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Intrinsic porosity of calcium phosphate cements and its significance for drug delivery and tissue engineering applications

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Abstract

One key point in the field of tissue engineering and drug delivery is to provide materials with an adequate porosity. Many events, including nutrient and waste exchange in scaffolds for tissue engineering, as well as the drug-loading capacity and control of the release rate in drug delivery systems, are controlled by the size, shape and distribution of the pores in the material. Calcium phosphate cements (CPCs) possess an intrinsic porosity that is highly suited for these applications, and this porosity can be controlled by modifying some processing parameters. The objective of this work was to characterize and control the intrinsic porosity of α-tricalcium phosphate (α-TCP) cements, and to investigate its role against adsorption of bovine serum albumin (BSA). Cements with different percentages of open porosity (35–55%) were prepared by modifying the liquid-to-powder ratio. In addition, two different TCP particles were used to yield cements with specific surface areas of ~20 and ~37 $m^2 g^{-1}$. Mercury porosimetry analysis on the set cements showed in most cases a bimodal pore size distribution which varied with the processing parameters and affected differently the adsorption and penetration of BSA. The peak occurring at larger pore dimensions controlled the penetration of BSA and was ascribed to the voids generated in between crystal aggregates, while the peak appearing at lower pore sizes was believed to be due to the intercrystallite voids within aggregates. It was found that, at the concentrations studied, the high intrinsic porosity in CPC does not ensure protein penetration unless there is an adequate pore size distribution.
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1. Introduction

It has been nearly 30 years since LeGeros [1] and Brown and Chow [2] first developed calcium phosphate cements (CPCs) for biomedical applications. The setting reaction in CPC requires mixing one or more solid calcium orthophosphates with an aqueous solution. The mixture first forms a paste which later sets and hardens through a dissolution–precipitation mechanism. The concomitant dissolution of the soluble reactants is followed by the precipitation of a highly stable phase. At physiological conditions, i.e. at neutral pH and 37 °C, the end-product of the cementitious reaction is usually calcium-deficient apatite which closely resembles the mineral phase in bone [3,4]. The ease of the processing of these materials, as well as their unique properties in terms of bioactivity, resorbability and the possibility of making them injectable, justify the large interest in these materials since their discovery.

The fabrication of CPC is a versatile process which yields a variety of tailor-made injectable pastes and set cement materials with different physicochemical and mechanical properties. The ultimate properties of the cement will depend on the characteristics of the solid and aqueous phase and the reaction conditions. One feature of special interest in cements is the fact that they are intrinsically porous. They have an important percentage of porosity within the nano/submicron size range. While porosity can be a limitation for the use of these materials in high-load bearing applications, e.g. vertebroplasty, it is

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vital for other applications. Porosity is sought to enhance a material's resorbability and the extent of bioactivity by increasing the surface area available for reaction. In the same way, their inherent porosity makes these materials good carriers for controlled drug delivery systems. However, although several studies have already been published about the use of CPCs in drug delivery systems, the approach up to now has been very empirical, and no detailed correlation between textural properties of the cements and loading and release kinetics has been established [5,6]. This contrasts with other ceramic carriers that have been proposed for drug delivery applications, such as the mesoporous materials, which have an ordered pore network very homogeneous in size (between 2 and 50 nm) that allows fine control of the drug load and release kinetics [7].

When using CPCs for drug delivery applications, the drug can be mixed in either the liquid- or solid-phase components of the cement [8], or alternatively it can be incorporated by adsorption on the set cement [9]. In either case, not only is the total interconnected porosity relevant for the loading and delivery of the drug, but the pore dimensions and pore size distribution within the cement, as well as its specific surface area (SSA) [6], are also important. Only a thorough characterization of these features will yield a sound knowledge and control of the kinetics of drug elution, ensuring reproducibility and reliability of CPC-based drug delivery systems. Moreover, in the field of bone tissue engineering, where CPCs have been proposed as starting materials to fabricate cell-supporting three-dimensional scaffolds [10], micro- and nanoporosity becomes crucial to allow entrance of nutrients and removal of waste residues generated by the cells [11].

While much is known about the overall properties of CPCs, little attention has been paid to the accurate characterization of their intrinsic porosity. The present paper aims to provide detailed information on the textural properties of α-TCP cements, and on the processing parameters that allow these properties to be controlled. Specifically, the effects of the liquid-to-powder (L/P) ratio and the particle size of the starting powder on the total porosity, pore size distribution and SSA are analyzed. As a first step to envisage the implications of the different textural properties on the interaction with proteins, a series of experiments are performed based on the adsorption of albumin, the most abundant protein in the blood plasma, which has a high affinity for apatite. In fact, albumin, which is a relatively small protein ($14 \times 4 \times 4$ nm^3), is present both in vivo and in the environments designed for in vitro tissue engineering applications, and moreover, is known for its role as carrier for drugs and molecules that are poorly soluble in water.

2. Materials and methods

2.1. Liquid- and solid-phase preparation

α-TCP was the only reactant used for the preparation of the cement's solid phase. It was obtained by heating in a furnace (Hobersal CNR-58), in air, an appropriate mixture of calcium hydrogen phosphate ($CaHPO_4$, Sigma–Aldrich C7263) and calcium carbonate ($CaCO_3$, Sigma–Aldrich C4830) at 1400 °C for 15 h followed by quenching in air.

Two different sizes of α-TCP powder were then prepared following two different milling protocols. The powder with smaller particle size (named as fine, F) was first milled in an agate ball mill (Pulverisette 6, Fritsch GmbB) with 10 balls ($d = 30$ mm) for 60 min at 450 rpm followed by a second milling for 70 min at 500 rpm with 100 balls ($d = 10$ mm). The powder with larger size (named as coarse, C) was first milled with 10 balls ($d = 30$ mm) for 20 min at 450 rpm and subsequently with 100 balls ($d = 10$ mm) for 10 min at 500 rpm. Precipitated hydroxyapatite (2 wt.%; Alco ref. 1.02143) was added as a seed in the powder. The particle size distribution of both F and C powders was analyzed by laser diffraction (LS 13 320 Beckman Coulter). To minimize aggregation during the measurement the samples were dispersed in ethanol in an ultrasonic bath. The SSA of the powders was analyzed by nitrogen adsorption following the Brunnauer–Emmet–Teller method (BET; ASAP 2020 Micromeritics). Helium pycnometry was performed to evaluate the skeletal density of the powder (AccuPyc 1330 Micromeritics).

The liquid phase consisted of an aqueous solution of 2.5 wt.% disodium hydrogen phosphate, Na_2HPO_4 (Panreac 131679.1210), added to accelerate the setting reaction of the cement.

2.2. Cement preparation

Cements with different L/P ratios of 0.35, 0.45, 0.55 and 0.65 ml g^{-1} were prepared using both the F and C α-TCP powders. The powder and liquid phase was first mixed in a mortar for about 1 min and then transferred into cylindrical molds 6 mm in diameter and 12 mm high. The cements were then allowed to set in Ringer's solution (0.15 M sodium chloride solution) for 7 days at 37 °C. The hydrolysis reaction that led to formation of a calcium-deficient apatite can be written as:

$$3Ca_3(PO_4)_{2(s)} + H_2O_{(l)} \rightarrow Ca_9(HPO_4)(PO_4)_5(OH)_{(s)} \quad (1)$$

Hereafter, the different cements will be named with the letter F or C, depending on the type of α-TCP powder used, followed by a number indicating their specific L/P ratio. The sequence of the different cements is the following: F35, F45, F55, F65, C35, C45, C55 and C65.

Prior to characterization, the cements were thoroughly rinsed with MilliQ water and allowed to dry in air. The cement's SSA was measured according to the BET method (ASAP 2020 Micromeritics). The cement's phase composition was evaluated by X-ray diffraction analysis (XRD, Philips MRD). Scanning electron microscopy (SEM, JEOL JSM-840) and field emission SEM (FE-SEM, Hitachi H-4100FE) were used to investigate the surface and

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