

Scaffolds for Bone Tissue Restoration from Biological Apatite

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A great deal of effort has been exerted to design scaffolds for bone tissue restoration from naturally-derived biomaterials that mimic the composition and structure of natural bone. This article reports a simple method for processing biological apatite (BAP) from bovine bone. Chemical and low temperature heat treatments were employed for the processing of BAP scaffold. Phase composition, microstructure, and compressive strength were examined by various analytical methods. The results indicated that the phase composition and crystalline structure of BAP correspond to the hydroxyapatite (HAP) phase. The SEM observation showed that the BAP possesses porous morphology and the pores are in the range of micrometers to nanometers in diameter. Despite the porous structure, the scaffold possesses adequate mechanical strength required for low-weight bearing orthopedic applications. The overall experimental results therefore suggest that the BAP may be used as scaffolds for bone tissue restoration.

Introduction

Scaffolds play a key role in bone tissue restoration. They are used either to induce new bone formation from the host tissues or to deliver cells or growth factors at the defective sites where bone regeneration is required. In this regard, the scaffolds should possess some specific characteristics to facilitate faster bone growth (Table 1). Importantly, the scaffolds should mimic the composition and structure of the host bone to allow osteointegration and soft tissue in-growth into the scaffolds. HAp, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, is one of the well characterized biomaterials, widely used in bone tissue restoration because of its chemical composition and structural similarity to natural bone minerals. Besides, it possesses most of the qualities required for bone tissue restoration, in particular biocompatibility, bioactivity, and osteoconductivity [1-3]. A number of methods for processing HA, either from natural sources (coral exoskeleton and animal bone) or from synthetic sources (inorganic chemical synthesis), have been reported [4-10],

but it is very difficult to justify which method is the best because each method has its own merits and demerits. Since most of the investigations on bone tissue restoration deal with the HAp, it is worth mentioning that the bone mineral is not only constituted of Hap, but also comprised of other ions, mainly of CO_3^{2-} and traces of Na^+ , Mg^{2+} , Fe^{2+} , Cl^- , F^- , etc [11]. Although these ions are significantly low in the bone mineral, they play an important role in the bone metabolism. In this regard, the scaffolds made of BAP are perceived to be more beneficial than stoichiometric HAP for bone tissue restoration because they may inherit some of the properties of raw bone such as chemical composition, crystalline phase, morphological structure, and mechanical strength, which are all prime determinant factors in the performance of scaffolds.

A number of techniques have been developed for processing porous scaffolds for bone tissue restoration, but most of them are involved typically incorporation of volatile organic particles in the inorganic HAP powders, gel

Table 1 Characteristics of scaffolds for bone tissue restoration.

Characteristics	General remarks
Biocompatible	Biologically compatible to host tissue i.e. should not provoke any rejection, inflammation, and immune responses.
Bioactive	To facilitate a direct biochemical bonding to host tissue.
Biodegradable	The rate of biodegradation has to be adjusted to match the rate of bone tissue formation.
Mode of degradation	Bulk or surface erosion.
Osteoconductive	Capable of supporting in-growth of sprouting capillaries, perivascular mesenchymal tissues, and osteoprogenitor cells from the recipient host into the three dimensional structure of a graft that act as a scaffold.
Vascular supportive	Should provide channels for blood supply for fast and healthy bone regeneration.
Porous structure	To maximize the space for cellular adhesion, growth, ECM secretion, revascularization, adequate nutrition and oxygen supply.
Three dimensional structure	For the assistance of cellular in-growth and transportation of nutrition and oxygen.
Adequate mechanical strength Sterilizable	To withstand <i>in-vivo</i> stimuli during bone formation. To avoid toxic contamination.
Cost-effective	Affordable to all.

casting of foams, three-dimensional printing, and replication of polymer sponge or foams [12-14]. This article reports a simple method for processing a porous BAp scaffold for bone tissue restoration from bovine bone by chemical and low temperature heat treatments.

Experimental Section

Processing of BAp scaffold

The BAp scaffold was processed from chemically and thermally treated bovine bone. The experimental method is briefly given as follows: first, cortical portion of the bovine bone was sliced into required size under running water. The soft tissues were removed by cleaning with 2% NaCl solution and immersed in acetone-ether mixture (3:2) for 24 h for degreasing. Second, the chemically-processed bone was heated at 500°C for 12 h under atmospheric pressure and ambient humidity. The

heat-treated bone was named as BAp scaffold by evaluating its physio-chemical characteristics.

Characterization

X-ray diffraction (XRD) method was employed to characterize the phase composition and crystallographic structure of the BAp using a powder X-ray diffractometer (Shimadzu XRD-600, Japan). The experiments were performed on both unprocessed bovine bone and processed bovine bone (BAp scaffold) after making them into fine powders. The measurements were done with Cu K α radiation at the wavelength of 1.5406 Å over the range of 20-60° (2 θ) in Guiner geometry. The surface morphology and microstructure of the BAp scaffold was observed by a scanning electron microscopy (SEM) (JSM 5600, JEOL, Japan), after the scaffold was coated with gold under argon atmosphere to a thickness of 20-30 nm. Compressive strength was measured for BAp by a universal testing machine (Instron).

Results and Discussion

The key intention of this investigation is to process a porous BAp scaffold from bovine bone for bone tissue restoration. The bovine bone was used in this experiment to extract BAp. The preliminary investigations confirmed that the low temperature heat treatment performed at 500°C for 12 h, under atmospheric pressure and ambient humidity, removed all the organic matters associated with the bovine bone [15].

As phase composition and crystal structure are critical for the biomaterials to be intended for bone tissue restoration, the XRD method was employed to evaluate these properties for the BAp. Fig. 1 shows the XRD patterns of unprocessed bovine bone and processed bovine bone (BAp scaffold). The results indicate that the unprocessed bovine bone (see Fig. 1a) is amorphous, due to the presence of organic substances, whereas the BAp (see Fig. 1b) is relatively crystalline, which is characteristically similar to crystallographic geometry of the human bone mineral [16] and also matches ionic-substituted HAp [17]. However, the overall diffracted peaks of BAp show poor crystallinity compared to that of HAp [10, 18]. There are two possible reasons for the occurrence of low crystallinity: (i) low temperature processing method, and (ii) presence of CO_3^{2-} ions associated with the bovine bone. The amount of CO_3^{2-} was determined by CHN analyzer and it was found to be 3 wt%, which confirms the presence of CO_3^{2-} in the scaffold and the results are in accordance with the FTIR observations (not shown).

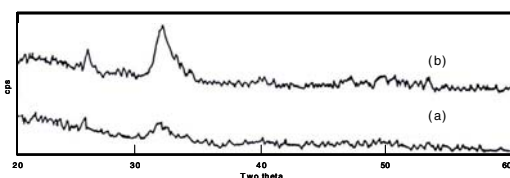


Fig. 1 XRD patterns of bovine bone (a) before processing, (b) after processing.

It is worth mentioning that the natural bone also contains the CO_3^{2-} in the range of 3-8 wt% depending on the age and other factors. Since the total amount of the CO_3^{2-} present in the scaffold lay within the range of natural bone, it may be con-

cluded that the scaffold mimics the human bone minerals to some extent. It is also reported that the CO_3^{2-} improves the mechanical strength and solubility of the implants [17, 19]. Since the mechanical property is also the key determinant factor for the better performance of the scaffold, the compressive strength was measured by an Instran made universal testing machine. The result (see Table 2) shows that the compressive strength of the scaffold is 132 MPa, which is also comparable to HAp and natural bone. It should be noted that despite having the porous structure, the scaffold possesses adequate mechanical strength required for bone tissue restoration, but only in the low weight-bearing bone sites.

Table 2 Compressive strength of BAp scaffold

Materials strength	Compressive (MPa)
BAp	132
HAp*	10-130
Cortical bone*	83-164

* from Ref. [20].

It is always preferred that the scaffolds for bone tissue restoration should have a porous structure without compromising their mechanical strength in order to withstand biological stimuli during bone formation. In this study, porous BAp scaffold was made by a simple method without adding any foreign agents to create pores on the scaffold. The surface morphology of BAp is shown in Fig. 2.

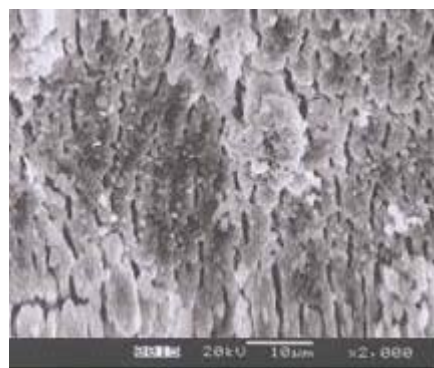


Fig. 2 - SEM micrograph of BAp scaffold

The results indicate that the scaffold has porous structure with occasionally interconnected pores. It is worth mentioning that the interconnected pore structure is one of the essential characteristics of a perfect scaffold because it allows the bone tissue in-growth as well as blood and nutrient supplies for cells to be alive. As it can be seen from the gross morphology, the pores are in the range of a few micrometers to nanometers in diameter. It is believed that the pores are created due to the removal of organic substances associated with the bovine bone and most of them are not homogeneous in shape and size. This porous structure may be compared to human trabecular structure to a certain extent. It has also been reported that the porous structure of the scaffold greatly influences cell adhesion, proliferation, and differentiation required for bone tissue restoration [14]. Therefore, it may be expected that the porous structure of the BAP scaffold could be useful to facilitate tissue in-growth during bone formation.

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Conclusions

The present study provided experimental evidence for the feasibility of processing porous scaffold from bovine bone by a simple, reproducible, and cost effective method. The chemical and low temperature heat treatments assisted the extraction of BAP. The rest of the organic matters associated with the bovine bone completely disappeared at 500°C. The chemical composition and structure of the BAP are perceived to be beneficial if it is used as a scaffold for bone tissue restoration.

Acknowledgement

This work was supported in part by the grant of Singapore Millennium Foundation.

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