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Sol–gel synthesis and characterization of macroporous calcium phosphate bioceramics containing microporosity

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Abstract

Amorphous calcium phosphate powders were precipitated from calcium metal and phosphoric acid in ethanol. Depending on the quantity of reagent, the CaP powders had different chemical compositions and, after heating, formed beta-tricalcium phosphate (β-TCP), hydroxyapatite (HA) or BCP mixtures. Dilatometric measurements indicated that shrinkage of compacted CaP powders occurred first at around 650 °C and continued up to 1200 °C. The amorphous CaP powders were mixed with urea beads, compacted under isostatic pressure at 140 MPa and sintered at 1100 °C for 5 h. Scanning electron microscopy indicated that macro–microporous ceramics were produced. The ceramics had spherical macropores of 700–1200 μm in diameter, with limited interconnections and a macroporosity of 42% as determined by microcomputed tomography. The micropores ranged from 0.1 to 1 μm in diameter. These ceramics made of HA, β-TCP or BCP exhibiting both macroporosity and microporosity can be used as bone fillers.

Keywords: Calcium phosphate; Sol–gel processing; Amorphous powders; Sintering; Ceramics

1. Introduction

Calcium phosphates (CaP) are the principal inorganic constituents of hard tissues in vertebrates (bones and teeth). In these biological tissues, CaP crystals are intimately associated with macromolecules such as collagen and proteins, forming mineral–organic composites with excellent mechanical properties [1]. Bone tissue is a "living material" as it is constantly resorbed by osteoclastic activity and formed by osteoblastic cells [2]. Although bone is able to repair and heal itself in most of the cases, there are many instances where biomaterials are needed in order to restore its function. For many years, scientists have attempted to mimic this natural material or the biological processes leading to its formation.

CaP ceramics are used increasingly as bone substitutes in orthopaedic and maxillofacial surgery [3]. These ceramics, composed of hydroxyapatite (HA), beta-tricalcium phosphate (β-TCP) and combinations of the two, known as biphasic calcium phosphate (BCP), are biocompatible, bioactive and osteoconductive [4]. After filling a bone defect, these bioceramics partly dissolve into body fluids, leading to the precipitation of biological apatite on to their surface [5]. Osteoblast cells colonize and produce the collagenous extracellular matrix, which mineralizes to form osteoid tissue in contact with the ceramic filler. The woven bone is gradually remodelled by osteoclastic cells into mechanically strong bone tissue. Although they have good biological properties, bioceramics degrade poorly and are not completely replaced by bone tissue during the healing and remodelling phases [6]. Furthermore, bioceramics generally lack the osteoinductive properties needed to regenerate large bone defects.

The structure of ceramics plays a critical role in their osteointegration [6,7]. It has been shown that open porosity with macropores ranging from 100 to 800 μm favours body fluid invasion, cell colonization, vascularization and bone tissue ingrowth. The macroporosity is usually obtained by introducing organic compounds into the compacted

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green bodies. During sintering, these organic compounds burn out, forming pores in the CaP ceramic [5]. The challenge is to produce open, interconnected porosity while maintaining sufficient strength for handling. Microporosity with pores in the 0.1–5 μm range is also an important parameter, as it determines the surface area, protein adsorption and dissolution properties of bioceramics. Microporosity is usually obtained by using low sintering temperatures. Microporous CaP ceramics have been easily prepared starting from amorphous CaP powders [8,9]. These nanometer-sized particles have high surface energy and could be sintered from the onset of thermal crystallization (ca. 600 °C) to around 1100 °C producing microporous ceramics. On the other hand, compacted crystalline HA powder has shown shrinkage and the formation of grain boundaries only at 1100 °C. These previous studies using compacted amorphous CaP powders focused on the preparation and characterization of HA ceramics avoiding macroporosity.

Several studies have recently demonstrated that microporous CaP ceramics exhibit osteoinductive properties as they are able to form mineralized bone tissue within 6–24 weeks after implantation into the muscles of large animals [10–12]. It has been postulated that these microporous ceramics concentrate endogenous bone growth factors on their surface through a dissolution–precipitation process [10,13]. These growth factors may induce the differentiation of circulating stem cells into osteoblasts that produce bone tissue. We have hypothesized that microparticles released from poorly sintered ceramic lead to an inflammatory reaction with the release of cytokines that may trigger circulating stem cells to form bone tissue [14,15]. The process may be similar to that observed in the healing of fractures, where bone debris is usually degraded by macrophages and osteoclasts [16]. It has been shown that microporous ceramics have superior osteogenic properties than dense ceramics when implanted in critical-sized bone defects [17,15].

The purpose of this study was to prepare and characterize macroporous ceramics by using nanometer-sized, amorphous CaP powders sintered at 1100 °C. CaP powders with different compositions were precipitated in ethanol by mixing $Ca(OEt)_2$ and H_3PO_4. The chemical composition, crystal phases and thermal behaviour of the precipitates were studied as a function of temperature. The powders were mixed with urea beads, compacted and sintered into ceramics composed of HA, β-TCP or BCP and exhibiting both macroporosity and microporosity.

2. Materials and methods

2.1. Sol–gel synthesis of calcium phosphate powders

Calcium metal (99%, Aldrich) and phosphoric acid crystals (98+%, Aldrich) were used for the preparation of calcium phosphate powders [8]. All the experiments were performed using conventional glassware with vacuum manifolds for air-sensitive products. The calcium diethoxide $Ca(OEt)_2$ was prepared by reacting the appropriate amounts of calcium metal with pure ethanol according to the following reaction

$$Ca + 2EtOH \rightarrow Ca(OEt)_2 + H_2 \uparrow$$

Several precautions were taken during the synthesis of the $Ca(OEt)_2$. The most important was to exclude moisture and air because $Ca(OEt)_2$ is very moisture- and air-sensitive. The glassware, calcium metal and ethanol used were dried and the synthesis was carried out under nitrogen gas. These measures were to prevent the precipitation of the hydrolysed product, i.e. $Ca(OH)_2$, and the carbonate salt, i.e. $CaCO_3$. The appropriate amount of calcium metal shavings (5.1 g; 0.128 mol) and 400 ml of dry ethanol were put in a 1000 ml, three neck, round bottom flask with a heater–magnetic stirrer and a condenser in a vacuum. The ethanol was refluxed for 4 h until all the metallic calcium had disappeared. A solution of orthophosphoric acid was prepared by dissolving the appropriate amounts of anhydrous H_3PO_4 crystals (7.4–8.7 g; 0.075–0.088 mol) in 200 ml of dried ethanol with stirring at room temperature. Given these amounts of calcium metal and phosphoric acid, the molar Ca/P ratios ranged from 1.45 to 1.70. These different Ca/P molar ratios corresponded to those of TCP ($Ca_3(PO_4)_2$; Ca/P = 1.5), HA ($Ca_{10}(PO_4)_6(OH)_2$; Ca/P = 1.67) and BCP. The orthophosphoric solution was added quickly through a nozzle to the vigorously stirred calcium ethoxide solution. A gelatinous white precipitate of calcium phosphate was immediately obtained. This precipitate was stirred for 10 min with refluxing. After cooling, the excess ethanol was removed by evaporating in a vacuum (Büchi, rotavap). The precipitate was dried in a vacuum at room temperature overnight and crushed in an agate mortar. About 20 g of a fine white powder was obtained for each batch.

2.2. Characterization of the calcium phosphate powders

All batches of CaP powder were analysed by X-ray diffraction and infrared spectroscopy before and after heating an aliquot of 1–2 g at 1100 °C for 5 h in air. Powder X-ray diffraction (XRD; Philips PW 1830) was performed using a Cu K_α source operated at 40 kV and 30 mA. The XRD patterns were recorded from 3° to 60° in 2θ with a step angle of 0.02°. XRD traces were compared to JCPDS standard files (HA #9-432, β-TCP #9-169). After checking the absence of other CaP phases, the quantities of HA and β-TCP phases were measured by the respective intensities of their 100% diffraction lines according to the ISO 10993-1 standard. The experimental HA/β-TCP weight ratios and Ca/P atomic ratios were calculated and plotted against the theoretical Ca/P molar ratios. Infrared (IR) spectra were obtained over the 4000–400 cm^{-1} region using a Fourier transform infrared (FTIR) spectrometer (Nicolet, Magna-IR 550). Transparent pellets were made by compacting at 14 tons about 1 mg of the sample mixed