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Porous bioceramics reinforced by coating gelatin

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Abstract Porous bioceramics with high porosity for bone tissue engineering were fabricated by the foam impregnation technique, but their mechanical strength was poor, only a mean compressive strength of 1.04 ± 0.15 MPa and an mean elastic modulus of 0.1 GPa. In order to reinforce porous ceramics, the ceramic samples were immerged in 5% gelatin solution and gelatin coatings were formed on the inter-surface of their pores. It was found that the mean compressive strength value and the mean elastic modulus value of porous samples coated with gelatin were improved to 5.17 ± 0.17 MPa and 0.3 GPa respectively without sacrificing their porosity greatly. Moreover composite samples were not as fragile as sintered ceramics. The results indicated that the gelatin coatings on the inter-surface of pores reinforced porous bioceramics effectively.

Introduction

Calcium phosphate bioceramics have received considerable attention as bone-graft substitutes primarily because of their excellent biocompatibility, bioactivity, and osteoconduction characteristics. They were fabricated in porous, granular, and dense forms. Among them, porous bioceramics are required as scaffolding materials for bone tissue engineering in substituting for and regenerating damaged bones. A porous structure promotes cell attachment, proliferation, and differentiation, provides pathways for transport of biofluids, nutrients and metabolic waste, and allows in growth of bone tissue to achieve full integration with the living bones [1–4].

In addition to compositional and porous structural requirements, scaffolds for bone tissue engineering should possess a strength compared to that of cancellous bone. A material generally weakens as its porosity increases, which poses a major challenge in developing porous scaffolds. Because of their natural brittleness, bioceramics such as HA and β-TCP, in a porous form, have very low strength and toughness. For example, the compressive strength of the macroporous CPCs changed from 3.7 to 0.6 MPa when the macroporosity varied from 31% to 62% [5]. According to Almirall et al. [6], macroporous hydroxyapatite originated from the hydrolysis of α-TCP had compressive strengths of 1.4–2.7 MPa in the macroporosity range of 36–27%. Despite their favorable biological properties, the poor mechanical properties of these ceramic materials have severely hindered their clinical applications, especially in bone tissue engineering [7, 8].

Coating organic and polymeric coatings on the reticular structure of porous bioceramics could be one of the methods to improve their mechanical properties. Miyazaki et al. [9] indicated that the compressive strength of a porous α-TCP increased after coated with organic polymers including hydroxypropylcellulose (HPC) and sericin, a kind of silk protein. Miao et al. [10] investigated the interpenetrating HA/TCP/PLGA composite with PLGA as an additive. The compressive strength of the porous HA/TCP coated with PLGA was about four times as that of the parent porous HA/TCP.

Gelatin is a biocompatible polymer, it is completely resorbable in vivo and is obtained by thermal denaturation

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or physical and chemical degradation of collagen. Thus, the addition of gelatin is expected to improve the mechanical properties of the bioceramics [11]. The purpose of this paper is to reinforce sintered porous bioceramics by coating gelatin film. The process of experiment involved two steps. Firstly, porous bioceramics were fabricated via the foam impregnation technique using HA and TCP as raw materials, and $Mg(H_2PO_4)_2$ and $Al(H_2PO_4)_3$ as binders. Secondly, gelatin as an interpenetrating phase was coated on the porous ceramics to improve the compressive strength. The morphologies of the ceramics before and after coated were observed with SEM and the compressive strength and the elastic modulus measured.

Materials and methods

Raw materials

Hydroxyapatite and tricalcium phosphate powders, used as raw materials in this study, were produced in our laboratory with an average particle size of 0.67 μm and 0.93 μm, respectively. Magnesia (MgO) and alumina (Al_2O_3) of analytical reagent reacted with superfluous phosphoric acid (H_3PO_4, analytical reagent), their reactants, $Mg(H_2PO_4)_2$ and $Al(H_2PO_4)_3$ were used as binders to synthesize porous ceramics. Polyurethane foam (the Nanjing Chemical Factory, China) was chose for carrier of soaking slurry.

Preparation of sintered porous bioceramics

HA, TCP, $Mg(H_2PO_4)_2$ and $Al(H_2PO_4)_3$ were mixed via 4:4:1:1 (mass ratio). Distilled water was added to prepare ceramic slurries with 75 wt.% solid content, ceramic slurries were deagglomerated by ball milling for 4 h. To prepare porous scaffolds, polyurethane foams were cut into desired shapes and immerged into the slurries under vacuum to force the ceramic slurries infiltrating into their pores. The green samples were dried in air for 24 h and heated at a rate of 1 °C/min to 300 °C in an electric furnace and remained for 1 h to burn out the polyurethane foams. Finally the samples were heated at a rate of 3 °C/min to 1150 °C and sintered for 2 h.

Coating porous bioceramics with gelatin

To improve the mechanical properties of the sintered porous bioceramics, gelatin was introduced into the remaining open pores and to form a coating on the pore walls of samples. For the infiltration of gelatin, the gelatin was dissolved in distilled water at the temperature of 50 °C to form 5% gelatin solution. The porous ceramic was then immersed into the gelatin solution, followed by vacuum infiltration. The process of immersion and drying was repeated for three times to increase the thickness of coatings.

Characterization

Morphological investigation of the samples before and after coating with the gelatin solutions was performed using a FEI SIRION scanning electron microscope. The samples were sputter-coated with gold prior to examination.

The porosity of samples was measured by the Archimedes method, using distilled water, with values calculated from the following equation [12, 13]:

$$\varepsilon = (V_1 - V_3)/(V_2 - V_1) \tag{1}$$

where ε is the porosity of the sample, V_1 the volume of water before the sample was added, V_2 the volume of water after the sample was added and V_3 the volume of water after the liquid had been pressed into the pores of the sample and the sample removed from the liquid.

The compressive strength was measured with a WD-10A electronic universal material testing machine, using a load of 10 kN and a pressing velocity of 0.5 mm/min. The specimens being examined were of cylindrical shape, with diameter 8.5 mm and length 15 mm [2, 12]. The compressive elastic modulus was calculated from stress–strain curves, according to Hooke's law. Five samples of each type were tested for mechanical properties.

Results

The porous structure of samples before and after coated with gelatin is shown by SEM image in Fig. 1. Figure 1a reveals the interconnected and uniform macroporous structure of the sintered ceramics with pore size ranged in 300–500 μm. After coated with gelatin, some differences in the morphology of samples were found, there are gelatin membranes on the surface of pores, which were marked by "⇨" in the Fig. 1b.

Figure 2 shows the cross-section morphologies of pore walls in samples before and after coated with gelatin. Comparing with the sintered ceramic, the morphology of pore walls changed obviously, and the pore walls were enwrapped by smooth gelatin coatings after coating with gelatin (Fig. 2a, b). The SEM image in higher amplification also showed that a gelatin coating with a thickness of about 5 μm adhered to the sintered ceramic tightly, and there was no apparent gap between them (Fig. 2c). Actually samples became porous composites including sintered ceramic and gelatin.

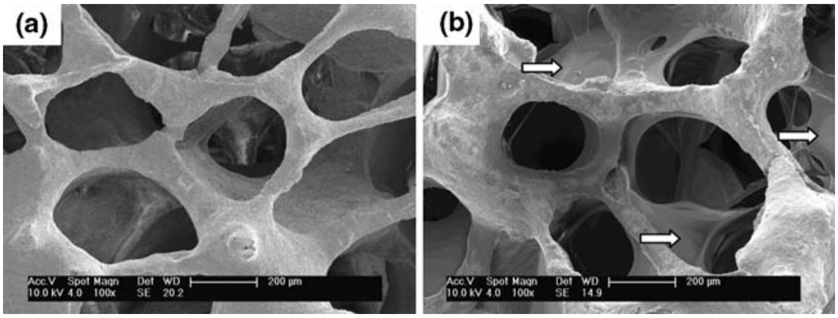

Fig. 1 The porous structure of samples before (**a**) and after coated with gelatin (**b**)

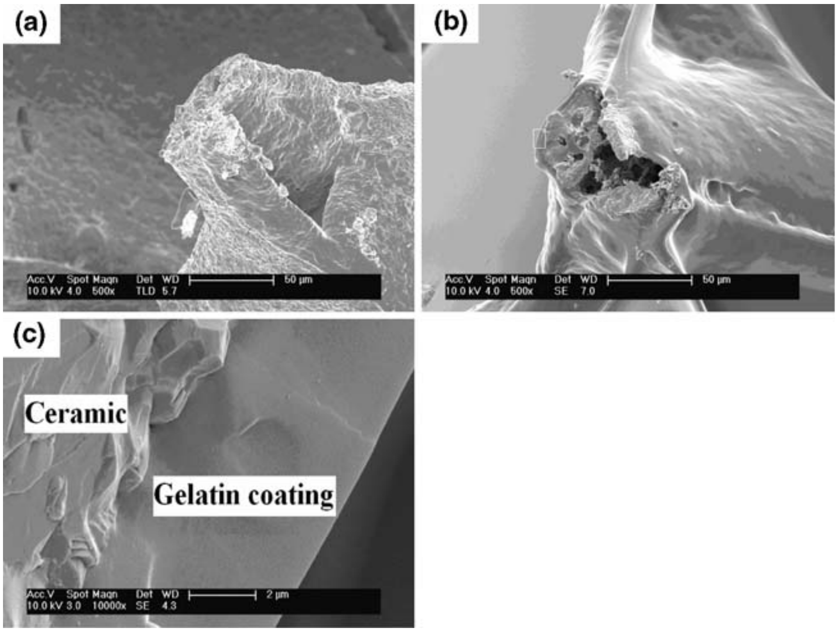

Fig. 2 The cross-section morphology of samples before (**a**) and after coated with gelatin (**b**) and (**c**)

The porosity of samples was calculated by the Eq. 1, the average porosity of the sintered porous ceramics was about 85.9 ± 1.6%. After coated with gelatin, the average porosity of porous composites was about 83.3 ± 1.8% and slightly less than that of the sintered ceramics.

Figure 3 reports data obtained from compressive tests. After coated with gelatin, the compressive strength of all the samples is higher than that of the sintered ceramics, and the stress values become five times greater than that of the sintered ceramics. The mean compressive strength values of samples before and after coated with gelatin are 1.04 ± 0.15 MPa and 5.17 ± 0.17 MPa, respectively.

Figure 4 shows the two typical stress–strain curves from compressive tests. The slope of the stress–strain curves in the linear elastic region gave elastic modulus E. Larger slope of the stress–strain curve indicates that the elastic modulus of sample after coated with gelatin is higher than that of the sintered porous ceramics. The mean elastic modulus values of samples before and after coated with gelatin are about 0.1 GPa and 0.3 GPa, respectively.

Discussion

In present study, calcium phosphate porous ceramics which had interconnected and uniform macroporous structure with pore size in 300–500 μm and mean porosity of 85.9 ± 1.6% were fabricated by the foam impregnation technique firstly. The properties of highly porous structure likely allow a high density of seeded cells into materials and facilitate proliferation of cells, vascular ingrowth, and internal mineralized bone formation in vivo according to plenty studies of researchers [7, 8, 14, 15]. In previous study [16], we have fabricated porous ceramic with $Mg(H_2PO_4)_2$ and $Al(H_2PO_4)_3$ as binders as the same as this study and their biological properties were evaluated in vitro. The results indicated that the porous ceramics exhibited no toxicity and good biocompatibility with this method.

It is well known that proper mechanical property is one of the most important characteristics that a scaffold material must possess. Although there is no clearly defined

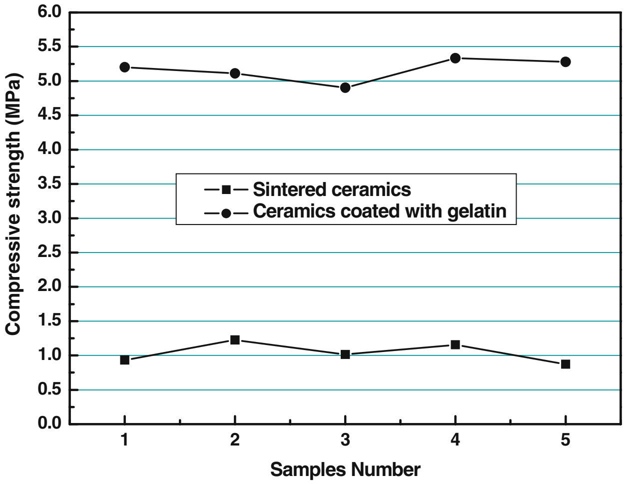

Fig. 3 Compressive strengths of samples

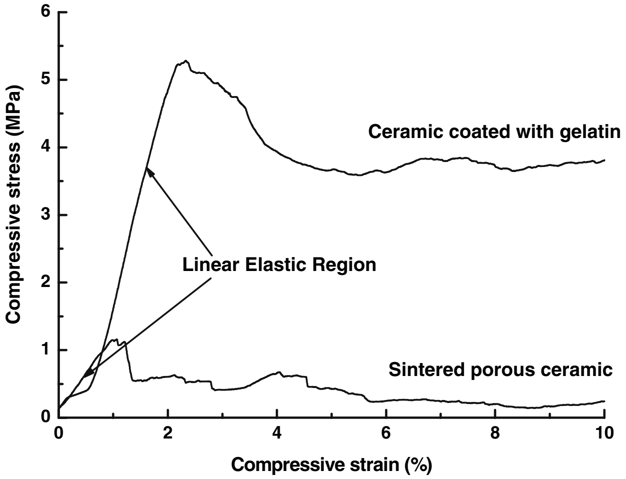

Fig. 4 Stress–strain curves obtained from compression testing

criterion in mechanical property required by bone tissue engineering, it is generally accepted that the scaffolding material should have mechanical strength as close as the strength of the human cancellous bone [10, 12, 17, 18]. The mean compressive strength value and elastic modulus value of the sintered porous ceramics are only 1.04 ± 0.15 MPa and 0.1 GPa, respectively, because its highly porous structure which is preferred in favor of cell growth was achieved at the expense of mechanical strength. If porosity was taken into consideration, these results were acceptable in comparison with the results of Barralet and Almirall [5, 6]. However, these data were still lower than those of the cancellous bone, which shows a compressive strength of 2–12 MPa and an elastic modulus of about 0.3 GPa, respectively [10, 18].

An increased compressive strength (5.17 ± 0.17 MPa) and a higher elastic modulus (0.3 GPa) were gained after the sintered porous ceramic was reinforced by coating their pore walls with 5% gelatin solution. Although the mechanical properties of the porous composites were still not sufficient to load-bearing applications, these mechanical data can be comparable to those of the cancellous bone. In addition, the infiltration of gelatin did make porous ceramics tougher, as evidenced by the fact the samples could did not shatter when cut mechanically. This mechanical integrity would provide convenience for implantation of the samples.

The mechanism for reinforcement brought by coated with gelatin may be the following two aspects. Firstly, gelatin coating which have a defined toughness dispersed partial crack energy and supported the whole material against deforming when samples were loaded. Secondly, gelatin coating on the inter-surface of porous samples enwrapped the pore walls tightly, which formed a composite structure and made the pore walls thicker and stronger. Nevertheless, the increasing thickness of the pore walls resulted to slight decreasing of porosity.

Many other methods and polymers had been adopted and proved to be very effective in reinforcing porous ceramic. For example, Miao [10] improved the compressive strength of porous HA/TCP from 8.4 MPa to 32 MPa by coating with PLGA. Bigi [11] reported the compressive strength value of apatite CPCs increased from 2.8 MPa to 10.7 MPa by casting a gelatin aqueous solution containing α-tricalcium phosphate and $CaHPO_4 \cdot H_2O$. However, porosity and interconnectivity of samples are very low, even not indicated in these researches. Although the compressive strength values reported in this paper are lower compared to these results, high porosity (>80%) and good interconnectivity (Fig. 1) were still maintained after coated with gelatin. In conclusion, the results in this study demonstrate that the gelatin coating process may be a potential method for reinforcing porous ceramics.

Conclusions

The method introduced in this study can form gelatin coatings on the surface of pore walls of porous ceramics, and the gelatin coatings made the sintered porous ceramics stronger and tougher. The mean compressive strength value of samples was improved from 1.04 ± 0.15 MPa to 5.17 ± 0.17 MPa on condition that the porosity of porous ceramics was not sacrificed greatly. Moreover their mean elastic modulus was improved from 0.1 GPa to 0.3 GPa. The method used in this study may be a potential method for reinforcing porous ceramics.

References

1. J.-M BOULER, R. Z. LEGEROS and G. DACULSI, *Biomed. Mater. Res.* **51** (2000) 680
2. R. Z. LEGEROS, S. LIN and R. ROHANIZADEH et al., *J. Mater. Sci., Mater. Med.* **14** (2003) 201
3. C.-K HSU, *Mater. Chem. Phys.* **80** (2003) 409
4. H.-W KIM, J. C. KNOWLES and H.-EE KIM, *Hyoun-Ee Kim. J. Biomed. Mater. Res.* **72** (2005) 136
5. J. E. BARRALET, L. GROVER and T. GAUNT et al., *Biomaterials* **23**(15) (2002) 3063
6. A. ALMIRALL, G. LARRECQ and J. A. DELGADO et al., *Biomaterials* **25** (2004) 3671
7. K. A. HING, S. M. BEST and W. BONEFIELD, *J. Mater. Sci. Mater. Med.* **10** (1999) 135
8. J. F. De OLIVERIRA, P. F. De AGUIAR and A. M. ROSSI et al., *Int. Soc. Art. Org.* **27** (2003) 406
9. T. MIYAZAKI, C. OHTSUKI and H. IWASAKI et al., *Mater. Sci. Forum.* **4** (2003) 426
10. X. MIAO, W.-K. LIM and X. HUANG et al., *Mater. Lett.* **59** (2005) 4000
11. A. BIGI, B. BRACCI and S. PANZAVOLTA, *Biomaterials* **25** (2004) 2893
12. H. R. R. RAMAY and M. ZHANG, *Biomaterials* **25** (2004) 5171
13. N. OZGUÈ R ENGIN and A. CUÈ NEYT TAS, *J. Eur. Ceram. Soc.* **19**, (1999) 2569
14. S. NI, J. CHANG and L. CHOU, *J. Biomed. Mater. Res.* **76A** (2006) 196
15. E. WINTERMANTEL, J. MAYER and J. BLUM et al., *Biomaterials* **17**, (1996) 83
16. Y. DONG, B. LIU and P. LIN et al., *Key Eng. Mater.* **288–289** (2005) 565
17. Y. FUJISHIRO, K. TAKAHASHI and T. SATO, *J. Biomed. Mater. Res.* **54** (2001) 525
18. Y. ZHANG and H. H. K. XU, *J. Biomed. Mater. Res.* **75A** (2005) 832