Acta Biomaterialia xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Multiple characterization study on porosity and pore structure of calcium phosphate cements

David Pastorino, Cristina Canal, Maria-Pau Ginebra *

Biomaterials, Biomechanics and Tissue Engineering Group, Department of Materials Science and Metallurgy, Universitat Politècnica de Catalunya. BarcelonaTech (UPC), Av. Diagonal 647, 08028 Barcelona, Spain
Centre for Research in Nanoengineering, Technical University of Catalonia (UPC), c/Pascual i Vila 15, 08028 Barcelona, Spain

ARTICLE INFO

Article history:
Received 29 May 2015
Received in revised form 8 September 2015
Accepted 15 September 2015
Available online xxxx

Keywords:
Calcium phosphate cement
Porosity
Nitrogen sorption
Mercury intrusion porosimetry
Thermoporometry
Pore size distribution
Synthetic bone graft
Nanostructured materials

ABSTRACT

Characterization of the intricate pore structure of calcium phosphate cements is a key step to successfully link the structural properties of these synthetic bone grafts with their most relevant properties, such as *in vitro* or *in vivo* behaviour, drug loading and release properties, or degradation over time. This is a challenging task due to the wide range of pore sizes in calcium phosphate cements, compared to most other ceramic biomaterials. This work provides a critical assessment of three different techniques based on different physical phenomena, namely mercury intrusion porosimetry (MIP), Nitrogen sorption, and thermoporometry (TPM) for the detailed characterization of four calcium phosphate cements with different textural properties in terms of total porosity, pore size distribution (PSD), and pore entrance size distribution (PESD). MIP covers a much wider size range than TPM and Nitrogen sorption, offering more comprehensive information at the micrometer level. TPM, and especially Nitrogen sorption, are non-destructive techniques and, although they cover a limited size range, provide complementary information regarding pore structure associated with crystal shape at the nanoscale, recording both PSD and PESD in a single experiment. MIP tended to register smaller sizes, especially at low L/P ratios, due to the network effect, which has a strong influence on the outcome of this technique.

Statement of significance

The detailed characterisation of the porosity of calcium phosphate cements is of paramount importance, since it is a key parameter influencing some of the most relevant features, like mechanical properties, degradation rate or drug loading and release kinetics. However, this is a challenging task because, once hardened, calcium phosphate cements present an intricate morphology, consisting of a network of precipitated crystals, which generate a high intrinsic micro/nano porosity, with pore sizes covering six orders of magnitude. This work provides for the first time a critical assessment of the advantages and limitations of three different techniques, namely mercury intrusion porosimetry, Nitrogen sorption and Thermoporometry, for the characterisation of the porosity of four calcium phosphate cements with different textural properties.

1. Introduction

Porosity has become a key feature in the design of biomaterials for bone regeneration. Some crucial aspects, like the rate of resorption and the extent of tissue colonization, depend not only on the intrinsic properties of the material but also on the amount, size, and shape of the pores of the biomaterial [1,2]; the relevance of porosity is even higher when the material is designed to act, in addition, as a substrate for the local and controlled delivery of drugs or cells, since its performance will depend on some physical phenomena like permeability or diffusion, which are directly linked to porosity [3]. The physical and biological behaviours of a porous material are strongly affected by the way in which the pores of various sizes are distributed within the solid, in addition to the effects of total porosity. Hence, it is important to

* Corresponding author at: Biomaterials, Biomechanics and Tissue Engineering Group, Department of Materials Science and Metallurgical Engineering, Universitat Politècnica de Catalunya. BarcelonaTech (UPC), Av. Diagonal 647, 08028 Barcelona, Spain.

E-mail addresses: david.pastorino@upc.edu (D. Pastorino), cristina.canal@upc.edu (C. Canal), maria.pau.ginebra@upc.edu (M.-P. Ginebra).

http://dx.doi.org/10.1016/j.actbio.2015.09.017
1742-7061/

characterize the porosity and textural properties of the material through the full-scale range. Whereas this can be relatively straightforward in the case of high-temperature sintered ceramics, the situation becomes more complex in biomimetic calcium phosphate materials, where nanometer-sized crystals are obtained, with intricate microstructures and high specific surface areas [4].

Calcium phosphate cements (CPCs) are good examples of nanostructured calcium phosphates [4,5]. These synthetic bone substitutes present attractive properties, such as similarity to the mineral phase of bone, the capacity to harden within the body, and injectability. Once hardened, the material consists of a network of nanometric and micrometric precipitated crystals, which generates a high intrinsic micro/nano porosity with a very wide pore size range, from 1 nm to 1 mm, i.e., six orders of magnitude [6,7]. This allows the design of materials inspired by the nanostructured nature of native bone [4,7], with distinct advantages in terms of bioactivity, resorbability, permeability, and the adsorption and release of active compounds [3–5,8,9]. Despite this, their intrinsic complexity makes the detailed evaluation of the porosity of CPCs a challenging task; therefore, the selection of the most adequate method for its characterization is not trivial.

Pycnometry, based on the Archimedes principle, can be used to determine the total pore volume but provides only limited or no information on pore structure parameters, like pore size distribution and specific surface area.

Mercury intrusion porosimetry (MIP) has proven to be a powerful technique to characterize porous materials with pore sizes ranging in several orders of magnitude [10]. MIP is based on the intrusion of a non-wetting liquid, mercury, into a material, and the possibility of relating the pressure needed to force mercury into the specimen with the equivalent pore sizes [11]. MIP is the most widely used method for determining the pore size distribution of hardened cement pastes for civil engineering applications, and it has also been successfully applied to the characterization of CPCs [5]; however, some concerns have been raised recently [12–14] related to the ink-bottle effect [15] and the high pressures applied there, which are speculated to be potentially harmful for the material. Moreover, a drying step is required prior to the experiment, which might affect its outcome. Other drawbacks are related to the toxicity of mercury and that MIP is a destructive method, since the sample cannot be reused after part of the mercury remains irreversibly trapped in the pores.

An alternative technique is gas adsorption, which is one of the most popular methods for the study of pore structure in materials that contain micropores (<2 nm) and mesopores (between 2 and 50 nm). In the field of CPCs, however, gas adsorption has generally been limited to the characterization of the specific surface area [1,16–18] using the Brunauer–Emmet–Teller (BET) theory [19], which involves a model for the adsorption of a monolayer of gas onto the surface of a material. However, capillary condensation measurements allow also the correlation of vapour pressure with pore dimensions, providing information on the pore size distribution in the material.

Thermoporometry (TPM), also called thermoporosimetry, is based on the decrease in freezing point of a probe liquid confined in the pores of the material tested. The method, first described by Brun and Lallemand, allows correlation of the shifts of the freezing and melting points with the pore size distribution of the material [21]. One of the main advantages of TPM is that, unlike most other techniques, it allows the characterization of wet samples and there is no need for specialized instruments, since a differential scanning calorimeter can be used to perform the measurements. TPM has already been applied in Portland cements with success [23]. Many probe liquids may be used, such as water [23], cyclohexane [24], or acetonitrile [25]; the only requirement is that the liquid must present a solid–liquid phase transition, while the methods used to track the phase change are many [22]. In this study, water was chosen as probe liquid and the phase transition (solid–liquid) was recorded by a micro calorimeter; the advantages and drawbacks of that choice will be discussed.

The three methods described, i.e., MIP, N_2 adsorption, and TPM, have access only to open pores and are applicable to different ranges of pore sizes, as presented in Fig. 1, together with the IUPAC classification of pore sizes [22,26]. However, not only are the ranges of pore sizes different, the specific structural features measured by each technique vary. Thus, MIP evaluates the pore entrance size [12], the gas adsorption technique allows one to measure both the pore size and pore entrance size, using the adsorption and desorption curves, respectively [20,27], and TPM allows the determination of the size of the cavity of the pore during the melting cycle and the pore entrance size during the freezing cycle [21,27].

The objective of this work is to perform a critical assessment of the suitability of the different techniques described, i.e., gas sorption, mercury intrusion, and TPM, for the characterization of the porosity and pore structure of apatitic CPCs obtained by hydrolysis of α-tricalcium phosphate (α-TCP) [28–30]. Four cements with a range of different porosities were compared to evaluate the information that can be obtained from them and the advantages and limitations of each technique.

2. Materials and methods

2.1. Materials

α-TCP was used for the preparation of the cement's solid phase and was obtained by heating in a furnace (Hobersal CNR-58) in air at a 2:1 M ratio a mixture of calcium hydrogen phosphate ($CaHPO_4$, Sigma Aldrich) and calcium carbonate ($CaCO_3$, Sigma Aldrich) at 1400 °C for 15 h, followed by quenching in air. Four cements with different microstructures and porosities were prepared by varying the initial particle size and liquid-to-powder ratio (L/P), as described in a previous paper [5] (Fig. 2). To this end, the α-TCP obtained was milled in a planetary mill (Pulverisette 6, Fritsch GmbH) using an agate bowl (10 cm diameter) and balls, following two different protocols to prepare two different granulometries: (i) a coarse (C) powder, obtained by milling with 10 balls (d = 30 mm) for 15 min at 450 rpm; and (ii) a fine (F) powder, obtained by milling in three steps: first with 10 balls (d = 30 mm) for 60 min at 450 rpm, followed by 40 min at 500 rpm, and a last step with 100 balls (d = 10 mm) for 60 min at 500 rpm. 2 wt% of precipitated hydroxyapatite (Merck, US) was added as a seed to the powder.

Cements were prepared with two L/P ratios, 0.35 and 0.65 mL/g, using both F and C α-TCP powders. The powder phase was mixed with water in a mortar for about 1 min and then transferred into 6 mm diameter × 12 mm height cylindrical Teflon moulds. The samples were allowed to set at 100% relative humidity for 7 days at 37 °C. CPCs obtained were coded as C35, C65, F35, and F65, where C or F stands for coarse or fine powder, respectively, and 35 or 65 corresponds to the L/P.

2.2. Phase composition and microstructure characterization

The hardened CPC samples were dried at 60 °C for 24 h and the phase composition was assessed by X-ray diffraction (XRD) using a PAN-Alytical X'Pert powder X-ray diffractometer. Scanning was performed in the Bragg–Brentano geometry using CuKα radiation with the following conditions: 2θ scan between 20° and 70°, with a scan step of 0.016°, and a counting time of 50 s per step at 45 kV and 40 mA. To observe the inner microstructure of the material, the specimens were fractured and the fracture surfaces were

Please cite this article in press as: D. Pastorino et al., Multiple characterization study on porosity and pore structure of calcium phosphate cements, Acta Biomater. (2015), http://dx.doi.org/10.1016/j.actbio.2015.09.017