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Nanoscale surface characterization of biphasic calcium phosphate, with comparisons to calcium hydroxyapatite and β-tricalcium phosphate bioceramics

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ABSTRACT

Objectives: It is our aim to understand the mechanisms that make calcium phosphates, such as bioactive calcium hydroxyapatite (HA), and biphasic calcium (BCP) and β-tricalcium (β-TCP) phosphates, desirable for a variety of biological applications, such as the filling of bone defects.
Methods: Here, we have characterized these materials by X-ray photoelectron spectroscopy (XPS), X-ray diffraction (XRD), scanning electron microscopy (SEM), Fourier-transform infrared (FTIR), time-of-flight secondary ion mass spectroscopy (TOF-SIMS) and laser granulometry.
Results: SEM shows clearly that BCP is a matrix made of macro-organized microstructure, giving insight to the specially chosen composition of the BCP that offers both an adequate scaffold and good porosity for further bone growth. As revealed by laser granulometry, the particles exhibit a homogeneous size distribution, centered at a value somewhat larger than the expected 500 μm. XPS has revealed the presence of adventitious carbon at all sample surfaces, and has shown that Ca/P and O/Ca ratios in the outer layers of all the samples differ significantly from those expected. A peak-by-peak XPS comparison for all samples has revealed that TCP and BCP are distinct from one another in the relative intensities of their oxygen peaks. The PO_3^-/PO_2^- and CaOH+/Ca+ TOF-SIMS intensity ratios were used to distinguish among the samples, and to demonstrate that the OH- fragment, present in all the samples, is not formed during fragmentation but exists at the sample surface, probably as a contaminant.
Conclusions: This study provides substantial insight into the nanoscale surface properties of BCP, HA and β-TCP. Further research is required to help identify the effect of surfaces of these bioceramics with proteins and several biological fluids.
Clinical relevance: The biological performance of implanted synthetic graft bone biomaterials is strongly influenced by their nanosurface characteristics, the structures and properties of the outer layer of the biomaterial.

1. Introduction

In the process of bone regeneration by synthetic grafting, the bioceramics most widely used as filling materials are hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$), β-tricalcium phosphate (β-TPC, $Ca_3(PO_4)_2$), and biphasic calcium phosphate (BCP, a mixture of HA and β-TCP), [1–3] due to their properties of biocompatibility, biodegradability, bioresorption and osteoconduction [4–6]. Each bioceramic, however, differs in its ability to participate with the dynamic physiological environment and to achieve a degree of chemical equilibrium with the host tissue, without fibrous capsule formation [7].

HA is the main component of the rigidity of vital tissues, such as bone, and has an ability to drive the further growth of bone at its surface [8,9]. Thus, the identification and distinction of different phases of bioceramic are crucial for understanding their biological effect [10–14].

The mechanical behaviors of bioactive ceramics are well enough known, and their physicochemical surface properties may now be understood in terms of their structure [15]. Both HA and β-TCP are biocompatible, nontoxic, resorbable, non-inflammatory, cause

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neither immune nor irritating responses, and have excellent osteoconductive abilities [16,17]. They differ in composition and degradation rates: β-TCP shows good ability to biodegrade and to bioresorb up to 10–20 times faster than HA, but in an unpredictable manner, so it may not provide a solid scaffolding for new bone formation [18,19]. In fact, according to Wiltfang et al. [18], the accelerated initial ceramolysis of ß-TCP ceramics did not hinder the bone-healing process, which follows the principles of a primary angiogenic reossification; in the early days, chemical impurities present in the TCP ceramics led to varying degradation times.

Biphasic calcium phosphate (BCP) is composed of a controlled mixture of HA and β-TCP [20]. According to the manufacturer's description, it is a fully synthetic bioactive, osteoconductive bone substitute, available in powder form and is already used clinically [21]. This biphasic calcium phosphate ceramic, composed of 60% HA and 40% TCP, has a chemical composition close to that of bone. It is able to gradually degrade, leaving room for natural bone [22]. The results of its implantation indicate good biocompatibility and bioresorbability, when firmly packed into the bone [23,24].

Solubility appears to be the characteristic of primary importance in the remineralization process; results showed that β-TCP has the highest solubility, followed by BCP, then HA [17,25]. In addition, the biodegradation rate increases with increasing specific surface area (powders > porous solid > dense solid), and with decreasing crystallinity and grain size [1]; this includes contaminant chemical substituents, such as F in HA or Mg in β-TCP [26]. In biphasic calcium phosphate, the limiting factor is the HA:β-TCP ratio [27].

Bone colonizes bioceramics more easily when their surfaces contain both micro- and macropores [3,4,28]. Porosity also influences biological material behavior, and is an essential parameter for a satisfactory clinical outcome [7,29,30]. For good tissue development, the pore size and interconnectivity affect fluids, nutrients and oxygen diffusion and protein adsorption, as well as cell migration and their attachment, differentiation and proliferation [20,31]. The presence of macropores (diameter >100 μm) gives the bioceramic its osteoconductive properties, and promotes cell colonization by providing a scaffold for blood vessel proliferation [32,33]. The presence of micropores (diameter <100 μm) increases the exchange surface area required for fluid penetration into tissues, and promotes the adhesion of macromolecules and proteins for selective adsorption; this presents a more suitable geometry for improved cell anchoring and cellular differentiation [15,34]. It is for this reason that the synthesis process of bioceramics is crucial.

The objective of this article is to identify and distinguish the nanoscale physicochemical features of different phases of three bioceramics, HA, β-TPC and BCP (Straumann BoneCeramic®, 60% HA–40% β-TCP), to better understanding their biological effects and to clarify the success of their use in implantation.

2. Materials and methods

2.1. Samples tested

Experiments were performed on microparticles that provide a greater exchange surface with surrounding fluids and lead to a more rapid bioceramic dissolution and, consequently, to a rapid change in the local stimulated fluid composition. The three synthetic bone substitutes analyzed were differed in their Ca:P ratios.

Hydroxyapatite (HA: $[Ca_{10}(PO_4)_6(OH)_2]$, Ca/P ratio: 1.67) was obtained from Sigma–Aldrich Inc.; β-tricalcium phosphate (β-TCP: $[Ca_3(PO_4)_2]$, Ca/P ratio: 1.5) was obtained from GFS Chemicals, and BCP (Straumann BoneCeramic 500®, 60% HA–40% β-TCP, Ca/P ratio: 1.61) was furnished by Straumann, Inc.

2.2. Protocol

All the samples were initially characterized as dry powders, referred to as the initial state. Particle dimensions were determined by laser granulometry. The surface states of the tested materials were then chemically characterized by X-ray photoelectron spectroscopy (XPS), time-of-flight second ion mass spectrometry (TOF-SIMS) and Fourier transformed infrared spectroscopy (FTIR); their crystallinities and crystal sizes were characterized by X-ray diffraction (XRD), and their morphologies by scanning electron microscopy (SEM).

2.3. Sample characterization

a. Particle size by laser granulometry:

A Coulter LS Particle Size Analyzer was used to determine the particle size distributions: the samples were dispersed in water and sonicated, as instructed by the instrument manual. The instrument provides graphical outputs of the volume, number and surface areal percentages of the particles, over a spherical equivalent diameter size range of 0.4–2000 μm.

b. Surface chemical composition by XPS:

The XPS analyses were performed using a VG ESCALAB 3 MK II; Al Kα radiation ($h\nu$ = 1486.6 eV, with an instrument resolution of 0.85 eV) was used, at a pressure below 1×10^{-9} torr. Powders were pressed into a 1×2 cm sample holders. The elements detected were observed using both survey and high-resolution, spectra, with element-dependent probe depths of ~4–5 nm. The XPS binding energy (BE) values were charge-corrected to that of uncharged adventitious carbon at 285.0 eV. This analysis method gives the energy distribution of electrons emitted as a result of the interaction between the biomaterial and incident X-rays. Their analysis gives qualitative (elements present) and quantitative (the relative concentration of each spectral peak component) information. This information permits us to evaluate the Ca:P and O:Ca ratios at the sample surfaces.

c. Crystal structure and size by XRD:

XRD was used to determine crystallinity of the samples, using a fixed incident beam angle of 5° and a detector angle range of $5° < 2\theta < 80°$. The X-ray diffractometer (PANalytic X'Pert MPD) used Cu Kα (1.54184 Å) radiation at a voltage of 50 kV, a current of 40 mA, a step size of 0.02° and a scan rate of 1.2°/min. The diffraction patterns were processed using the X'Pert High Score software. This method permits distinguishing products with the same gross chemical composition but different crystal structures (e.g., different crystal structures of calcium phosphate). Further, it permits the determination of nanocrystal size through the use of the Scherrer formula on an appropriate diffraction peak.

d. Microstructure and topography by SEM:

A JEOL JSM-7600TFE scanning electron microscope was used to image the surface structure and topography. Accelerating voltages for HA and β-TCP, using the LEI detector, were 1 and 2 kV, respectively; for BCP, using the Compo detector for the 1 μm scale and the LEI detector for the 100 μm scale, the accelerating voltages were 5 kV and 2 kV, respectively.

e. Bulk composition by FTIR:

Infrared spectra were obtained at a resolution of 4 cm^{-1}, using an M-TEC M300 photoacoustic cell mounted on a Digilab FTS700