Journal of Molecular Structure 1098 (2015) 101–109

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: http://www.elsevier.com/locate/molstruc

Structure of chitosan gels mineralized by sorption

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ARTICLE INFO

Article history:
Received 2 February 2015
Received in revised form
19 April 2015
Accepted 1 June 2015
Available online 9 June 2015

Keywords:
Chitosan thermosensitive gel
Mineralization
Scaffold

ABSTRACT

The paper presents the structural studies of mineralized chitosan hydrogels. Hydrogels produced by using sodium beta-glycerophosphate (Na-β-GP) as a neutralizing agent. Mineralization was performed method "post loading", which consisted in sorption to the gels structure Ca ions. In order to obtain – in the structure of gels – compounds similar to the hydroxyapatites present naturally in bone tissue, gels after sorption were modified in: pH 7 buffer and sodium hydrogen phosphate. In order to determine the structural properties of the gels, the following methods were used: infrared spectroscopy with Fourier transformation, FTIR, X-ray diffractometry, XRD, scanning electron microscopy, SEM.

1. Introduction

Studies on the production of new materials for use as scaffolds are issues of high priority in tissue engineering. The purpose of these materials is the restoration of tissue (in vivo or in vitro) with a structure similar to that of native tissue and functionally compatible with it. An interesting form of such scaffolds are hydrogels, due to the presence of water in the structure, which makes them similar to living tissue: Moreover, they are soft and flexible, which minimizes damage to the surrounding tissue during their implantation and protects the functional and morphological characteristics of the regenerated tissue. They also provide an environment which ensures more efficient and faster cell growth, due to the fact that water present in the structure ensures permeability to oxygen and other water-soluble metabolites.

One of the promising materials for use in tissue engineering is chitosan, especially in the form of a hydrogel. In the context of this application the cationic nature of chitosan is very important, as this allows interaction with the negatively charged glucosaminoglycans (GAG) and proteoglycans. These properties allow the introduction of GAG-chitosan complexes into the scaffolds which inhibit the release of beneficial substances secreted by the cells. Furthermore, chitosan is structurally similar to GAG and, according to Kim's tests, plays a significant role in creating and cell differentiation [1]. Chitosan hydrogels can be prepared by mixing with other water-soluble non-ionic polymers such as poly (vinyl alcohol) (PVA) [2,3]. Thermosensitive (capable of self-organization under heat) chitosan gels may be produced by adding polyols, in the form of the disodium salt of glycerol phosphate [4], or by grafting on chitosan poly (ethylene glycol) (PEG) [5].

Chitosan gels are also produced by the polycation–polyanion reaction. Polyanions forming chitosan gels were proteins: gelatin, collagen, keratin, albumin and fibroin [6–8], anionic polysaccharides: hyaluronic acid, alginate, pectin, heparin, xanthan, dextran sulphate, chondroitin sulphate [9–12], cellulose and glycosaminoglycans carboxymethylcellulose [13] synthetic polyanions – as poly (acrylic acid) (PAA).

In order to give scaffolds osteoinductive properties, calcium and phosphorus compounds are introduced into the structure to form composite systems. The method of production of mineralized scaffolds consists mainly in the introduction of micro-or nano-hydroxyapatite granules into chitosan salt, followed by the generation of a 3D structure by the phase separation method or by freezing or freeze drying or both processes sequentially [14–18]. To produce

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http://dx.doi.org/10.1016/j.molstruc.2015.06.001
0022-2860/

chitosan structures containing hydroxyapatite, an ion-exchange process can also be used. The scaffold is placed in a chamber between the two cation and anion exchange membranes. In the chamber from the side of the anode and a cation exchange membrane there is calcium nitrate (V) solution $Ca(NO_3)_2$, or calcium chloride $CaCl_2$, whereas in the chamber from the side of anion exchange membrane and the cathode there is sodium dihydrogen phosphate (V) solution NaH_2PO_4. Under the influence of the applied voltage an ion exchange takes place and hydroxyapatite is precipitated in the structure of the scaffold. To allow the movement of ions the scaffold is soaked with NaCl, which at the same time serves as a blowing agent.

There are also produced chitosan scaffolds where hydroxyapatite is formed during scaffold formation. In this case the reactions of phosphoric acid H_3PO_4 and calcium hydroxide $Ca(OH)_2$ are used – phosphoric acid is added to a solution of chitosan salt (chitosan acetate) and then added to calcium hydroxide to form a suspension [19]. Hydroxyapatite in the structure is also obtained by introducing the following to a solution of chitosan salt: calcium nitrate $Ca(NO_3)_2$ and ammonium hydrogen phosphate (V) $(NH_4)_2HPO_4$ or calcium nitrate $Ca(NO_3)_2$ and phosphoric acid H_3PO_4, or potassium dihydrogen phosphate KH_2PO_4. Generating the scaffold requires freezing of the solution, freeze-drying and in some cases neutralization in NaOH and final washing [20–22]. The adsorption processes as a method for mineralization of chitosan gels have not been used in the literature so far. This is due to the fact that research shows that during the study of the adsorption process in hydrogel chitosan granules for calcium ions Ca^{2+} the maximum sorption capacity has not been achieved - in spite of research in a wide range of concentrations [23]. For the equilibrium concentrations of 15 mmol/dm^3, the amount of adsorbed ions is within the limit of experimental error. So probably calcium ions are adsorped by physical processes only, which is confirmed by calorimetric tests (measurable thermal effect has not been achieved).

The paper presents the structural studies of mineralized chitosan hydrogels. Hydrogels produced by using sodium beta-glycerophosphate (Na-β-GP) as a neutralizing agent (their gel sol transition occurs at the physiological temperature of the human body) [24]. Mineralization was performed method “post loading”, which consisted in sorption to the gels structure Ca ions. The mineralization was conducted due to the fact that phosphorus is present in the structure of chitosan hydrogel, which alters the nature and properties of the adsorbent. In order to obtain - in the structure of gels – compounds similar to the hydroxyapatites present naturally in bone tissue, gels after sorption were modified in: pH 7 buffer and sodium hydrogen phosphate.

2. Materials and methods

2.1. Structural studies

In order to determine the structural properties of the gels, the following methods were used: infrared spectroscopy with Fourier transformation FTIR, X-ray diffractometry XRD, scanning electron microscopy SEM.

FTIR studies were performed using a Nicolet 6700 spectrometer from Thermo Nicolet equipped with a snap Photoacoustics MTEC model 300. Samples for photoacoustic testing were placed in a special snap holder.

The X-ray diffraction patterns were determined using a wide-angle X-ray Simens D5000 diffractometer and Kα, Cu radiation.

The resulting diffractograms were identified on the basis of patterns of the International Centre for Diffraction Data (JCPDS):

The structure of gels was observed using scanning electron microscopy FEI QUANTA 200 F and atomic force microscopy Nanoscope IIIa, Veeco.

2.2. Preparation of chitosan hydrogels

Thermosensitive chitosan gels were prepared according to the method described by Chenite [25]. To prepare hydrogels shrimp chitosan (Sigma Aldrich) of a degree of deacetylation SD ~ 79.5%, and molecular weight of 86 kD was used. 0.4 g of chitosan was dissolved in 16 ml of 0.1 M HCl (Sigma–Aldrich). The chitosan solution thus prepared was left on the mechanical shaker for approximately 12 h to ensure complete dissolution. At the same time 2 g of sodium β-glycerophosphate (Na-β-GP) (Sigma–Aldrich) was dissolved in 2 ml of deionized water (18 μS). The resulting chitosan salt solution was cooled to 4 °C. Subsequently at a low temperature, i.e. in the conditions of crushed ice to the chitosan

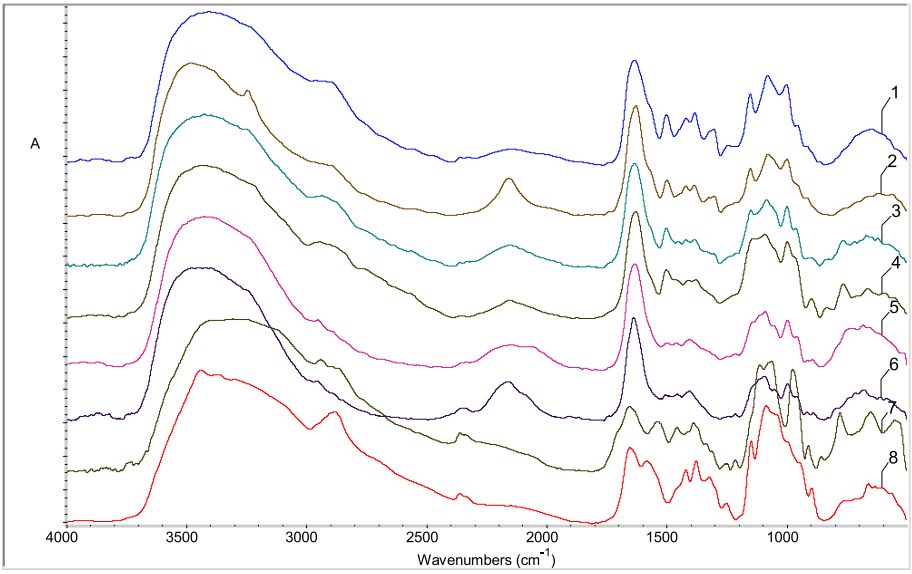

Fig. 1. Comparisons of photoacoustic spectra FTIR in the range of 4000–500 cm^{-1} for chitosan gels **after sorption** of calcium from solutions of $CaCl_2$ at concentrations of: 1–10; 2–20; 3–50; 4–70; 5–80; 6–90 g_{Ca}/dm^3, 7 – hydrogel spectrum immediately after preparation, 8 – chitosan.