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Synthesis and characterization of bioactive biodegradable chitosan composite spheres with shape memory capability

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ABSTRACT

Chitosan (CHT) spheres incorporating bioactive glass nanoparticles (BGNPs) were prepared to obtain a composite system able to induce the deposition of an apatite layer upon immersion in a biological-like environment. Spheres were synthesized with different concentrations of BGNPs obtained from a sol–gel route and genipin (GNP, the crosslinking agent). Biomimetic superhydrophobic surfaces were used to support droplets of chitosan-based solutions that after crosslinking enabled to produce well developed spherical particles with controlled sizes. From SEM and EDS analysis it was observed the successful formation of bone-like apatite on the surface when the spheres were immersed in a simulated body fluid (SBF). Lower GNP concentration promoted more apatite formation. The spheres presented shape memory behaviour triggered by hydration with high values of shape fixity and shape recovery. This effect was used to introduce these spheres in a bone defect showing a good geometrical accommodation in the implanted site. The bioactive spheres allowed the incorporation of a drug model and its effective release. Overall the developed nanocomposite spheres showed great potential for bone tissue engineering in particular as a device to be implanted using minimal invasive procedures.

1. Introduction

In bone tissue engineering it is important to a have good interfacial bonding between the bone and the implant. Without this bonding, a formation of a fibrous tissue is likely to occur, osteointegration is compromised, and the implanted material eventually fails [1,2]. It is known that bioactive glasses are compatible with bone tissue because they develop an apatite surface layer upon implantation providing favourable substrate conditions for bone growth and bone matrix production, which leads to a good interfacial bonding [3–5]. Also, these glasses can promote the recovery of damaged tissues by acting on intra and extracellular metabolisms that are responsible for tissue growth. In particular, bioactive glass nanoparticles (BGNPs) and biomimetic nanocomposite derivatives have shown a potential to be applied in a variety of biomedical applications, including bone tissue engineering and periodontal regeneration [2,6,7]. The use of such BGNPs possesses some advantages once they maximize the surface area, enhancing the dissolution of the ions responsible for the precipitation of apatite [8–10].

A common strategy to induce bioactivity in non-bioactive polymeric biomaterials is to incorporate BGNPs in the polymer matrix, where the macromolecular fraction acts as a continuous medium for the immobilization of the nanoparticles [2]. This combination creates a nanocomposite material with increased osteocondutive properties that have been explored, for example, as porous scaffolds for bone tissue-engineering applications or membranes with applications in the dental field [6,11–14].

Chitosan (CHT), an aminopolysaccharide polymer, is obtained by the alkaline deacetylation of chitin and is composed by glucosamine (deacetylated units) and N-acetyl-glucosamine (acetylated units) units randomly distributed. It has been extensively used for tissue engineering and other biomedical applications since it is from natural origin, biodegradable, non-toxic, biocompatible, and is well known for its susceptibility to chemical modifications [15–17]. It is known that the crosslinking of CHT composite systems will favour the formation of stable matrices as 3D supports [18,19]. Genipin (GNP) is a natural crosslinking agent with low cytotoxicity [20,21] that has been used to crosslink amino group contained in biomaterials such as CHT [22,23]. It has also been reported that GNP binds with biological tissues [24] and biopolymers [22] leading to matrices with good mechanical properties, reduced swelling extent and good biocompatibility.

Injectable systems have been proposed in orthopaedic applications to carry and deliver therapeutic molecules and cells through minimally invasive procedures [25]. It minimizes the risk of complications from a common surgery, diminishing the recovery time, and offers less pain

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to the patient. In this way, shape memory polymers (SMPs) represent a technologically important class of stimuli-responsive materials that offer mechanical and geometrical action triggered by an external stimulus [26]. Such a device can be deformed and subsequently fixed into a temporary shape, which remains stable unless it is exposed to an appropriate external stimulus that triggers the polymer to recover its permanent shape. We showed before that CHT-based biomaterials have shape memory properties triggered by hydration [27,28].

This advanced functionality makes such SMPs suitable and promising biomaterials for diverse technological applications, such as systems able to be implanted using minimally invasive surgery procedures. It is interesting to consider the addition of other functionalities to those biomaterials, such as the inclusion of therapeutic molecules.

In this work, we propose the synthesis of shape memory bioactive nanocomposite spheres with drug release capability. The application of microspheres can be advantageous as they can cumulate as organized 3D sphere scaffolds with interconnecting porous structures in which the interstitial spaces between the spheres may act as pores that allow oxygen and nutrient access and cellular invasion [29]. Spheres were composed by a CHT polymeric matrix, crosslinked with GNP, and reinforced with BGNPs. The crosslinking reaction before implantation in small volumes of polymer will assure a more homogeneous and controlled crosslinking reaction of the matrix. This will prevent unwanted migration of non-crosslinking material from the defect area and facilitate the surgical procedure, which are problems found in in situ crosslink of gels possessing slower or faster crosslinking times, respectively [30].

The combination of CHT and the bioactive glass nanoparticles aims at designing biocompatible spheres promoting the formation of a calcium-phosphate layer at the nanocomposite surface, thus enhancing the osteoconductivity behaviour of the biomaterial.

2. Materials and methods

2.1. Materials

CHT of medium molecular weight (Mw = 190,000–310,000, 75–85% degree of deacetylation, viscosity 200–800 cps, code: 448877, batch: SLBH2747V) was purchased from Sigma Aldrich and was purified by a "reprecipitation" method before use. Tetraethyl orthosilicate (TEOS, 99.90% pure), citric acid monohydrate (99–100%), ammonium phosphate dibasic, calcium nitrate tetrahydrate (99%), ethanol absolute, ammonium hydrogen phosphate (98%, maximum of 33% NH_3), 1H,1H,2H,2H-Perfluorodecyltriethoxysilane (PFDTS), polyethylene glycol (PEG), congo red (CR), ammonium hydroxide solution, acetic acid and all chemicals for SBF preparation were purchased from Sigma-Aldrich. Polystyrene (PS) was reagent grade (158 K, Styrolution, France) and was used as received. Genipin (GNP) was purchased from Wako chemical.

2.2. Superhydrophobic surface preparation

The preparation of the PS superhydrophobic substrate followed a phase inversion method described by Song W. et al. [31]. A PS solution (70 mg ml^{-1}) in tetrahydrofuran was prepared and mixed with ethanol (2:1.3 v/v). The solution was dispensed onto smooth commercial PS substrates for a few seconds, which were then immersed on ethanol for 1 min and dried at room temperature. This step gives roughness to the substrate at both micro and nano-length scales, confering the superhydrophobic behaviour. The surfaces were modified with PFDTS (1% (v/v) in ethanol) after argon plasma treatment for 20 s at 30 W (Plasma Prep5, Gala Instruments, Germany), to enhance surface hydrophobicity. The water contact angle of the produced surfaces was measured in a OCA 15+ goniometer from DataPhysics Corporation (U.S.A.) at room temperature with a 5 μl water droplet.

2.3. BGNP preparation

To prepare the BGNPs with the composition SiO_2:CaO:P_2O_5 (mol.%) = 55:40:5, a previously described protocol was used consisting in sequential reagent dissolutions that resulted in hydrolysis and polycondensation reactions [32–35]. Briefly, TEOS (99.90% pure) was used as the Si precursor, ammonium hydrogen phosphate as the P precursor, calcium nitrate tetrahydrate as the Ca precursor, citric acid monohydrate to promote hydrolysis, ethanol, ammonium hydroxide as the jellifying agent and PEG as the surfactant. The mixture of precursor's solutions (7.639 g of calcium nitrate in 120 ml of distilled water, and 9.167 g of TEOS in 60 ml of ethanol/30 ml of citric acid 10% (w/v)) was added drop-by-drop to an aqueous solution containing the phosphorus precursor (1.078 g of ammonium hydrogen phosphate in 1500 ml of distilled water). The pH was adjusted at 11.5 with ammonium hydroxide addition. The precipitate obtained was stirred for 48 h, followed by a resting period of 24 h. The precipitate was washed three times with distilled water. A 200 ml amount of an aqueous solution of poly(ethylene glycol) 2% (w/v) was added to the precipitate, followed by freeze-drying. Finally, the gel powder was calcined at 700 °C for 5 h.

2.4. Bioactive sphere preparation

Spheres were produced by a biomimetic fabrication process for hydrogel sphere production over a superhydrophobic substrate previously described [36–38]. CHT was dissolved in an aqueous acetic acid solution 2% (v/v) to a concentration of 1% (w/v) for preparation of the spheres. Then, different contents of GNP (1, 5, 10 and 20% w/w relatively to CHT) were added to crosslink the droplets. BGNPs were also added with various contents to induce the growth of apatite (0, 5, 10, 20, 40, 50 and 60% w/w relatively to CHT). Thus, all the nomenclature percentages presented in this work regarding the BGNP content or GNP content are expressed in function of CHT weight. Also for spheres where the content of BGNPs was varied, the content of GNP remained 10% w/w; in the case where the content of GNP was varied, the content of BGNPs remained 20% w/w. Then, a controlled volume of each formulation was dropped in a superhydrophobic surface allowing the control of the final dimensions of the spheres (Fig. 1). Then, the crosslinking process occurs for 24 h in a water-saturated environment, and the droplets turn into solid spheres. The occurrence of the crosslinking was indicated by the change from transparent to deep blue colour of the spheres.

2.5. Scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS)

A NanoSEM-Fei Nova 200 (FEG/SEM) scanning electron microscope was used to study the composition and morphology of the surfaces. A Pegasus X4M instrument was used to perform the EDS experiments. Images were selected to be representative of the spheres. For EDS mapping, histological cuts of 50 μm were performed and the slices were analysed. All samples were fixed by mutual conductive adhesive tape. After immersion in SBF, the samples were again analysed in order to visualize the appearance of a calcium-phosphate layer on the surfaces.

2.6. Dynamic mechanical analysis (DMA)

The viscoelastic measurements were performed using a TRITEC8000B DMA from Triton Technology (UK), equipped with the compressive mode. The measurements were carried out at room temperature. The composites were immersed in a phosphate buffered saline (PBS) solution bath placed in a Teflon® reservoir; this hydrated environment allows the assessment of the mechanical properties in more realistic conditions [39,40]. The geometry of the hydrated samples was measured and the samples were clamped in the DMA apparatus and immersed in the PBS solution. After equilibration, the DMA spectra were obtained during a frequency scan between 0.1 and 10 Hz. The experiments were performed

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