Materials Letters 121 (2014) 181–184

Contents lists available at ScienceDirect

Materials Letters

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

Preliminary evaluation of bone graft substitute produced by bone of duck beak

Jun Sik Son [a,*,1], Soon Ho Jang [a], Tae-Yub Kwon [b], Kyo-Han Kim [b], Seong Soo Kang [c], Seok Hwa Choi [d,**,1]

[a] Korea Textile Development Institute, Daegu 703-712, Republic of Korea
[b] Department of Dental Biomaterials, School of Dentistry, Kyungpook National University, Daegu 700-412, Republic of Korea
[c] College of Veterinary Medicine, Chonnam National University, Gwangju 500-757, Republic of Korea
[d] Veterinary Medical Center, Chungbuk National University, Cheongju 361-763, Republic of Korea

ARTICLE INFO

Article history:
Received 29 August 2013
Accepted 25 January 2014
Available online 1 February 2014

Keywords:
Bone
Duck beak
Xenograft
Bioceramic
Bone regeneration

ABSTRACT

This paper studied the production and properties of bioceramics bone graft substitutes from duck beak bone, as a natural source. Duck beak bone particles were fabricated by de-fatting, followed by heat-treatment at 1000 °C for 3 h in an air atmosphere. It was confirmed that heat-treated beak bone particles were highly porous in their structure, with a rough surface, and the Ca/P atomic ratio value was 1.65, similar to that of human bone. In addition, the heat-treatment process of beak bone particles resulted in single phase hydroxyapatite (HA) with high crystallinity. *In vivo* performance of beak bone particles using rat calvarial defects showed a significantly higher bone volume than that of bovine bone particles at 4 weeks post implantation. This study demonstrated that duck beak bones can be prepared as economical bone graft substitutes of natural, biological origin HA.

1. Introduction

Bone defects often occur as a result of trauma, bone tumors, resection, and metabolic diseases. These defects are typically reconstructed using either a natural bone graft (autograft, allograft, xeonograft) or an artificial synthetic bone graft (alloplast). Autografts have the distinct advantage of histocompatibility without the risks of disease transfer, and are the best materials for bone repair, but are often restricted to filling small defects, since they are limited by the amount of tissue that can be harvested [1]. Although allogenic bone grafts have better availability than autografts and avoid the need for a second surgical procedure to obtain an autograft, allogenic bone grafts may transmit diseases and cause immune responses, leading to graft failure [2]. Alloplastic bone grafts are synthetic bioceramics, and include hydroxyapatite (HA) and β-tricalcium phosphate (TCP), which have good biocompatibility, but a high possibility of infection, and do not exhibit bone-inducing capacities. Additionally, their long term stability has not been thoroughly tested [3,4].

The use of xenografts, especially those made of bovine or porcine bone, has recently increased not only in ridge preservation procedures, but also other bone augmentation procedures [5,6]. Xenograft bone has major advantages over autografts, especially the much greater availability [7]. However, the downsides of the use of bovine bone include its slow resorbability and healing with fibrous encapsulation. Furthermore, the risk of transmittable diseases, such as porcine foot-and-mouth disease and bovine spongiform encephalopathy, are major concerns in the clinical use of materials of this type.

Livestock ducks are mainly used for food production, but the duck heads are usually discarded or used to produce natural organic fertilizer and animal feeds. The top part of the beak is called the upper mandible, and the bottom part, the lower mandible. The duck beak is composed of keratin, and contains two major bones in each side of the lower mandible and upper mandible, with a small nose bone affixed to the skull. These wasted duck beak bones have potentially valuable uses in biomaterial fields, especially in bone graft substitutes [8]. To our knowledge, no one has previously developed bone graft substitutes from bones of duck beaks.

In this study, natural HA bioceramic as bone graft substitute was developed using duck beak bone for bone regeneration. The characteristics and *in vivo* performance of produced bone particles were investigated.

* Corresponding author. Tel.: +82 53 560 6587; fax: +82 53 560 6759.
** Corresponding author. Tel.: +82 43 261 3144; fax: +82 43 267 2595.
E-mail addresses: sonjk1@empas.com (J.S. Son), shchoi@cbnu.ac.kr (S.H. Choi).
[1] These two authors contributed equally to this work.

http://dx.doi.org/10.1016/j.matlet.2014.01.141
0167-577X

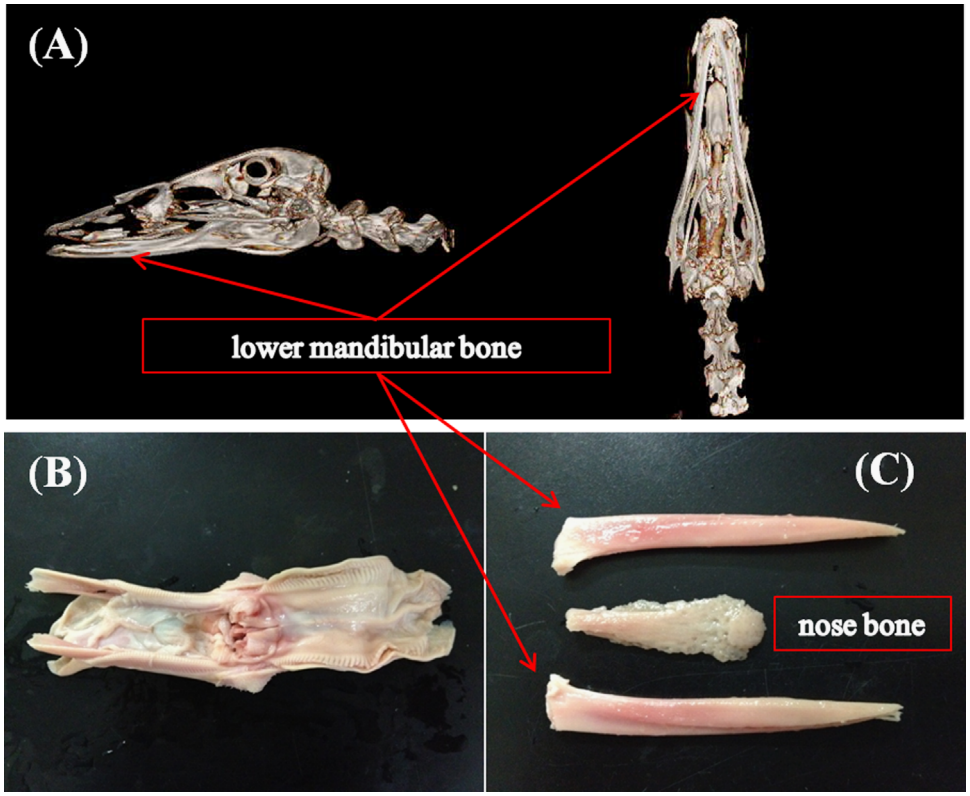

Fig. 1. Three-dimensional CT image of duck head (A) and digital pictures of duck beak (B) and separated bones (C).

2. Experiment

Nature HA particles were derived from duck beak bone by a series of thermal processes. To collect suitable stock material of duck beak bone tissue from livestock ducks (cherry valley, 6–8 weeks old), harvested duck beak was surgically extracted and all soft tissue was removed of all. The collected bone samples were thoroughly cleaned to remove macroscopic adhering impurities and boiled in distilled water for 8 h for easy removal of bone marrow and tendons. After the cleaning process, the bones were deproteinized by continued boiling in distilled water for 8 h. The boiled bone samples were then dried for 48 h in an oven at a temperature of 60 °C. The deproteinized bone samples were cut to pieces of 2 cm^3 or less and heat-treated in an electric furnace at a temperature of 1000 °C. The temperature was maintained for 3 h to remove the organic matrix and to eliminate any disease-causing agents. The heat-treated duck beak bone pieces were subsequently milled into particles of 500–700 μm.

The three-dimensional images of duck heads were generated using a CT scanner (Siemens emotion 16, Forchheim, Germany) at a scan parameter thickness of 1 mm. The morphological features of heat-treated duck beak bone particles were analyzed using a scanning electron microscope (SEM, JSM-6700F, JEOL, Japan). The calcium and phosphorus ratio was measured with an energy dispersive spectrometer (EDS, Inca x-sight, Oxford Instruments, UK). The porosity and the pore size of bone particles were measured using a mercury porosimeter (Autopore IV 9500, Micromeritics, USA). The crystallinity of the bone particles was analyzed with an x-ray diffractometer (XRD, Max-2500, Rigaku, Japan), and the chemical composition of the surfaces was identified using x-ray photoemission spectroscopy (XPS, Quantera SXM, ULVAC-PHI, Japan).

The *in vivo* studies of heat-treated duck beak bone particle were carried out using a calvarial defect model of Sprague–Dawley rats. The study and housing protocols were approved by the Animal Research Committee for Chonnam National University (CNU IACUC-YB-R-2013-31) and were in accordance with the international guidelines for care for laboratory animals. Two 8 mm defects were created using a trephine drill bit, and the created defects were either filled with duck beak bone, bovine bone (Bio-Oss, Geistlisch Pharmaceutical, Switzerland) or unfilled as a control. Rats were sacrificed at 4 weeks post-surgery and the samples were collected and placed into 10% neutral buffered formalin. To evaluate new bone formation, the *in vivo* samples were scanned using micro-computed tomography (micro-CT, Skyscan, Aartselaar, Belgium) at a spatial resolution of 8.77 μm. The total volume of bone ingrowth was computed using micro-CT software.

3. Results and discussions

Fig. 1 shows three-dimensional computed tomography images of duck head and digital pictures of duck beak bone. In Fig. 1 (A) and (B), CT images and digital picture reveal that there are two main bones in lower mandibular beak and a small nose bone in upper mandibular beak. The lower mandibular bone was simple and cleanly separated from duck beak without soft tissue on its surface. The length of lower mandibular bone was about 5–8 cm and the nose bone was 3–5 cm, as shown Fig. 1(C).

Fig. 2(A) shows the SEM image of heat-treated duck beak bone particles. The prepared bone particles were an irregular oblong shape and had many pores of macro- and micrometer size, due to the evaporation of organic compounds (collagen, blood vessels, proteins, polysaccharides and lipids) by heat-treatment processing. The pores were observed to be regularly arranged with elongation along the duck beak bone. In addition, the surfaces of beak bone particles were observed to have rough, compact and dense structures within interconnected mesopores distributed over its entire surface, as shown in Fig. 2(B). The porosity and