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Nanogels based on alginic aldehyde and gelatin by inverse miniemulsion technique: synthesis and characterization

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ABSTRACT

Nanogels were developed from alginic aldehyde and gelatin by an inverse miniemulsion technique. Stable inverse miniemulsions were prepared by sonication of noncontinuous aqueous phase (mixture of alginic aldehyde and gelatin) in a continuous organic phase (Span 20 dissolved in cyclohexane). Cross-linking occurred between alginic aldehyde (AA) and gelatin (gel) in the presence of borax by Schiff's base reaction during the formation of inverse miniemulsion. The effects of surfactant (Span 20) concentration, volume of the aqueous phase and AA/gel weight ratio on the size of the alginic aldehyde–gelatin (AA–gel) nanoparticles were studied. Nanogels were characterized by DLS, FT-IR spectroscopy, TGA, SEM and TEM. DLS, TEM and SEM studies demonstrated nanosize and spherical morphology of the nanogels. Hemocompatibility and *in vitro* cytocompatibility analyses of the nanogels proved their nontoxicity. The results indicated the potential of the present nanogel system as a candidate for drug- and gene-delivery applications.

1. Introduction

Nanosized hydrogel particles, also known as nanogels, formed from physically or chemically cross-linked polymer networks have bagged growing interest in recent years due to their unique features such as small size, large surface area, colloidal stability and high drug-loading capacity (Kabanov & Vinogradov, 2009; Raemdonck, Demeester, & De Smedt, 2009; Rejinold et al., 2012; Shah, Desai, Patel, & Singh, 2012). Nanogel particles have been developed by different approaches such as physical self-assembly, homogeneous or heterogeneous polymerization of monomers, cross-linking of preformed polymers, microfluidics, micromolding, photolithography, precipitation polymerization and inverse emulsion polymerization (Kabanov & Vinogradov, 2009; Oh, Drumright, Siegwart, & Matyjaszewski, 2008). An alternative, less energy-intensive method for the formation of nanogels is inverse miniemulsion. Nanomaterials such as polymeric nanoparticles, drug nanocrystals, semiconductors and magnetic particles have been prepared by this technique (Munshi, De, & Maitra, 1997; Nesamony & Kolling, 2005; Sato, Ohtsu, & Komasawa, 2000; Sims et al., 2002). Inverse miniemulsion can be obtained very easily by gentle mixing of appropriate amounts of water, oil and surfactant(s). The ease with which miniemulsion can be achieved makes it a preferred technique for nanoparticle synthesis. Spontaneously formed miniemulsions constitute of uniform-sized droplets or particles and possess narrow size distribution and spherical or near-spherical shape (Pileni, 1997).

Nanogels have been prepared from synthetic as well as natural polymers through different routes and their versatile applications have been illustrated (Daoud-Mahammed et al., 2009; Nesamony, Singh, Nada, Shah, & Kolling, 2012; Sasaki et al., 2011). Even though nanogels from synthetic polymers were investigated extensively, biopolymer-based nanogels assume significance due to their biodegradability, biological origin, abundance in nature, nontoxicity and the presence of large number of functional groups for possible conjugation with cell-targeting agents and drug molecules (Oh, Lee, & Park, 2009). Among the natural polymers, polysaccharides and proteins are preferred due to their solubility, biocompatibility and biodegradability. Self-assembled nanogels from hydrophilic biopolymers, namely pullulan, chitosan and dextran were prepared by attaching hydrophobic moieties such as cholesterol, deoxycholic acid and bile acid to the polymer backbone (Hirakura, Nomura, Aoyama, & Akiyoshi, 2004; Lee & Akiyoshi, 2004; Na, Park, Jo, & Lee, 2006). Daoud-Mahammed et al.

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(2007) reported nanoassemblies of lauryl-modified dextran and β-cyclodextrin polymers. Jayakumar and co-workers prepared chitin nanogels by precipitation method and explored the feasibility of using these nanogels for drug delivery to cancer cells, biosensing and bioimaging (Jayakumar, Nair, Rejinold, Maya, & Nair, 2012; Mangalathillam, Rejinold, Nair, Lakshmanan, Nair, & Jayakumar, 2012; Rejinold, Chennazhi, Tamura, Nair, & Rangasamy, 2011).

The present work deals with preparation of cross-linked nanogels from sodium alginate and gelatin. Alginate is a polyanionic and biodegradable polysaccharide composed of β-D-mannuronic acid and α-L-guluronicacid units and is a proven biomedical polymer. Its inert hydrophilic matrix can incorporate drugs, proteins and genes (Li et al., 2013; Maciel et al., 2013). In the presence of divalent cations, alginate forms gels in aqueous medium and these gels are used in drug-delivery applications. Alginate–chitosan nanoparticles have been developed and used as carrier for DNA and for protein delivery exploiting the gelling property of alginate in presence of calcium chloride (Douglas & Tabrizian, 2005; Li, Shi, Du, & Tang, 2007). However, the loss of the cations and the resultant release of the components may pose problems in ionically cross-linked systems. Li et al. (2013) prepared bioreducible alginate-poly(ethylenimine) nanogels through electrostatic interactions and used as an antigen-delivery system. Alginate can also form covalently cross-linked nanogels with hyaluronic acid in polyion complex nanoreactors (De Santis, Diociaiuti, Cametti, & Masci, 2014).

Gelatin, a versatile protein, is being utilized as a drug carrier system because of its appealing physical and chemical properties such as biocompatibility, safety and biodegradability. It is widely used in tissue engineering, gene delivery and wound dressing applications (Kanokpanont, Damrongsakkul, Ratanavaraporn, & Aramwit, 2012; Lim et al., 2011; Tseng et al., 2008). However, poor mechanical properties may limit its potential in certain applications (Boanini, Rubini, Panzavolta, & Bigi, 2010). To overcome this limitation and to improve stability in aqueous medium and mechanical properties, gelatin is subjected to cross-linking by different cross-linking agents (Imani, Rafienia, & Hojjati Emami, 2013; Kim, Jun, Shin, Jang, Kim, & Shin, 2010). Gelatin nanoparticles have been explored and are used for various biomedical applications (Lee, Yhee, Kim, Kwon, & Kim, 2013; Ofokansi, Winter, Fricker, & Coester, 2010; Xu, Gattacceca, & Amiji, 2013). Cross-linking agents such as carbodiimide and glutaraldehyde are being employed for the preparation of cross-linked gelatin nanoparticles (Ethirajan, Schoeller, Musyanovych, Ziener, & Landfester, 2008; Qazvini & Zinatloo, 2011).

Even though gelatin nanogels are prepared by the routes mentioned above, the researchers are interested to develop methods by which the toxic cross-linking agents can be avoided. The latest development in this direction is the preparation of gelatin nanogels using miniemulsion technique by cross-linking of gelatin by genipin, a naturally occurring cross-linking agent for proteins (Choubey & Bajpai, 2010). But, the very high cost of genipin may limit the applicability of the process. Even though reports are available on nanoparticles prepared individually from gelatin and sodium alginate, as mentioned above, the possibility of preparing nanogels by self-cross-linking of these two biopolymers are yet to be investigated. Hence in the present work, we have attempted to prepare alginic aldehyde cross-linked gelatin nanogels using inverse miniemulsion technique. Introduction of aldehyde functionality on alginate and utilizing it for gelatin cross-linking offers a facile route for the preparation of cross-linked AA–gel nanogels. Cross-linking leading to nanogel formation can occur between aldehyde groups in AA and free amino groups in gelatin in the presence of borax (Balakrishnan & Jayakrishnan, 2005). Balakrishnan et al. have developed injectable *in situ* forming alginic aldehyde–gelatin hydrogel scaffolds in this route for wound dressing applications by avoiding extraneous cross-linking agents. In the present work, since the cross-linking takes place in inverse miniemulsion, the process would result in the formation of cross-linked nanogels instead of the macroscopic hydrogels. To the best of our knowledge, this is the first report on cross-linked alginate aldehyde–gelatin nanogels *via* inverse miniemulsion technique. Through this method, we are able to prepare cross-linked nanogels without using any external cross-linking agents. Inverse miniemulsion was prepared by pouring a mixture of AA and gelatin under sonication to the organic phase in which surfactant was dissolved. Physicochemical characterizations of the nanogels were performed by FT-IR spectroscopy, SEM, TEM and DLS. Nontoxic nature of the nanogels was proved by *in vitro* hemocompatibility and cytotoxic studies.

2. Materials and methods

2.1. Materials

Sodium alginate (medium viscosity) and gelatin (Type A) were obtained from Sigma Aldrich, Saint Louis, USA. Sodium metaperiodate, sodium tetra borate (borax), Span 20, sodium chloride, disodium hydrogen phosphate, sodium dihydrogen phosphate, hydroxyl amine hydrochloride, methyl orange, minimum essential medium (MEM), isopropanol, sodium hydroxide, cyclohexane and acetone were obtained from Merk (Mumbai, India). Dialysis tubing (3500 MWCO) was procured from Spectrum Laboratories Inc., CA, USA.

2.2. Methods

2.2.1. Preparation of alginic aldehyde (AA)

Sodium alginate was oxidized to alginic aldehyde by a previously reported procedure (Balakrishnan & Jayakrishnan, 2005). Briefly, into 10 g (0.058 mol) of sodium alginate dissolved in ethanol–water mixture (1:1, v/v), sodium periodate dissolved in minimum amount of water (3.28 g, 0.015 mol, required for 30% oxidation) was added. The reaction mixture was stirred at 20 °C for 6 h in dark. Purification was done by dialysis using dialysis tube of MWCO 3500 for 3 days against distilled water. After the complete removal of periodate, dialysate was frozen and lyophilized. Alginic aldehyde was characterized by FT-IR spectroscopy and the aldehyde content was evaluated by titrimetry (Manju, Muraleedharan, Rajeev, Jayakrishnan, & Joseph, 2011).

2.2.2. Preparation of alginic aldehyde–gelatin (AA–gel) nanogel

Nanogels were prepared by an inverse miniemulsion technique with appropriate modification to the reported procedure (Ethirajan, Schoeller, Musyanovych, Ziener, & Landfester, 2008). A typical procedure for nanogel preparation is as follows. Span 20 (0.2 g) was dissolved in 10 ml of cyclohexane and a mixture of 250 μl of alginic aldehyde (10%, w/v, solution in 0.1 M borax) and 250 μl of gelatin (10%, w/v, solution in water) were added to the solution under sonication over a period of 5 min. The nanogel emulsion was precipitated by drop-wise addition into acetone (50 ml) while stirring with a magnetic stirrer. The precipitate was collected by centrifugation (5000 rpm, for 15 min) and washed three times with water and dried under reduced pressure to obtain the nanogel in powder form. Nanogel samples were prepared with different weight fractions of the water phase and surfactant concentrations to investigate the impact of these factors on the size of the nanogels.