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Chitosan based hydrogel assisted spongelike calcium phosphate mineralization for in-vitro BSA release

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ABSTRACT

New chitosan-g- poly (3-sulfopropyl methacrylate), CHI-g-P(SPMA), hydrogel was prepared by free radical polymerization process and investigated as a template for biomimetic spongelike calcium phosphate mineralization in a solution mimicking physiological condition. Infrared spectroscopy, scanning electron microscopy, X-ray diffraction and transmission electron microscopy confirmed the predominant formation of rod-like hydroxyapatite. The swelling behavior of the nanocomposite was evaluated at different pHs and different saline concentrations. Bovine serum albumin (BSA), as a model protein drug, was loaded in the CHI-g-P(SPMA)/calcium phosphate hybrid. The BSA release behavior was investigated and the results suggested CHI-g-P(SPMA)/calcium phosphate hybrid as controlled release carrier. These results suggest that next generation of polysaccharides based hybrid materials could be interesting for biomedical applications.

1. Introduction

Hybrid materials have recently attracted interest due to their potential applications in biology and biomaterials science [1,2]. In addition, hybrid materials prepared from polymers and calcium phosphate are important materials in bone and teeth regeneration, design of implants and drug delivery systems [3,4]. Up to now, the vast majority of the studied polymers, induced calcium phosphate mineralization, discussed the role of synthetic polymers. For example, Poly(2-hydroxyethyl methacrylate) hydrogel was investigated as a template-driven nucleation to design biomimetic composite materials [5]. Also, Andriy Shkilnyy et al. investigated poly(ethylene imine) crosslinked hydrogels as efficient templates for calcium phosphate mineralization with spongelike morphology which presented as interesting material for implants [6]. One of the key challenge in hybrid biomaterials progress is the need for the development of new alternatives based on renewable and sustainable polymers. In the trend towards greener and more sustainable chemistry, polysaccharides have recently emerged as a desirable candidates for studying the biomimetic inorganic mineralization due to their low cost, non-toxicity and bio-degradability [7,8]. However, the low bioactivity of most polysaccharides arising from their low functionality has drawn the attention of researchers to explore new functionalized polysaccharides [9,10].

Hydrogels have received increasing attention for inorganics, especially calcium phosphate mineralization in bulk aqueous solution. Among them, polysaccharides based hydrogels are particularly interesting for hybrid materials development due to their promising applications in biomaterials [11]. The network structure of the hydrogels can be created by numerous protocols making them as insoluble and swellable materials. Moreover, the similarity of these materials to biological tissues proposes them as fascinating materials for biomedical applications [12]. Constructing new polysaccharides based hydrogels are promising strategy to increase the sustainability, biodegradability and biocompatibility of the formed network. Moreover, we can overcome the deficiency associated with the synthetic hydrogels such as poor mechanical properties [13]. The combination of mineralized inorganic and hydrogel networks can produce novel advanced materials with enhanced performance and new properties to the classic hydrogels. In the past few years, significant efforts have been put into the development of nanocomposite hydrogels for preparing drug delivery or tissue/bone replacement [14]. Surprisingly, only a few studies have dealt with the bioactivity of polysaccharides based materials as growth modifier to promote calcium phosphate or calcium carbonate mineralization [1]. For example, Falini, et al. studied the formation of different calcium phosphate phases into a chitin matrix [15]. A recent study has also reported calcium phosphate/chitin nanofiber hydrogel for bone tissue engineering [16]. However, the limited solubility of chitin in most solvents makes its handling and processing very difficult. As a result, chitosan,

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which is soluble in acidic aqueous solutions, has been evaluated as new alternative in biomaterial. Chitosan/inorganic hybrid materials are highly attractive due to their broad applications in areas such as drug delivery and healing damaged bone tissue; these developments have recently been reviewed [17]. The main criteria for selecting chitosan as an interpenetrating polymer in the preparation of hydrogel is due to its biodegradability and protecting properties [18].

In the biomimetic mineralization process, acid function groups, such as carboxylic groups, have the ability to induce nucleation of apatite [19]. Sulfonated polymers have recently applied as templating agents for metal oxides and inorganic mineralization [20,21]. It has been reported that calcium phosphate was deposited on polyamide film containing sulfonic groups in a simulated body fluid. The study showed that the sulfonic groups can act as an effective functional groups for apatite deposition [22]. Tobias Mai et al. prepared water soluble block copolymers on the basis of Poly (ethylene oxide) and poly (3-sulfopropyl methacrylate) blocks. The authors reported that the block copolymer delay the precipitation of calcium phosphate but, upon precipitation, assists monodisperse hydroxyapatite spheres [23].

In view of the need for new sustainable and bioactive hybrid materials, acidic hydrogels are highly interesting candidates for further rationalizing calcium phosphate crystallization because they resemble the structure of noncollagenous proteins, that is, the water-soluble fraction of proteins [24]. The current study thus represents a novel trial for studying the role of polysaccharides containing sulfonic groups for calcium phosphate mineralization from bulk aqueous solution. CHI-g-P (SPMA) hydrogel was prepared and characterized as a new chitosan based hydrogel for calcium phosphate biomimetic mineralization. At the same time, the swelling behavior and BSA release profile of the hydrogel and the hybrid were investigated.

2. Experimental

2.1. Materials

Chitosan low molecular weight (CHI), 3-Sulfopropyl methacrylate potassium salt (SPMA), N, Ní-methylenebisacrylamide (MBA) and potassium persulphate (KPS) were purchased from Sigma-Aldrich. The other chemicals are of analytical grade and used as received without further purification.

2.2. Preparation of CHI-g-P(SPMA) hydrogel

CHI-g-P(SPMA) hydrogel was synthesized by adding 0.5 gm CHI to 25 mL 2% acetic acid solution in a three-neck reactor to prepare 2% (w/v) solution. The solution placed in a thermostated water bath adjusted at 70 °C and purged with nitrogen for 30 min. Potassium persulphate (0.1 gm) was added, followed by the addition of SPMA (5 gm) then the crosslinker (0.05 gm). The temperature was kept at 70 °C and maintained for 2 h to reach complete crosslinking. To remove the unreacted molecules, the final product was cut into small pieces, about 2–5 mm, and washed several times with distilled water. The chopped hydrogel was freeze dried from water.

2.3. Mineralization process

Biomimetic calcium phosphate mineralization was carried out as described in our previous study [25]. Doubly concentrated simulated body fluid (2xSBF) is a well-established system to accelerate the calcium phosphate formation. The biomimetic mineralization process was carried in Falcon tubes for7 days. 1 g of CHI-g-P (SPMA) hydrogel was immersed in 100 mL 2xSBF with continuous renewed every 24 h and the pH was checked regularly and maintained at 7.4 over the entire course of the mineralization to minimize the problems associated with SBF preparation and stabilization. The mineralized CHI-g-P (SPMA) hydrogel was washed with water then dried in a freeze-drier.

2.4. Characterization

Fourier transform infrared spectroscopy (FT-IR) was done on a Mattson 5000 FTIR spectrometer using KBr discs in the range of 4000–500 cm^{-1}. Scanning electron microscopy was done on Model Quanta 250 FEG (Field Emission Gun) attached with EDX unit (Energy Dispersive X-ray Analyses), with accelerating voltage 20 K. The samples were initially fixed on a carbon tape and then coated with gold by conventional sputtering techniques. Transmission electron microscope (TEM) images were taken with a JEOL JEM-2100 electron microscopy at 100k × magnification, with an acceleration voltage of 120 kV. TEM sample was prepared by placing one dilute drop of prepared hybrid material, dispersed in ethanol using ultrasonic, onto a copper grid and allowing it to dry well. X-ray diffraction (XRD) patterns were recorded with an Empyrean Powder Diffractometer (Cu K_α, 0.154 nm) between 5 and 70° 2θ with a step size of 0.01°/sec. Samples were mounted on a silicon support.

2.5. Swelling properties

The swelling% of the synthesized CHI-g-P(SPMA) hydrogel before and after calcium phosphate mineralization was measured in distilled water, acidic, basic and in saline solutions (0.3, 0.6, 0.9 and 1.2 NaCl wt%). The swelling percent was calculated by the following equation:

$$Swelling\% = \left[(W_t - W_o)/W_o\right] \times 100$$

where W_o is the initial weight and W_t the weight of the hybrids at time t [25].

2.6. Drug loading and release studies

BSA loading process was carried out by immersing 50 mg of dry CHI-g-P (SPMA) hydrogel and CHI-g-P (SPMA)/calcium phosphate hybrid in BSA solution (10%) for 3 days with continuous stirring. Then the swollen samples were washed and allowed to dry at room temperature. The in vitro release of the entrapped BSA was measured by soaking the drug loaded samples in 10 ml of buffer solution and the results were measured by UV-spectral measurement.

3. Results and discussion

The current article is the first trial toward design CHI-g-P (SPMA) hydrogel via free radical polymerization technique using SPMA as monomer, MBA as cross-linker and KPS as initiator. Biomimetic mineralization technique was used to evaluate the capability of the amino and the sulfonic groups on the surface and interior of cross-linked CHI-g-P (SPMA) hydrogel to promote the nucleation and growth of calcium phosphate. Scheme 1: Illustrates the steps involved for the preparation of CHI-g-P (SPMA) hydrogel which followed by preparation of CHI-g-P(SPMA)/calcium phosphate hybrid materials to form bonelike biomaterial. The presence of graft copolymer containing sulfonic groups in the resulted hydrogel are expected to act as active sites for promoting the nucleation and growth of calcium phosphate on the surface along with extensive calcification of the hydrogel interior. The mineralization process starts with the nucleation step which involves binding of calcium and phosphate ions to the sulfonic and amino groups in the hydrogel by ionic or hydrogen bonds. Afterwards, additional ions from