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Manipulating the bioactivity of hydroxyapatite nano-rods structured networks: Effects on mineral coating morphology and growth kinetic☆

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ABSTRACT

Background: Nano-hydroxyapatite particles have better bioactivity than the coarse crystals. So, they can be utilized for engineered tissue implants with improved efficiency over other materials. The development of materials with specific bioactive characteristics is still under investigation.
Methods: The surface properties of four hydroxyapatite materials templated by different micelle-polymer structured network are studied. The synergistic interaction of each block copolymer in contact with CTAB rod-like micelles results in crystalline HAp nano-rods of 25–50 nm length organized in hierarchical structures with different micro-rough characteristics.
Results: It was observed that the material in vitro bioactivity strongly depends on the surface structure while in a minor extent on their Ca/P ratio. So, MIII and MIV materials with Skewness parameter $R_{sk} > 2.62$ favored the formation on their surfaces of net-like phase with a high growth kinetic constant; while MI and MII ($R_{sk} \leq 2.62$) induced the appearance of spherulitic-like structures and a growth rate 1.75 times inferior. Material biocompatibility was confirmed by interaction with rat calvarial osteoblasts.
Conclusions: The different structures growth is attributed to a dissimilar matching of crystal planes in the material and the apatite layer formed. In specific synthesis conditions, a biocompatible material with a Ca/P ratio close to that for the trabecular bone and a morphology that are considered essential for bone-bonding was obtained.
General significance: The creation of implantable devices with a specific bioactive characteristic may be useful to manipulate the attachment of cells on mineral coating directly affecting the stability and life of the implant. This article is part of a Special Issue entitled: Protein trafficking & Secretion.

1. Introduction

Synthetic hydroxyapatite (HAp, $Ca_{10}(PO_4)_6(OH)$) has a chemical similarity to the inorganic component of bone matrix and exhibits strong affinity to host hard tissues [1]. The most significant advantages of synthetic HAp are its biocompatibility, slow degradation in situ, and good osteoconductive/osteoinductive capabilities [1,2] making it an ideal candidate for the construction of orthopedic and dental implantable devices. Synthetic HAp has been widely used in bone repair, bone augmentation, as well as in coatings of metal implants or acting as a filler in bone or teeth [3,4]. However, the low mechanical strength of typical HAp ceramics, especially in a wet environment, generally restricts its use in low load-bearing applications [5]. Recent advances in nanoscience and nanotechnology have reinserted attention to the development of nanosized HAp materials and the study of their properties at the nanoscale. Current applications of nano-HAp include surface modifications of HAp to modulate their colloid stability [6–8], prevent dissolution in the case of low pH to avoid inflammation and as an intermediate layer to allow strong bond formation between HAp-polymer matrices. HAp nanoparticles have also served as non-viral carriers for drug delivery and gene therapy because of their recognized biocompatibility, ease of handling and well-known adsorption affinity [9–13]. Furthermore, HAp nanoparticles can be stably loaded with radioisotopes [11]. After loading with genes or drugs by adsorption, HAp nanoparticles provide a protective environment that shields them from degradation while providing a convenient pathway for cell membrane penetration and the controlled release of the gene/drug [12]. The research results indicate the potential of nano-HAp in gene delivery and as drug carriers [12,13] Nanocrystalline HAp powders exhibit sinterability and enhanced densification due to their greater surface area, which may improve fracture toughness as well as other mechanical properties [14]. Moreover, nano-HAp, compared to coarse crystals is expected to have better bioactivity. Thus, nano-HAp particles can be utilized for engineered tissue implants with improved bioactivity over other materials [1].

In consideration of its multiple applications and even when many HAp materials with multiform morphologies were prepared by a variety of techniques [15–19]; it is of great importance to develop new nano-HAp synthesis methods focused on the precise control of

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particle size morphology and chemical composition. Bio-inspired by bio-mineralization, morphogenesis has been emerging as an important environmentally friendly route to generate inorganic materials with controlled morphologies by using self-assembled organic superstructures, inorganic or organic additives, and/or templates with complex functionalization patterns [20]. As an alternative strategy, using water soluble polymers as crystal modifiers for controlled crystallization is widely increasing and becomes a benign route for controlling and designing multiple inorganic architectures. These additives do not act as supramolecular templates which predefine the latter hybrid material structure, but usually act as a soluble species at various hierarchy levels of the forming mineral hybrid similarly to what occurs in nature.

Here we present a study that involves different hexadecyltrimethylammonium bromide (CTAB) micelles–block copolymer organized networks to create bioactive superstructures resulting from HAp nano-rods associations. At the synthesis conditions CTAB forms rod-like micelles of 47 nm length [21] that template the deposition of PO_4^{-3} and Ca^{2+} ions favoring the formation of bone dimensioned HAp nano-rods. The interaction with block copolymers restrings the crystals' growth and directs their association inducing the final structure arrangement. The structure set-up and evolution relies on a synergistic effect of the mutual interactions of the confined reaction media environment (block copolymer network) in contact with the external template (CTAB rod-like micelles). The manipulation of such interactions permits the alteration of the chemical and/or the surface properties on the synthesized materials and their subsequent bioactivity, including mineral coating morphology and growth kinetic. The proposed method lets us obtain bioactive and biocompatible materials which may allow us to replicate to some extent the bone structure, HAp nanorods of 25–50 nm length organized in hierarchical structures. Such characteristics are desirable to reproduce in a synthetic material thinking of its future use as nanoscopic building units of tissue engineered scaffolds designed to mimic the structural and biological functions of native extracellular matrix (ECM).

2. Experimental

2.1. Materials

Hexadecyl-trimethyl ammonium bromide (CTAB, MW = 364.48 g mol^{-1}, 99% Sigma), poly(ethylene glycol) 400 (PEG 400, Sigma-Aldrich, MW = 380–420 g mol^{-1}, δ = 1.126 g cm^{-3} at 25 °C), poly (propylene glycol) (PPG, Sigma-Aldrich, MW = 425 g mol^{-1}, δ = 1.004 g cm^{-3} at 25 °C), Poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol) (PEG–PPG–PEG, PEG 10 wt.%, Sigma-Aldrich, MW = 2800 g mol^{-1}, δ = 1.018 g cm^{-3}), Octylphenyl-polyethylene glycol (IGEPAL® CA 630, $(C_2H_4O)_nC_{14}H_{22}O$, Sigma-Aldrich, MW = 603 g mol^{-1}, δ = 1.06 g cm^{-3} at 25 °C), sodium phosphate (Na_3PO_4, MW = 148 g mol^{-1}, 96% Sigma), calcium chloride ($CaCl_2$, MW = 91 g mol^{-1}, 99% Sigma) and sodium nitrite ($NaNO_2$, MW = 69 g mol^{-1}, 97%) were used without further purification (as a reference their structures are shown in Scheme 1). For solution preparation, only triple-distilled water was used.

2.2. HAp material synthesis

Four materials (denoted as I, II, III and IV) were prepared using a modification of the method proposed by Liu et al. [22]; descriptions of the selected synthesis condition are shown in the electronic supplementary material (ESM). Material I: first, 350 mL of a 3.13 mM CTAB aqueous solution was mixed with 20 mL of PPG and stirred at 500 rpm for 10 min. Second, 200 mL of 2 M sodium nitrite aqueous solution and 2.2 g calcium chloride were incorporated in sequence. Finally, 200 mL of 0.14 M of Na_3PO_4 aqueous solution was added to the above mixed solution drop by drop at room temperature under magnetic stirring at 500 rpm. Finally 20 mL of 2 M sodium nitrite aqueous solution and 0.22 g calcium chloride were incorporated in sequence. After the integration of all reactants, the solution was magnetically stirred for 1 h. The resulting gels were left for 24 h in an autoclave at 100 °C. The obtained materials were filtered, washed with triple-distilled water to remove impurities. Finally, the surfactant was completely removed by acidic solvent extraction technique [23,24].

For samples II, III and IV the preparation protocol was similar to that described for sample I with the exception of 20 mL of PEG, PEG–PPG–PEG and IGEPAL® CA 630 respectively instead of PPG.

2.3. Field emission scanning electron microscopy (FE-SEM)

Surface morphology was evaluated using a field emission scanning electron microscope (FE-SEM ZEISS FE-SEM ULTRA PLUS). To acquire all the SEM images a Secondary Electron Detector (in lens) was used. The accelerating voltage (EHT) applied was 3.00 kV with a resolution (WD) of 2.1 nm. Local compensation of charge (by injecting nitrogen gas) or the sample shading was not necessary. The associated energy-dispersive spectrophotometer provided qualitative information about surface elemental composition. The topography of samples was quantified from SEM microphotographs using different software packages [25], uncertainty of 5%.

2.4. Transmission electron microscopy (TEM)

Transmission electron microscopy was performed using a Philips CM-12 transmission electron microscope equipped with a digital camera MEGA VIEW-II DOCU and operated at 120 kV with magnification of 730,000×. Observations were made in a bright field. Powdered samples were placed on copper supports of 2000 mesh. High resolution transmission (H-TEM) microphotographs were taken using a Libra 200 FE OMEGA transmission electron microscope operated at 200 kV with magnification of 100,0000×. Observations were made in a bright field. Powdered samples were placed on carbon supports of 2000 mesh.

2.5. X-ray powder diffraction

Powder X-ray diffraction (XRD) data were collected with a Philips PW 1710 diffractometer with Cu K_α radiation (λ = 1.5418 nm) and Graphite monochromator operated at 45 kV; 30 mA and 25 °C. The mean crystalline size (δ) of the particles was calculated from XRD line broadening measurement using the Scherrer equation [26]:

$$\delta = \frac{0.89\lambda}{\beta \cos\theta} \quad (1)$$

where λ is the wavelength (Cu K_α), βis the full width at the half maximum of the HAp (211) line and θ is the diffraction angle. The fraction of crystalline phase (X_c) of HAp powders was evaluated by the following equation [26]:

$$X_c = \frac{1-\upsilon_{112/300}}{I_{300}} \quad (2)$$

where I_{300} is the intensity of (300) diffraction peak and $\upsilon_{112/300}$ is the intensity of the hollow between (112) and (300) diffraction peaks of HAp. The estimated uncertainties are about 20%.

2.6. FT-IR spectroscopy

The experiments were done in a Nicolet FT-IR Nexus 470 Spectrophotometer. To avoid co-adsorbed water, the samples were dried under vacuum until constant weight was achieved and diluted with KBr powder before the FT-IR spectra were recorded.

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