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A new approach to fabricate bioactive silica binary and ternary hybrid microspheres

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ABSTRACT

Bioactive microspheres represent an extremely developing field in biomedical applications, such as bone tissue engineering and bone pathologies (metabolic bone disease, trauma or bone cancer). Their innate osteogenic properties have turned them to biomaterials with improved added value. The aim of this study was to prepare binary and ternary hybrid silica microspheres with enhanced bioactive properties according to our previous synthetic procedure. In brief, the synthetic approach based on the emulsifier free-emulsion polymerization method, by which polystyrene (PS) microspheres were produced and used as core template for the sol–gel coating method. During the coating reaction an inorganic shell was fabricated by silane and phosphate precursors (tetraethoxysilane, trimethylphosphate). The final microspheres were treated by different catalyst concentrations, during the coating process, which resulted in the formation of diffused voids (a porous-like structure). The in vitro bioactivity of the resultant microspheres was studied by treatment in simulated body fluids (SBF). The bioassay evaluation indicates the deposition of a bone-like apatite layer on microspheres' surface with enhanced bioresorbability, which verifies their bioactivity and permits their application in the treatment of bone pathologies.

1. Introduction

The designing of novel biomaterial that will be applied in the treatment of bone pathologies and will present enhanced biocompatibility and osteogenic (osteoconductive, osteoproductive and osteoinductive) properties is the key feature of modern biomedical research. Particularly, bone pathologies including metabolic bone disease, trauma or bone cancer ablation are responsible for various abnormalities or deformations of normal bone. In recent decades, bone pathologies present a major problem since they may result in severe bone pain and extended loss of bone density [1]. In general, bones are living tissues that provide rigid support and consist of a protein matrix (the osteoid) and crystal complexes of calcium and phosphate (the hydroxyapatite).

In bones the main nutritious activity is carried out by the osteocytes and the primary metabolic activity is promoted by the osteoblasts, in which the protein matrix known as osteoclasts is formed. Thus, the bone pathologies not only resulted in reduced bone density, but also directed the inhibition of cellular function [1–3]. Under these circumstances, the ideal biomaterial (also called scaffold) for application in bone therapies should accomplish certain requirements, such as i) the ability to be resorbed, ii) display enhanced repair and reconstruction of the bone, iii) the ability to promote the bone's regeneration process, iv) exhibit mechanical properties comparable to the defective bone site, v) allow the attachment of osteoprogenitor cells, vi) promote the differentiation of osteoblasts and vi) exhibit osteogenic properties. Despite the significant research interest for the ideal biomaterial, the development of this ideal scaffold is yet under consideration [5–7,10–17].

So far, the bioactive glasses and glass-ceramics were a successful option for scaffolding materials, which possessed innate osteogenic properties [1]. The positive role of bioactive glasses may be summarized to the ionic dissolution products from their surface, which highly enhanced the development of bone-like hydroxyapatite layer, during contact with physiological fluids. At present it is known that, this apatite layer stimulated the prompt bone bonding to the glass surface and further enhanced the bone growth, repair and regeneration process providing substantial help to the vascularization and bone formation. Upon their use in bone applications (bone pathologies and tissue engineering), the bioactive glasses were applied as granules of at least 100 μm or as solid structures, which revealed excellent anti-inflammatory properties [8]. However, their irregular size and poor mechanical properties constituted a major obstacle to their further clinical implementation. Currently, the main research objective is the development of microspherical scaffolds with potential osteogenic properties and the perspective to be used as injectable systems that stimulate bone growth [3]. One of the most interesting alternatives of bioactive

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glasses was the glassy microspheres, which displayed inherited osteogenic properties. Moreover, the spherical structure of the microspheres promoted their homogeneous distribution in cellular suspensions, which resulted in enhanced cell adhesion [3,4]. Another motivating alternative was provided by the sol–gel chemistry which enabled the intimate mixture of organic polymers with inorganic glassy structures. The incorporation of bioactive sol–gel glasses in polymeric matrixes (natural [6] or synthetic [10,11]) enabled the in vitro bioactivity and biocompatibility of the hybrids [10–17]. An excellent example was also provided by the organically modified silicates (ormosils), which belong to hybrid materials with combined properties of traditional inorganic glasses (elasticity, chemical reactivity) and organic polymers (hardness, strength, thermal stability) [1,3,17].

However, the resultant properties were determined by synergistic effects between the constituents (organic–inorganic), consequently the hybrid structures may present poor mechanical strength, due to the inorganic phase, and/or low hydrophilicity, due to the organic phase. Additionally, the problem of reduced hydrophilicity greatly affected the bioactivity of the hybrid structures, since during contact with physiological fluids the ionic dissolution was postponed. As a result, the final osteogenic properties were also detained [1,3]. This problem has been tackled recently by the incorporation of less hydrophobic organic polymers. An example was provided by the synthesis of hybrid silicate microspheres with poly(styrene) and poly(methyl methacrylate) polymers [15]. The bioactive hybrid microspheres exhibited bone-like apatite formation combined with enhanced loading and release of doxorubicin, directing to the action in bone cancer disease. In the present study, the above problem was approached through the synthesis of binary silicate and ternary phosphate–silicate hybrid structures, which were formed during the coating reaction of poly(styrene) template microspheres. Specifically, the coating reaction resulted in one-step formation of hybrid microspheres with simultaneous partial decomposition of the organic core. The comparative study of physicochemical properties of the above hybrids was followed with respect to the morphological characteristics (surface modification and size variations), which were evaluated by SEM and their structural characterization by FT-IR analysis. The in vitro bioactive behavior was assessed in simulated body fluids (SBF), wherein the formation of bone-like apatite was examined, in detail. The ionic dissolution products from the microsphere surface ultimately affected the hydroxyapatite (HA) layer development during contact with simulated body fluids. This effect may be understood by the variation in pH of the surrounding SBF [9,12]. Thus, the in vitro bioassay behavior of the hybrid microspheres was accessed through the examination of the SBF pH, in real time for a period of 20 days. This study resulted in hybrid PS@Silica microspheres with suitable bone regeneration properties (enhanced bioactivity and bone-like apatite formation) aiming at bioactive microspheres with the prospect to be applied in therapies of bone pathologies (bone disease, trauma or bone cancer ablation).

2. Experimental procedure

2.1. Materials and reagents

All chemicals used herein were of analytical grade. Aqueous ammonia (NH_3 aq. 30%), anhydrous ethanol, tetraethoxysilane (TEOS, $Si(OC_2H_5)_4$ purity > 98%), potassium persulfate (KPS), calcium nitrate tetrahydrate ($Ca(NO_3)_24 \times H_2O$), natrium nitrate ($NaNO_3$), and trimethylphosphate (TMP, $(CH_3)_3PO_4$ purity > 98%) were purchased from Aldrich and used without further purification. Styrene (St, 99%) was double distilled under reduced pressure before use.

2.2. Preparation of PS@silica binary and ternary microspheres

Polystyrene microspheres which were used as the core material (template), were prepared as described in previous study [15] by surfactant-free emulsion polymerization. Briefly, a mixture of styrene/water/KPS = 1/10/3 (ml/ml/mg) at 70 °C was used for polymerization, in which a conversion of 88% was accomplished after 24 h. The suspension was centrifuged at 6000 rpm for 5 min at 10 °C for several times. The size of the resultant microspheres was about 330 ± 30 nm in diameter. Then the second step was followed, in which the silicate shell was fabricated. Initially, 100 mg of PS spheres was suspended in a solution of ethanol/water = 9/1 (ml/ml) at 50 °C. Subsequent, 2.4 ml of aq. NH_3 30% was added and within 5 min 0.6 ml of TEOS was added dropwise. After another 20 min 0.4 ml of TMP was added in case of ternary microspheres. This process ensured the formation of a smooth coating, preventing the fabrication of silica byproducts [15]. Since the reaction was based on a sol–gel process, to assure hydrolysis and the progression of condensation, the mixture was sustained for about half hour. Following, proper solutions of calcium or natrium nitrate were added and the mixture was left to suspend for complete homogenation for about 2 h [14]. Then, another 2.4 ml of aq. NH_3 30% was added and the reaction was accomplished in 2 h. The catalyst concentration (aq. NH_3 30%) was varied between 4.8, 1.4 and 1.0 ml. The final hybrid microspheres were obtained by centrifugation of several times (1500 rpm for 5 min at 10 °C).

The synthetic route about the silicate and phosphate–silicate network formed on the PS templates was summarized in Scheme 1.

2.3. Instrumentation

The morphological characteristics and the diameter of the hybrid microspheres before and after SBF treatment were investigated by scanning electron microscopy (SEM), using a Zeiss35 VP microscope with field emission electron gun (resolution 1.7 nm) 30 KV, coupled with an energy dispersive X-ray analyzer (EDX) for element analysis, using a Philips Quanta Inspect (FEI Company, Eindhoven, the Netherlands) microscope with a W (tungsten) filament 25 KV. The microspheres were characterized by Fourier transform infrared spectroscopy (FT-IR) using a Perkin Elmer Precisely Spectrum 100 Spectrometer equipped with an attenuated total reflectance (ATR). Infrared transmission spectra (FT-IR) were recorded from 400 to 4000 cm^{-1} using the KBr method.

Hybrid microspheres were studied before and after incubation in SBF solution by X-ray powder diffraction (XRD) and the patterns were recorded from 10° to 70° 2θ at a scan rate of 0.02°/min. To specify the crystallite size the peak broadening of XRD reflection was used based on Scherrer's formula as follows [12]: $X_S = (0.9\lambda) / (FWHM\cos\theta)$, where *Xs* is the crystallite size (in nm), λ is the wavelength of X-ray beam (λ = 0.15406 nm for Cu Kα radiation), FWHM is the full width at half maximum for the diffraction peak under consideration (in rad), and θ is the diffraction angle (°). The fraction crystalline phase was evaluated by $X_C = 1\text{-}V_{112/300}/I_{300}$, where I_{300} is the intensity of (300) diffraction peak and $V_{112/300}$ is the intensity of the void between (112) and (300) diffraction peaks of hydroxyapatite, which completely disappeared in non-crystal materials.

2.4. In vitro assay

In vitro bioactivity was accessed using SBF solution with an ionic concentration 2.0-fold higher than human blood plasma (2.0 SBF: Na^+:284.0, K^+:10.0, Mg^{2+}:3.0, Ca^{2+}:5.0, Cl^-:295.6, HCO_3^-:8.4, HPO_4^{2-}:2.0, SO_4^{2-}:1.0), following Ohtsuki methodology, aiming at evaluating the hydroxyapatite formation [13]. Sealed polyethylene containers with mass to volume ratio 1 mg microspheres/1 ml SBF were placed at 37 °C for 2 weeks. Then, the specimens were collected by filtration, gently washed with water and subsequently vacuum-dried at room temperature. After incubation in 2.0 SBF the microspheres were evaluated in order to determine the extent of bone-like apatite layer formation and crystallization by FT-IR, XRD spectroscopies and the morphology by SEM microscopy.