O3-4 **Polysaccharides modified in solid-state for encapsulation of bioactive molecules**

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INTRODUCTION AND OBJECTIVES

Polysaccharides are natural polymers that are offer great potential for medical and pharmaceutical applications. One of them – chitosan - is the most popular polycations used for preparing stable microcapsules. Chitosan is metabolised by certain human enzymes, especially lysozyme, and is con-sidered as biodegradable. Due to its positive charges at physiological pH, chitosan is also bioadhesive, promotes wound-healing and has bacteriostatic effects. Use of chitosan as drug delivery agent or a biomaterial for scaffolds has drawn a considerable attention in the applications for the repair of articular cartilage. However due to their low solubility in water (at neutral pH) and organic medium chitosan is common used in oligo-forms or in a form of its derivatives.

In last years a great deal of interest received another polysaccharide - hyaluronic acid (HA) - anionic, non-sulfated glycosaminoglycan. Due to HA be found in many tissues of the body it is well suited to biomedical applications targeting these tissues. Cross-linking of HA is a promising way to prolong bioactives release and thus to enhance their applicability for biomedical and cosmetic applications.

Modifications of the polysaccharides by monomeric and polymeric substances (e.g. grafting) have of great practical and theoretical importance. They are better biocompatible polymers with desired properties of degradation, hydrophilic-hydrophobic balance, and other physico-chemical properties to meet the need of bioencapsulation technology.

Copolymers of chitosan and synthetic polyesters are the main objects of our recent research. Biocompatible and biodegradable poly(L-lactic acid) (PLLA) and poly(lactide-co-glycolide) (PLGA) are often using to design scaffolds for tissue engineering and carriers for prolonged or controlled drug delivery (Ding 2004, Bhattarai 2006). Cytocompatibility of chitosan-g- poly(lactide) copolymers was also recently approved (Yao 2003).

The novel approach to new polysaccharide-based materials in simple one-step procedure under conditions of shear deformation (a twin-screw extruder) - Solid-State Reactive Blending (SSRB) - was employed to obtain chitin and chitosan graft-copolymers with numerous synthetic monomers and polymers (Ozerin 2007) and cross-linked HA (Volkov 2009). The entire modification process proceeds in the solid state of the components, and does not require any solvents as reaction medium and any catalysts. This is desirable for biomedical applications and provides numerous possibilities to circumvent many processing obstacles typical for preparation of natural-polymers-based materials.

The objective of this research was to modify polysaccharides by biocompatible polymers or bioactive molecules under conditions of solid-state synthesis to enhance their applicability for biomedical application, bioencapsulation technologies and cell cultivation.

MATERIALS AND METHODS

Chitosan with low degree of acetylation (in range 0.03-0.10), low crystallinity and various (20-60 kDa) Mw prepared from crab chitin by SSRB was used. Poly(vinyl alcohol) (PVA), galactomannan, semi-crystalline polylactide and amorphous poly(lactide-co-glycolide) were used to prepare chitosan-based copolymers of different hydrophilicity.

The graft-copolymers of chitosan and PVA were prepared by solid state co-extrusion of chitin and poly(vinyl acetate) mixtures with sodium hydroxide under simultaneous action of pressure and shear deformation at elevated temperature (180°C for chitin deacetylation; 60°C for PVA preparation and grafting).

Solid-state synthesis of chitosan/polyester composites is carried out in twin-screw extruder at various temperatures 50-150°C (below melting point of components) and different weight ratio 40:60 wt-% and vice-versa.

Modification of chitosan by L,D-lactide at SSRB conditions was studied as well. The reactions were carried out at equal molar ration of components and various temperature (50-100°C).

Hyaluronic acid (Bio Sodium Hyaluronate HA20, Shiseido, Tokyo, Japan) was cross-linked at SSRB conditions in single-screw extruder in presence of bioactive molecules.

Conventional organic techniques (like sequential precipitation, viscometry, potentiometric methods etc) in combination with IR analysis in transmission and ATR

modes, X-ray diffraction measurements, GPC, DLS, DSC, DMTA and electron microscopy are the methods which were employed to characterize the bulk and surface properties of the materials obtained.

RESULTS AND DISCUSSION

The obtained graft-copolymers of chitosan unlike non-modified polysaccharide possess enhanced solubility in water at neutral pH as well as in organic solvents as compared with chitosan.

The solubility of chitosan-g-poly(vinyl alcohol) at physiological conditions (temperature below 40°C, pH=7.0) ensure successful operation with pH-sensitive cells, enzymes, proteins, and other biologically active substrates. These copolymers has been employed for design of alginate-(chitosan-PVA) microcapsules for bioencapsulation of tumor cells (Zaytseva-Zotova 2007). As it has been shown, chitosan–PVA copolymers demonstrate a possibility for generating multicellular spheroids using various tumor cell lines in these microcapsules.

The obtained graft-copolymers of chitosan with polyesters unlike non-modified polysaccharide possess amphiphilic properties and form micelle-like stable ultra fine dispersions in organic medium. The mean size of micelle in chloroform according to DLS data is in range from 200 nm (PLGA) to about 1 μm (PLLA), while the main size of chitosan particle according to TEM images is 50-400 nm (PLLA). The NMR data and IR-ATR analysis confirm that the polysaccharide chains are mainly internalized within a core of the chitosan-polyester nanoparticles generated within organic solvents. The physical, mechanical and relaxation properties were studied for the obtained chitosan-polyester compounds as well.

It has been previously shown (Tsoy 2009) that microcarries (mean size is in range 125-250 μm) obtained from PLLA with subsequent chitosan sorption on their surface have enhanced cell adhesion that one from neat PLLA. We decided to develop materials which can be used for preparing scaffold without additional steps like chitosan sorption.

Fractionation of the chitosan modified by L,D-lactide and study of separated fractions by using FTIR spectroscopy showed that the yield of reactions (both salification and acylation) is up to 90% and lactide associates with chitosan in dimer form. Elemental analysis the samples of acylated chitosan shows that sufficient temperature of the treatment is 90°C and degree of substitution of chitosan amino-groups is up to 0.40. Modified by L,D-lactide chitosan have enhanced water solubility at neutral pH.

At conditions of SSRB was obtained cross-linked HA with encapsulated tocopherol, ascorbic acid, retinol and folic acid (Volkov 2010). According to FTIR spectroscopy and fraction analysis the obtained HA samples incorporate about 95-99% of bioactives. Gels based on the modified HA are stable for months (up to half of year).

All of the obtained polysaccharide-based materials can be transformed into nano- and microparticles, fibers, etc and seem to be very promising materials for numerous applications, in particular, as carriers of bioactive compounds and cells.

CONCLUSIONS

A new efficient and ecologically safe process for syntheses of polysaccharide-based materials was proposed. The modified chitosan materials have enhanced solubility in water and organic solvents and can be used for encapsulation various bioactive molecules. Solid-state synthesis allows easily incorporating various bioactive components, without loss their activity in simple one-step procedure as it has been shown by the example of modified HA.

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