JOURNAL OF THE MECHANICAL BEHAVIOR OF BIOMEDICAL MATERIALS 48 (2015) 1–11

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/jmbbm

Research Paper

Mechanical and biological properties of the micro-/nano-grain functionally graded hydroxyapatite bioceramics for bone tissue engineering

Changchun Zhou[a,*,1], *Congying Deng*[b,1], *Xuening Chen*[a], *Xiufen Zhao*[b], *Ying Chen*[a], *Yujiang Fan*[a], *Xingdong Zhang*[a,*]

[a]*National Engineering Research Center for Biomaterials, Sichuan University, Chengdu 610064, China*
[b]*School of Manufacturing Science and Engineering, Sichuan University, Chengdu 610065, China*

ARTICLE INFO

Article history:
Received 4 November 2014
Received in revised form
31 March 2015
Accepted 1 April 2015
Available online 8 April 2015

Keywords:
Functionally graded materials
Two-step sintering
Biological property
Mechanical property
Bone tissue engineering

ABSTRACT

Functionally graded materials (FGM) open the promising approach for bone tissue repair. In this study, a novel functionally graded hydroxyapatite (HA) bioceramic with micrograin and nanograin structure was fabricated. Its mechanical properties were tailored by composition of micrograin and nanograin. The dynamic mechanical analysis (DMA) indicated that the graded HA ceramics had similar mechanical property compared to natural bones. Their cytocompatibility was evaluated via fluorescent microscopy and MTT colorimetric assay. The viability and proliferation of rabbit bone marrow mesenchymal stem cells (BMSCs) on ceramics indicated that this functionally graded HA ceramic had better cytocompatibility than conventional HA ceramic. This study demonstrated that functionally graded HA ceramics create suitable structures to satisfy both the mechanical and biological requirements of bone tissues.

1. Introduction

Biomaterials must simultaneously satisfy various requirements and possess certain properties such as non-toxicity, mechanical strength, and biocompatibility (Lavine et al., 2012; Mehrali et al., 2013). Natural tissues often possess functionally graded materials (FGM), which enable them to satisfy multiple requirements (Zhang et al., 2012). Human tissues have evolved to best adapt to their multiple functional requirements. For instance, the perfect design of natural bone with a dense, stiff external structure (cortical bone) and a porous internal structure (cancellous bone) demonstrates that functional gradation has been utilized for biological adaptation (Pompe et al., 2003). Generally, FGMs are characterized by a gradual change in material properties over volume. The gradient can be in composition and/or in

---

[*]Corresponding authors. Tel.: +86 28 85412757; fax: +86 28 85410246.
E-mail addresses: changchunzhou@scu.edu.cn (C. Zhou), zhangxd@scu.edu.cn (X. Zhang).
[1]These authors contributed equally to this study.

http://dx.doi.org/10.1016/j.jmbbm.2015.04.002
1751-6161/© 2015 Elsevier Ltd. All rights reserved.

microstructures (Cannillo et al., 2007). Recently, one remarkable characteristic feature of bone implants is the increasing acceptance of biologically inspired approaches (Zhu et al., 2014). Bio-inspired FGMs lend insight into the design concept of new biomaterials and open promising approach for bone tissue repair.

To better mimic the structure of nature tissues, FGMs with properly designed functional gradient may show unexpected properties, which differ from those of conventional materials (Park and Lu, 2008). With this concept, materials, including ceramics, polymers and metals, can be combined with a gradual change from one material to another (Chen et al., 2005; Watari et al., 2004). For example, carbon fiber (CF)-reinforced polylactic acid (PLA)/nanometer hydroxyapatite (HA) biomaterial had been prepared by Liao et al. (2009), in which CF was used as the reinforcement to improve mechanical properties, and the advantages of PLA and nano-HA were retained. Watari et al. (1997) developed a dental implant with functionally graded titanium (Ti) and HA. Maruno et al. (1994, 1991) developed a functional gradient HA composite containing glass-coated Ti and studied its microstructures, mechanical properties, and thermal properties. Kumar and Maruno (2002) proposed a HA–glass–titanium (HA–G–Ti) composite and implanted it in the femur of a dog to evaluate its bonding strength. These improved implants can provide sufficient mechanical strength. However, metal- and polymer-based implants usually lead to stress shielding, wear debris, delayed osseointegration, resorption, degradability or other biological complications. Therefore, new bone tissue implants should avoid these disadvantages and meet the multiple functional requirements of bone tissue (Campo et al., 2014; Kraaij et al., 2014).

Seeking for biomaterials with ideal biological properties, calcium phosphate ceramics, especially the bioactive nanostructured hydroxyapatite, have received considerable attention in recent years (Dorozhkin, 2010; Kandori et al., 2011; Zhou et al., 2013). *In vitro* and *in vivo* experiments have demonstrated that the nano-HA has excellent biological performances compared with conventional micrograin HA (Balasundaram et al., 2006; Kim et al., 2010). Nano-HA possesses exceptional biocompatibility and bioactivity with respect to bone cells and tissues, probably as a result of its similarity with the chemical component and mineral structure of bone tissues. Thus, nano-HA is beneficial to biomineralization in bone tissue regeneration (Choi et al., 2010). Furthermore, due to their small size and large specific surface, nano-HAs may not only promote ion exchange within a physiological environment, but also increase protein adsorption and cellular response as well as affect subsequent biological behavior. Given these special biological properties, nano-HA has been explored as filling powders, cements, component materials, or coating on orthopedic implants (Chen et al., 2013; Goenka et al., 2012; Wang and Tong, 2008; Zhu et al., 2009).

This study presents a novel FGM ceramic with both micrograin and nanograin HA crystals that can satisfy the mechanical and biological property requirements of bone implant. The composition of HA graded structures conforms to the need of mechanical property. Moreover, functionally graded HA ceramic with nanoscale surface topography shows better cytocompatibility than conventional HA ceramic.

2. Experiments

2.1. *Fabrication of the functionally graded HA bioceramics*

HA nanopowders were synthesized by the wet chemical method using reagent-grade calcium nitrate 4-hydrate [$Ca(NO_3)_2 \cdot 4H_2O$], and diammonium phosphate [$(NH_4)_2HPO_4$]. (Qu et al., 2004) The molar ratio of calcium to phosphorus in the reaction solution is 1.67. The pH of the reaction solution was maintained at 8 by adding ammonium hydroxide solution. The reaction solution was stirred for 4 h and aged for 24 h. Reaction precipitate was then collected, washed with deionized water, and dried. HA powders with an average diameter of 50 nm to 80 nm were obtained. The ceramic green body was fabricated using these nanopowders by a pressure forming method (8 MPa, 30 s) and then sintered by a two-step sintering schedule to form functionally graded nanoceramics. The prepared samples were $\Phi 10\ mm \times 3\ mm$ cylinders.

2.2. *Characterization*

Transmission electron microscopy (TEM; HITACHI H600-IV, Japan) was performed to characterize the synthesized HA nanoparticles. Differential scanning calorimetry (DSC) and thermal gravity (TG) measurements were performed using a Netzsch STA 449C (Germany) instrument to analyze the thermal properties of the HA nanopowders. The operations were performed in nitrogen protection at atmospheric pressure and rising temperature rate of 10 °C/min. The ceramics microstructure was observed with scanning electron microscopy (SEM; JSE-5900LV, Japan). The grain size of the ceramics was analyzed by an image-processing software, Image-pro Plus, based on SEM images. The crystalline phase was analyzed using X-ray diffraction (XRD; Philips X'Pert 1 X-ray diffractometer, Netherlands) with CuKα radiation at 20 mA current and 30 kV voltage. Scans were performed with 2θ values from 20° to 60° at a rate of 0.05°/sec. The obtained peaks were compared with standard references for HA (09-0432) in the JCPDS file available in the software. The mechanical properties of the graded HA ceramics were tested by the dynamic mechanical analysis (DMA; Precision Universal Tester Autograph AG-X, Japan). Compression test was done with the specimens of $3\ mm \times 3\ mm \times 10\ mm$. The experiments were performed at constant strain amplitude of 70 μm. A small preload was applied to each sample to ensure that the entire ceramics surface is in contact with the compression plates before the experiment was conducted and that the distance between plates is equal in the entire test.

2.3. *Culture of BMSCs*

Rabbit bone marrow mesenchymal stem cells (BMSCs) were used to assess the biocompatibility of the FGM HA ceramics *in vitro*. All animal studies have been approved by the Animal Care and Use Committee of Sichuan University and performed in accordance with the ethical standards. Four New Zealand white rabbits (male, 8 weeks, weighing 2.0–2.2 kg) were anesthetized by injection of 30 mg/kg sodium pentobarbital (Sigma Chemical, MO, USA). In the aseptic conditions, 4–5 ml bone marrow was extracted from the greater trochanter of femur and