ligands: (3) and (4). Thus, four types of DOX-loaded liposomal dispersions were obtained (Table 1). Physical-chemical FLPs parameters, such as mean diameter, ζ-potential and stability were measured. MTT-test was used for cytotoxicity assay with HeLa (a human cervical cancer), MCF-7 (a human breast adenocarcinoma), U-87 MG (human brain glioma) and C6 (rat brain glioma) cell lines which are known to differ in the folate receptors amount. The FLPs accumulation and localization in the cells and multicellular tumor spheroids was evaluated by confocal microscopy and flow cytometry.

Table. 1. Physical-chemical parameters of the obtained liposomal dispersions

Lipid composition, % w/w	D, nm	PI, %	ζ- potential, mV
(1) + (3) 98:2	254	87	+47
(1) + (4) 98:2	135	93	+51
(2) + DOPE + (3) 32,5:65,5:2	238	94	+16
(2)+ DOPE + (4) 32,5:65,5:2	270	98	+28

This study was supported by Russian Foundation for Basic Research (grant 18-34-00919 in part of liposomes, grants 17-54-33027 and 18-04-01087 in part of 3D tumor spheroids).

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POLY(N-VINYLPYRROLIDONE) NANOPARTICLES LOADED WITH DNA PLASMIDS ENCODING GLYCOPROTEINS AGAINST RVF VIRUS

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Rift Valley Fever (RVF) is a peracute transmissible and zooanthroponotic viral infection which is characterized by fever, hemorrhagic diathesis, damage of central nervous system, necrotizing hepatitis and/or gastroenteritis. Sheep, goats, cattle, horses, antelopes, monkeys as well as humans are susceptible to this infection. Presently, there is no licensed or commercially available vaccines neither for humans nor for veterinary. Therefore development of vaccines is of great importance. Entrapment of DNA plasmids in polymer nanoparticles (NPs) could provide:

(1) DNA protection from cleavage by nucleases; (2) an increased DNA delivery to cells; (3) an easy binding specific ligand(s) to the nanocarrier which allows targeted delivery via interaction with cell receptors; (4) preparation of a polyvalent vaccine containing several DNA plasmids or a mixture a DNA plasmid with a protein (antigen).

The aim of this study was to develop new formulation based on polymer NPs loaded with DNA plasmids encoding Gn и Gc proteins of RVF virus and to evaluate these NPs in vivo using a mouse model. Two amino acid derivatives of amphiphilic poly-N-vinylpyrrolidone (PVP), namely ß-alanine containing polymer Ala-PVP-OD3500 and glycine-containing polymer Gly-PVP- OD3500 were synthesized and characterized as candidates for DNA plasmids biocompatible nano-scaled carriers. Both copolymers had molecular weight of 3500 Da. The introduction of amino acid groups into amphiphilic polymer structure provided tight binding and entrapment of DNA molecules in self-assembled NPs. The mean NPs diameter was 200 nm, spherical morphology was confirmed by transmission electron microscopy and scanning probe microscopy. Cytotoxicity of the NPs was estimated by MTT-test using mouse fibroblasts cells (L929). To study immunogenicity of the DNA-loaded NPs, immunization of BALB/c mice of two experimental and 1 control group (10 animals in each group) was carried out. The experimental group 1 was intramuscularly immunized with a single dose of native pCI-neo/Gc/3 and pCI- neo/Gn/1 plasmids (25 μg of each plasmid/mouse). The group 2 was administered with NPs loaded with DNA plasmids (25 μg of each plasmid/mouse), and group 3 was injected with blank NPs. Neutralizing antibodies against RVF virus were determined by ELISA of blood sera samples in 7, 14, 25 and 40 days after immunization. Entrapment of plasmids into NPs resulted in enhancement of antibody titres, in particular from 1:250 (in case of native plasmids) to 1:250-1:750 (for plasmids in NPs) on day 14. Thus, entrapment of DNA plasmids into PVP-based NPs by self-assembly is a promising approach for development of novel nano-encapsulated DNA vaccines.

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COMPOSITE CROSS-LINKED HYDROGELS BASED ON POLYSACCHARIDES AND FIBROIN FOR REGENERATIVE MEDICINE

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Macroporous hydrogels based on natural biodegradable polysaccharide chitosan (Chit) and hyaluronic acid (HA) are promising biomaterials for tissue engineering. Chit has antibacterial and bioadhesive activity, while HA is a main extracellular component of a connective tissue being involved in growth, proliferation and differentiation processes. In order to improve mechanical properties of the hydrogels, a silk protein fibroin (Fb) could be proposed.

The work was aimed to obtain composite covalent cross-linked hydrogels based on Chit, HA and fibroin (Fb), to study structure and properties of these hydrogels, as well as their ability to support cell growth *in vitro.*

Hydrogels were produced by covalent cross-linking of Chit (MM 320 kDa), Fb and HA (30 kDa). HA was introduced to hydrogel composition in two ways: before and after cross-linking. Genipin was used as a cross-linking agent. When HA was introduced before Chit cross-linking: solutions of Chit, Fb and HA were mixed and cross-linked for 24 hs. In this case HA macromolecules were distributed mostly within the hydrogel volume. When HA was introduced after Chit cross- linking, the obtained cross-linked Chit hydrogel, containing Fb, was incubated in HA solution (2 mass. %) for 2 hs. As a result, the HA macromolecules were distributed mostly on the hydrogel surface. The structure of the hydrogels was studied by confocal laser microscopy. The cytotoxicity of hydrogels was evaluated by extract-test using mouse fibroblasts L929. Cultivation of the cells in the hydrogels was carried out in DMEM (10% FBS) for 7 days. Distribution, adhesion, and cell growth in the hydrogels were evaluated qualitatively by confocal microscopy, while the number of viable cells was determined by MTT-test. It was shown that the structure of the hydrogels changed with Fb introduction. The hydrogels were found to support adhesion, growth and proliferation of fibroblasts during their long-term cultivation.

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