G Model
JECS-11290; No. of Pages 7
ARTICLE IN PRESS
Journal of the European Ceramic Society xxx (2017) xxx–xxx
Contents lists available at www.sciencedirect.com
Journal of the European Ceramic Society
journal homepage: www.elsevier.com/locate/jeurceramsoc

Bio-inspired hydroxyapatite dual core-shell structure for bone substitutes

Shaan Chamary[a,*], Dominique Hautcoeur[b], Jean-Christophe Hornez[a], Anne Leriche[a], Francis Cambier[b]

[a] Laboratoire des Matériaux Céramiques et Procédés Associés (Université de Valenciennes et du Hainaut Cambresis), Bd. Charles de Gaulle, 59600 Maubeuge, France
[b] Belgian Ceramic Research Center, Av. du Gouverneur E. Cornez 4, 7000 Mons, Belgium

ARTICLE INFO

Article history:
Received 19 January 2017
Received in revised form 16 May 2017
Accepted 22 May 2017
Available online xxx

Keywords:
Hydroxyapatite
Bio-inspired
Ice templating
Polymeric template
Core/shell structure

ABSTRACT

A variety of processing methods has been developed and optimized to generate porous bio-ceramic structures close to that of the natural bone. Two different ceramic processing techniques were combined in order to generate a bio-inspired structure similar, in terms of composition and architecture, to that of the bone. Hydroxyapatite was manufactured through aqueous precipitation and complied with international standard ISO 13779's requirements. The impregnation of a polymeric scaffold by slurry enabled the manufacturing of a porous network reminiscent of spongy bone (the core) whereas ice templating generated the outer structures (the shell) very similar to compact bone. The core-shell structure was mechanically characterized thanks to longitudinal compression and the Brazilian test (traction test). According to porosity levels, the compression and Brazilian tests yielded values ranging from 30 down to 1 MPa and 4.6 down to 0.5 MPa, respectively. Porosity levels ranged from 36 up to 54%.

1. Introduction

Natural bone tissues are armed with a self-repair mechanism enabling them to heal lesions such as fractures with very limited human intervention. However, this ability can be insufficient or impaired leading to surgeries and sometimes, to the use of bone substitutes. A variety of both natural and synthetic materials are currently used with the intent of filling bone voids, consolidating critical zones and enhancing biological repair. The gold standard is the autograft where the donor and the host are the same individual. However, drawbacks, such as graft quantity and quality, donor site morbidity, increased recovery time, overall cost and post-operation chronic pain, can be expected. Allografts and xenografts are regarded as alternative methods. Allografts are transplanted tissues provided by a genetically different individual from the same species, whereas xenografts are taken from a different species. However, from these two techniques stems a number of inconveniences such as immunogenicity and pathogen transmission. Synthetic bone substitute addresses both issues. They are mainly made out of polymers and/or ceramics. Polymers can be biodegradable (polylactic acid or PLA) or not (poly- (methyl methacrylate) or PMMA). Bioceramics can be inert (alumina) or bioactive (calcium phosphates). Bioactivity is the ability of a material to induce a positive reaction favoring its integration to the surrounding tissues.

Historically, the main goal of bone substitutes has been to overcome and to reduce mechanical deficiencies. However, the improvement of our understanding of bone physiology over the last decades led to a paradigm shift: nowadays, more and more bone substitutes are bioinspired. Chemical composition and structure are closer to that of natural bone. Indeed, bone tissue is a combination of mineral matter that can be associated to a lacunar calcium phosphate apatite and an organic phase mostly made out of collagen, a protein polymer. Macroscopically, two structures can be identified: compact and spongy bone (Fig. 1). Compact bone is characterized by a porosity of 3–12% with pores ranging from 190 to 230 μm of diameter and stress at failure ranges from 90 to 230 MPa (mineral part only). Spongy (or trabecular) bone is highly porous, with porosities reaching values between 50 and 90% and pores range from 500 to 600 μm in diameter [1,2]. Stress at failure is very low and rarely exceeds a few MPa (2–45 MPa—mineral part only). Spongy bone constitutes around 20% (wt.) of bone tissues and is mostly found in long bones such as the tibia or the femur. As a result, calcium phosphates, such as hydroxyapatite (HA), tricalcium phosphates or biphasic systems, are widely used because of their

* Corresponding author.
E-mail address: shaan.chamary@univ-valenciennes.fr (S. Chamary).

http://dx.doi.org/10.1016/j.jeurceramsoc.2017.05.043
0955-2219/

Please cite this article in press as: S. Chamary, et al., Bio-inspired hydroxyapatite dual core-shell structure for bone substitutes, *J Eur Ceram Soc* (2017), http://dx.doi.org/10.1016/j.jeurceramsoc.2017.05.043

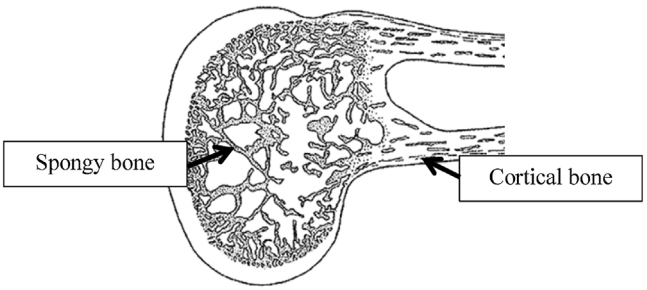

Fig. 1. Spongy and cortical bone of a long bone.

close chemical composition, good osteoconduction properties and bioactivity [3]. In this case, HA is chosen when a low resorption rate compared to other calcium phosphates is wanted.

In order to get closer to the bone structure, various shaping techniques have been optimized to generate porous material. The scaffold should exhibit a network of hierarchically distributed and interconnected pores which would promote cell invasion and proliferation, efficient vascularization and new bone formation. Porous scaffolds such as foams, sacrificial templates [4] and porogens [5,6] (water, polymers. . .), rapid prototyping methods [7], and ice templating. [8,9] are amongst the many processing techniques developed and optimized for ceramic shaping [10,11]. Most of these techniques yield spherical porosities suitable for bone substitution with dimensions ranging from a 20–1500 μm [1–5]. However, ice templating generates ellipsoidal pores with smaller diameter sizes and an aspect ratio ranging from 3 to 6 [10,11]. This angular pore shape has been shown to enhance the cell invasion especially vascular cells [12]. Moreover, this technique generates naturally a microporosity inside the walls (around 10% in volume) [13], potentially favorable to bone ingrowth [14,15].

The aim of this work was to combine two shaping methods, starting from HA powders: calcium phosphate slurry impregnation of a polymeric scaffold [6,16], generating porous networks very similar to spongy bone (the core), and ice templating generating long elongated structures close to compact bone (the rim) [17,18]. Individually, these 2 processing methods yield samples suitable and adapted to cell culture [19]. Indeed, commercial implants, filling media (Biolu, China) or osteotomic wedges (Biocetis Tissue Engineering, France) are currently produced from the impregnation of polymeric scaffolds. Cell invasion tests, with positive results, were also carried out on samples produced by freeze casting [19].

2. Material and methods

2.1. Hydroxyapatite synthesis and characterization

HA powder was synthesized through aqueous precipitation [20]. A di-ammonium phosphate solution $(NH_4)_2HPO_4$ (Carlo Erba, France) is added in a controlled manner to a calcium nitrate solution $Ca(NO_3)_2$ (Brenntag, France), in a jacket reactor, under mechanical stirring. An ammonia solution maintains the alkaline (pH 8) conditions needed in the reactor. The reaction (Eq. (1)) takes place at 50 °Cover 20 h. Over the course of the reaction, an apatitic phase will precipitate. The solution is then filtered and the solid matter is dried at 90 °C for 48 h.

$$10Ca(NO_3)_2(aq) + 6(NH_4)_2HPO_4(aq) + 8NH_4OH(aq) \rightarrow Ca_{10}(PO_4)_6(OH)_2(s) + 20NH_4NO_3(aq) + 6H_2O \quad (1)$$

Eq. (1): Hydroxyapatite chemical reaction when Ca/P = 1.667

The outcome of the reaction depends on the Ca/P ratio [21]. A calcium deficiency (1.667 >(Ca/P) > 1.5) leads to traces amount of β-tricalcium phosphate, $Ca_3(PO_4)_2$ (β-TCP), whereas a calcium excess leads to the formation of calcium hydroxide $Ca(OH)_2$ and subsequently to calcium oxide CaO (Fig. 2). Traces amount of β-TCP (<5%) is tolerated and sometimes beneficial for sintering purpose unlike CaO. Indeed, the presence of 2 wt.% of β-TCP improves the densification process [22].

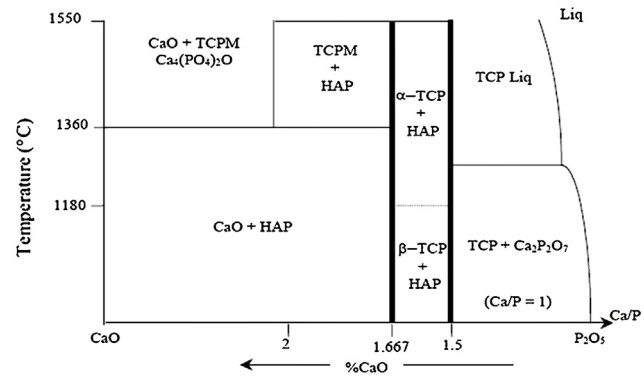

Fig. 2. CaO–P_2O_5 system phase diagram (adapted from [20]).

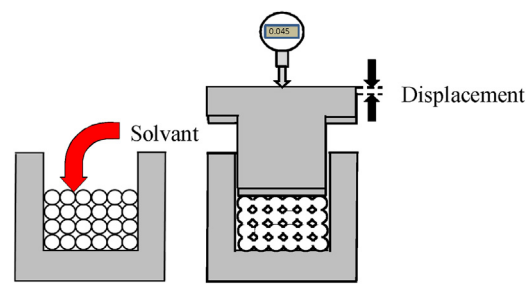

Fig. 3. Core manufacturing and displacement measurement.

The crystalline phase composition was verified by X-Ray powder diffraction [23]. The presence of calcium oxide, CaO was verified through the phenolphthalein test, i.e. the absence of CaO is characterized by the absence of pink coloration [24].

Once purity was assessed, the powder underwent a thermal treatment at 950 °C for 3 h and a grinding process (24 h in milling jars) in order to break the agglomerates. Grain population is bimodal with D50 = 2 μm. These last steps allow reducing the high specific surface area (> 60 m^2/g), obtained through aqueous precipitation, in order to match slurry requirements: about 12 m^2/g. The highest level of densification for the latter characteristics was reached thanks to the following cycle: 1250 °C/3 h at the rate of 5 °C/min.

2.2. Dual core shell structure manufacturing

2.2.1. Core manufacturing: slurry impregnation of a polymeric scaffold

The polymeric scaffolds were made out of partially fused polymethylmetacrylate (PMMA, Diakon TM Ineos Acrylics, Holland) beads that acted as the sacrificial phase. After being firmly packed in a mold, a solvent (acetone) was added, triggering the slow dissolution of the polymer which induced an overlapping between the individual bodies. This leads to the formation of bridges between PMMA beads and a significant shrinkage of the bead pile. For a known size range of beads, interconnection/bridge diameter can be related to this displacement (Fig. 3) via a series of equations: empirical (Eq. (2)) and/or a geometrical model based on the theoretical arrangement of spheres (Eq. (3)) [16].