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Silicon-zinc-glycerol hydrogel, a potential immunotropic agent for topical application

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ABSTRACT

Nanoparticles synthesized using sol-gel method are promising agents for biomedical applications, in particular for the therapy and diagnosis of various diseases. Using silicon and zinc glycerolates as biocompatible precursors we synthesized by the sol-gel method a new bioactive silicon-zinc-containing glycerohydrogel combining the positive pharmacological properties of the precursors. In the present work the structural features of silicon-zinc-containing glycerohydrogel and its immunotropic properties were studied. The advanced physical methods, including XRD, TEM, dynamic and electrophoretic light scattering, were used for studying the structural features of the gel. Hydrolysis of zinc monoglycerolate was investigated under gelation conditions. Evaluation of the efficiency of silicon-zinc-containing glycerohydrogel in providing immune functions was carried out using a model of the complicated wound process behind immunosuppression induced by hydrocortisone administration in the Wistar rats. It has been shown that zinc monoglycerolate exists in the state of amorphous nanoparticles in the cells of 3D-network formed due to incomplete hydrolysis of silicon glycerolates and subsequent silanol condensation. Zinc monoglycerolate is not hydrolyzed and does not enter 3D-network of the gel with the formation of Zn–O–Si groups, but it forms a separate phase. Immunotropic action of silicon-zinc-containing glycerohydrogel was revealed by the histology and immunohistochemistry methods. Amorphous nanoparticles of zinc monoglycerolate, water-soluble silicon glycerolates, and products of their hydrolytic transformations, which are present in a aqueous-glycerol medium, are in the first place responsible for the pharmacological activity of hydrogel. The results obtained allow us to consider silicon-zinc-containing glycerohydrogel as a promising immunotropic agent for topical application.

1. Introduction

Chemical substances of various classes in the form of nanoscale materials are of great interest for biomedical research due to the uniqueness of their morphological, physicochemical characteristics such as small size, large surface area, enhanced mechanical properties, tunable molecular and chemical structures, and variable surface functionalization. Nanoparticles of metals, oxides, polyfunctional polymers and composites, including drug–polymer conjugates, solid lipid nanoparticles, polymeric/silica nanoparticles, dendrimers, liposomes, polymeric micelles, *etc.*, were investigated as promising agents for biomedical applications, in particular for the therapy and diagnosis of various diseases. (Cao et al., 2015; Ikoba et al., 2015; Kessler et al., 2006; Langer and Tirrell, 2004; Peng et al., 2015; Sumer and Gao, 2008).

Normally, polymeric nanoparticles are used to deliver drugs to affected tissues and to regulate the kinetics of the release of the drugs in the body. Polymeric nanoparticulates provide extensive opportunities

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for the functionalization of drug delivery systems. Conjugation, entrapment, absorption, and encapsulation of drugs and diagnostic agents in polymeric particles can result in combined loading, ultimately achieving the function of theranostics at the molecular and cellular levels (Büyüktimkin et al., 2012; Gu et al., 2013; Muthu and Singh, 2009; Muthu and Feng, 2013).

Nanocomposites possess multifunctional properties and have many advantages for delivery of drugs and imaging agents (Peng et al., 2015).

Nanoparticles can be synthesized using the sol-gel method. Classic sol-gel method is a physicochemical process, based on hydrolysis and condensation reactions in the precursor solution leading to the formation of a new phase. These chemical reactions are closely connected with the physical colloidal transformations in the system, which lead to the subsequent formation of a gel or a precipitate. (Brinker and Scherer, 1990; Chen and Mao, 2007; Gawel et al., 2010; Hench and West, 1990; Levy and Zayat, 2015a; Levy and Zayat, 2015b; Pierre and Pajonk, 2002; Sakka, 2005). The mechanism of sol-gel transformation, especially for silica (alkoxy) precursors, has been well documented.

Polyolate precursors (element-containing derivatives of polyatomic alcohols), unlike alkoxy precurcors, have been much less investigated. The available data usually are of practical, mainly biomedical, importance (Brook et al., 2004; Brandhuber et al., 2005; Chen et al., 2006; Shchipunov et al., 2005; Shchipunov and Postnova, 2009) as polyatomic alcohols released in sol-gel processing do not cause denaturation and/or precipitation of biomacromolecules and the process takes place under mild conditions without using a catalyst and additional homogenizing solvent (Brandhuber et al., 2005; Shchipunov et al., 2005; Khonina et al., 2012; Khonina et al., 2015).

Using silicon and zinc glycerolates as biocompatible precursors we have synthesized a new bioactive silicon-zinc-containing glycerohydrogel (Chupakhin et al., 2014) combining the positive pharmacological properties that are characteristic for two essential elements (silicon and zinc) in a biologically active and accessible form.

Being a microelement the silicon performs different functions in a human organism, *e.g.*, in the composition of mucopolysaccharides and protein complexes it forms the skeleton of connective tissue and determines its mechanical strength, elasticity, and resilience, it provides the growth and strengthening of connective tissue during wound healing, it promotes the biosynthesis of collagen and bone formation, and it plays an important role in the metabolic processes (Nielsen, 2014).

Pharmacologically active silicon glycerolates obtained by transesterification of tetraethoxysilane in excess of glycerol followed by the elimination of ethanol, and hydrogels obtained on their base by sol-gel method are known (Khonina et al., 2008). These substances possess anti-inflammatory, wound-healing, regenerative, and transcutaneous activity. Silicon glycerolates and hydrogels on their base can be used both as separate medicines for topical use and also as component in pharmaceutical compositions for the treatment of skin, soft tissues and mucous membranes diseases of various etiologies (Charushin et al., 2008a; Charushin et al., 2008b; Chupakhin et al., 2012).

Zinc, like silicon, is an essential element necessary for the normal functioning of a human organism. Its biological role in the organism is varied, *e.g.*, as a coenzyme it is involved in enzymatic reactions and increases the resistance to infection diseases, it also provides the synthesis of proteins and nucleic acid metabolism, it is involved in the processes of cell division and differentiation, it directly takes part in the formation of T-cell immunity. As a component of retinol-transferring protein zinc prevents the occurrence of immune deficiencies stimulating the synthesis of antibodies and providing antiviral effect (Shankar and Prasad, 1998).

It is known that zinc monoglycerolate is effective in pharmaceutical compositions for topical treatment of skin diseases such as dermatitis, eczema, psoriasis, ichthyosis, and intertrigos (Cheong et al., 2012; Cheong et al., 2015). It can also be used in the treatment of herpetic lesions of the oral cavity (Gaby, 2006). Furthermore, zinc monoglycerolate exhibits antibacterial activity (Taylor and Brock, 1989).

The *in vivo* tests have shown that the combined silicon-zinc-containing glycerohydrogel is non-toxic; it shows up a pronounced healing and regenerative effect characteristic for silicon glycerolates and a moderate antibacterial activity inherent to zinc monoglycerolate (Chupakhin et al., 2014). Based on these results and published data related both to the effect of zinc microelement on the body's immune function (Shankar and Prasad, 1998) and to the efficiency of zinc monoglycerolate in the topical treatment of immune diseases (Cheong et al., 2015; Gaby, 2006) a further study of the pharmacological properties of silicon-zinc-containing glycerohydrogel, especially its immunotropic activity, has become interesting.

It should also be noted that, in spite of the practical interest in silicon-zinc-containing glycerohydrogel, a several details related to its structure remain unclear. Thus, it was shown (Chupakhin et al., 2014) that due to glycerol excess in the system the polymeric 3D–network of the hydrogel is formed in the course of the incomplete hydrolysis of the most active precursor in the sol-gel process (silicon glycerolates) and subsequent silanol condensation, which gives Si–O–Si bonds containing residual glyceroxy groups at the silicon atom as it was reported in ref. (Khonina et al., 2012). The presence of organic component (glyceroxy groups) was confirmed by elemental analysis and IR spectroscopy. The crystalline zinc monoglycerolate was detected (X-ray diffraction) in the gel solid phase isolated by exhaustive cold extraction in ethanol.

However, it is not clear whether zinc monoglycerolate is subjected to hydrolysis under gelation conditions, and whether Zn is a part of 3D-network of silicon-zinc-containing hydrogel and forms the Zn–O–Si groups as a result of co-condensation of Zn–OH and Si–OH groups. It should be also elucidated in what phase state (amorphous or crystalline) zinc monoglycerolate is present in the gel.

Thus, the objective of the present work was to study the structural features of silicon-zinc-containing glycerohydrogel and its immunotropic properties.

2. Material and methods

2.1. Methods

Elemental analysis (C, H) was carried out using a Perkin Elmer PE 2400 elemental analyzer (series II CHNS–O EA 1108). IR spectra were recorded at a Nicolet 6700 Thermo Scientific spectrometer in 400–4000 cm^{-1} range. Atomic emission spectroscopy (Si, Zn) was performed using an iCAP 6300 Duo Thermo Scientific optical emission spectrometer. Proton NMR spectra were registered by means of a Bruker AVANCE DRX-400 spectrometer at 400 MHz in dimethylsulphoxide-d_6. X-Ray diffraction analysis was carried out using a Shimadzu XRD 700 X-ray diffractometer (Cu K_α radiation).

Measurements of particle size of zinc monoglycerolate were carried out using a Brookhaven Zeta Plus universal analyzer by dynamic light scattering method (Tscharnuter, 2001). The laser wavelength was 659 nm, the scattering angle was 90°, water viscosity value was 0.890 cP, and refractive index was 1.330. The values of average effective diameter of the particles/aggregates in suspension were calculated as an average over 5 measurements lasting 1 min each. For this purpose glycerol-aqueous dispersion of zinc monoglycerolate was homogenized during 15 min using an ultrasonic processor Cole-Palmer CPX-750. Electrokinetic potential ξ (zeta-potential) measurements were performed using a Brookhaven Zeta Plus universal analyzer by electrophoretic light scattering method based on the measurement of Doppler shift of the frequency of the light scattered by particles moving in DC electric field (Ware and Flygare, 1972). All measurements were carried out at 25 °C with an accuracy of 2%.

Transmission electron microscope (TEM) observation was performed with a sample prepared by drying a puttied drop of the zinc monoglycerolate in glycerol and ultrasonically dispersed piece of