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Porous calcium phosphate ceramics modified with PLGA-bioactive glass

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Abstract

Porous calcium phosphate ceramics (mainly hydroxyapatite) with interconnected macropores (~ 1 mm) and micropores (~ 5 μm) as well as high porosities (~ 80%) were prepared by firing polyurethane foams that were coated with calcium phosphate cement at 1200 °C. In order to improve the mechanical properties such as compressive strength and compressive modulus and maintain the desirable bioactivity (i.e. the ability of apatite layer formation), the open micropores of the struts were infiltrated with poly(lactic-co-glycolic acid) (PLGA) to achieve an interpenetrating bioactive ceramic/ biodegradable polymer composite structure. The PLGA filled struts were further coated with a 58S bioactive glass (33wt%)-PLGA composite coating. The PLGA- bioactive glass modified porous calcium phosphate ceramics proved to be bioactive and exhibited compressive strengths up to 7.7 MPa and compressive moduli up to 3 GPa, which were comparable to those of natural spongy bones. The obtained complex porous bioactive/ biodegradable composites could be used as tissue engineering scaffolds for low-load bearing applications.

Keywords: hydroxyapatite; porosity; compressive strength; bioactive glass; poly(lactic-co-glycolic acid)

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1. Introduction

Porous biomaterials have proved to be important for bone replacement and regeneration. Many porous polymers and porous ceramics have been prepared for orthopedic applications. Porous titanium (Ti), tantalum (Ta) [1], and magnesium (biodegradable) [2] are also used as scaffolds for bone tissue engineering. A trend for scaffold materials is the development of compositionally and geometrically complex scaffolds to meet the strict property requirements. For instance, there are intensive studies on the surface-modified scaffolds, nano-composite scaffolds, and hybrid scaffolds. Some scaffolds are also modified with drugs, growth factors, and proteins, etc.

Specifically for porous bioceramics, tens of methods have been developed for the scaffold preparation. For example, Charriere et al. [3] successfully built precipitated hydroxyapatite cement scaffolds with controlled macropore size and shape using a solid freeform fabrication process. The current author [4] also produced porous calcium phosphate ceramics through the usage of calcium phosphate cement, which was coated on combustible polyurethane foams. Individually developed methods can also be combined to obtain better porous structures and mechanical properties. For

example, Li et al. [5] combined a foaming method and a dual-phase mixing method (involving HA slurry and polymethylmethacrylate (PMMA) resin) to prepare macroporous hydroxyapatite (HA). Similarly, Ramay et al. [6] used a technique that combined a gel-casting method with a polymer sponge method to prepare macroporous hydroxyapatite scaffolds.

While porous bioactive and/ or biodegradable ceramics are highly biocompatible, osteoconductive, and even osteoinductive, their mechanical properties such as fracture toughness and compressive strength are very poor especially when the porosities are high. To avoid the brittleness of porous ceramics, many porous biodegradable polymer / bioactive (or biodegradable) ceramic composite systems have been developed. For example, Maquet et al. [7] prepared highly porous poly(D,L-lactide)/ Bioglass composite scaffolds by a thermally induced phase separation process and by subsequent solvent sublimation. Lu et al. [8] also developed a three dimensional (3-D), porous composite of polylactide-co-glycolide (PLGA) and 45S5 bioactive glass for bone tissue engineering.

It should be mentioned that the above polymer/ ceramic composites were polymer matrix-based composites with dispersed ceramic phases. The amount of the ceramic phase was often limited by the processing method used. The bioactivity of a dispersed composite may not be maximized due to the isolation of the ceramic particles by the polymer matrix. In order to overcome these shortcomings of the randomly dispersed composites, one may need to develop interpenetrating ceramic/ polymer composites. For example, Li et al. [9] produced macroporous HA ceramics with struts of interconnected nanopores. Then a polymer phase, Polyactive™, was incorporated into the struts by vacuum impregnation. As a result, the mechanical properties of the porous composites with the interpenetrating organic/inorganic phases were found to improve significantly.

Another way of using the advantages and minimizing the problems of porous bioactive/ biodegradable scaffolds could be the usage of a bioactive polymer coating or a biodegradable polymer/ bioactive ceramic composite coating. For example, Kim et al. [10] coated hydroxyapatite (HA) porous scaffolds with an HA and polycaprolactone (PCL) composite coating. The PCL polymer, as a coating component, was able to improve the brittleness and low strength of the HA scaffolds. The HA particles in the coating were to improve the osteoconductivity and bioactivity of the coating layer.

In the present study, porous calcium phosphate (mainly hydroxyapatite) ceramics with both open macropores and micropores were prepared by sintering calcium phosphate cement that was coated on polyurethane foams. Bioactive glass–PLGA slurry was used to infiltrate the micropores in the struts and also coat the struts in order to improve the mechanical properties of the complex scaffolds without a loss of the bioactivity. While inspired by Kim et al.'s work, the present study differed from Kim et al.'s work in several aspects: our microporous calcium phosphate struts versus Kim et al.'s dense HA struts; our bioactive glass–PLGA coating versus Kim et al.'s HA-PCL coating; our higher compressive strength versus Kim et al.'s low strength due to the introduction of interpenetrating ceramic/ polymer struts.

2. Experimental procedure

2.1 Sample preparation

Preparation of TTCP powder

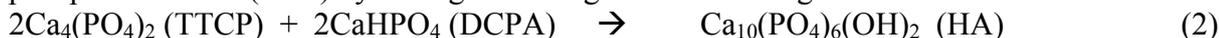
Pyro-calcium phosphate ($\text{Ca}_2\text{P}_2\text{O}_7$) powder was mixed with calcium carbonate (CaCO_3) powder in the weight ratio of 1.27: 1. For the mixing step, the starting powders were poured into an ethanol solution to produce a viscous paste. Then the mixed powder was dried and crushed using a mortar

and a pestle, followed by calcination in a platinum crucible at 1350°C for 5 hours in air and quenching in air to 25°C. Finally the calcined powder (TTCP phase) was ground into a fine powder. The chemical reaction for the TTCP powder was as follows:



Preparation of TTCP-DCPA mixed powder

The weight ratio of tetracalcium phosphate ($\text{Ca}_4(\text{PO}_4)_2\text{O}$; TTCP) powder to dicalcium phosphate anhydrous (CaHPO_4 ; DCPA) powder was 72.9: 27.1. These powders were mixed in dry state in a jar placed in a vibration mill (SPEX 8000). The mixed powder was used to prepare calcium phosphate cement (CPC) by setting according to the following reaction:



Preparation of porous CPC using polyurethane foams

First 0.5M Na_2HPO_4 was added into 1 liter of distilled water to produce Na_2HPO_4 aqueous solution, which was used as the liquid phase for the CPC. Then a flowable slurry for the CPC was obtained by mixing the liquid phase with the solid phase (i.e. the mixed powder mentioned above) in a ratio of 0.5ml:1g. This slurry was then used to coat the polyurethane foams of different pore sizes (2.3 mm, 1.6 mm, and 1 mm) through the actions of slurry dipping, squeezing, and blowing with air. The slurry-coated foams were then allowed to set (i.e., reaction (2) occurred in the presence of the liquid phase) and dry to produce a porous CPC.

Preparation of porous calcium phosphate ceramics (major hydroxyapatite)

To remove the polyurethane foams, the coated foams were fired in air in an electric furnace using a 4 stage schedule, which included (i) heating from 40°C to 600°C with a heating rate of 1°C/ min (to burn off the polyurethane), (ii) further heating from 600°C to 1200°C at 5°C /min., (iii) dwelling at 1200°C for 2 hours (to partially sinter the CPC), and (iv) finally cooling down to 40°C at the cooling rate of 5°C/ min.

Preparation of 58S bioactive glass powder

A sol-gel derived bioactive glass (coded as 58S) powder with particle sizes < 3 μm and of a composition of 58 mol% SiO_2 – 38 mol% CaO – 4 mol% P_2O_5 was prepared through the hydrolysis and condensation of a mixed solution of tetraethoxysilane (TEOS; $\text{Si}(\text{OC}_2\text{H}_5)_4$), triethylphosphate (TEP; $\text{OP}(\text{OC}_2\text{H}_5)_3$) and calcium nitrate tetra-hydrate ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$). An HCl solution was used as a catalyst for the hydrolysis and condensation reactions. The formed sol was then sealed in a beaker and aged in an oven at 60°C for 2 days. The formed gel was then dried in the oven at 60°C for another 2 days. The dried gel after crushing was further calcined at 700°C for 1 hour, followed by ball milling to obtain the 58S bioactive glass powder.

Preparation of bioactive glass-PLGA coating on porous calcium phosphate ceramics

Firstly, every 5g of 58S bioactive glass powder was mixed with 25ml of dichloromethane (CH_2Cl_2) solvent using a mortar and a pestle to disperse the fine glass particles. Secondly, the bioactive glass slurry was diluted with 45ml more of dichloromethane. Thirdly, 10g of PLGA (75LA: 25GA) was added so that the PLGA was dissolved completely. Lastly, the resulting flowable slurry was used to coat the sintered porous CPC through the actions of dipping, shaking, blowing, and evacuation.

Biomimetic apatite coating on the bioactive glass-PLGA coated porous samples

The bioactive glass-PLGA coated porous samples were immersed separately in glass containers that contained a simulated body fluid (SBF; the standard composition), and placed in an oven at a temperature of 37°C for 1 to 2 weeks. After the immersion testing, the samples were taken out, rinsed with distilled water, and dried for subsequent surface examination.

2.2. Sample characterization

Porosity measurement

The total porosity of each sintered porous CPC sample was determined using the following equations: bulk density (ρ_B) = weight of the sample/ volume of the sample; the theoretical density for HA (ρ_o) = 3.16g/cm³; relative density (R.D.) = (ρ_B / ρ_o) x 100%; and finally total porosity = 100 % – R.D. The dimensions and the weight of each sample were measured and recorded through a Vernier caliper and an electronic balance, respectively.

Optical microscopy

The overall morphologies (pore sizes and shapes) of the porous PU foams, the sintered porous CPC, and the bioactive glass-PLGA coated porous samples, were observed using a stereo-optical microscope (Leics MZ6).

X-ray diffraction (XRD)

XRD was used to identify the crystallographic phases of the reaction products such as the TTCP powder, the set CPC, and the sintered CPC. For the XRD analysis, the samples were ground into fine powders and each powder was mounted in a specimen holder for the diffractometer (6000 Shimadzu). Cu K _{α 1} ray ($\lambda = 1.5406 \text{ \AA}$) scanning was conducted using a 2θ angle of from 20° to 45°. The scan rate and the step size were 2.0° min⁻¹ and 0.02°, respectively.

SEM /EDS examination

The topographical images of the sample surfaces or the fracture surfaces were examined under a JEOL 5310 scanning electron microscope (SEM). In addition, the elements present in the samples, especially in the top layers, were analyzed using energy dispersive spectroscopy (EDS).

Compressive tests

For the measurements of the compressive strengths of the porous samples (i.e. the porous sintered CPC and the bioactive glass-PLGA coated porous sintered CPC), rubber pads were placed on the top and the bottom surfaces of each sample. The rubber-padded sample was then placed in an Instron tester (model 5567) to conduct a compressive test. The rubber pads were used to ensure a uniform distribution of the applied load onto the sample. A crosshead speed of 0.5mm/min. was used for the compressive tests.

3. Results and discussion

The commercially available polyurethane (PU) foams used for the study had high porosities, highly interconnected pores, and different pore sizes. Fig. 1 shows the PU foam with a pore size of 1 mm, as an example. When the foams were immersed in the CPC slurry, the ceramic particles carried by the aqueous solution were able to coat the struts of the PU foams. The excess ceramic slurry in the macropores must be driven away by hand squeezing, rolling with a roller, blowing with low pressure air, or centrifugation to prevent the blockage of the macropores by the ceramic slurry. Since the CPC slurry was made of TTCP and DCPA mixed powder plus the Na₂HPO₄ solution, the coating process should be completed before the setting reaction (i.e. the reaction between TTCP and DCPA in the presence of Na₂HPO₄ to form poorly crystallized hydroxyapatite). After the setting reaction, the slurry-coated PU foams could be dried in air at the room temperature. The setting reaction to form the hydroxyapatite phase made the CPC framework strong enough to stand freely without being sagged or distorted by the weight.

Due to the lack of biocompatibility, the commercial PU foams must be removed by slow burning in air. The PU foams were thus used only as a template to form the ceramic framework. During the firing step, the struts of the PU foams experienced thermal expansion, melting, and evaporation.

Since the ceramic particles were joined together due to the setting reaction, the CPC framework would not be damaged by the removal of the PU, resulting in intact hydroxyapatite porous bodies. For example, Fig. 2 shows a sintered porous CPC body with a macropore size of 0.7 mm. On the other hand, the firing process would also change the microstructure of the set CPC. Specifically, in the set CPC, while the hydroxyapatite phase was present in the form of whiskers, as reported before [4], some residual TTCP and DCPA were still left behind. The set CPC was also porous with a large amount of micropores. Nevertheless, after sintering at 1200 °C for 2 hours, equal-axed hydroxyapatite grains were formed although microporosity remained in the struts (Fig. 3). In addition, the residual TTCP phase was depleted to a great extent for the formation of hydroxyapatite. Some β -TCP was also found in the sintered CPC, indicating some degree of thermal decomposition of the hydroxyapatite phase into the tricalcium phosphate phase, as shown in Fig. 4. Another feature of the sintered porous CPC was the presence of large defects (pores) inside the struts, which were due to the removal of the struts of the PU foams. Thus, the sintered porous CPC contained mainly hydroxyapatite phase with macropores (as a replica of the macropores in the PU foams) in the overall structure and open micropores in the struts.

The sintered porous CPC was rather weak mechanically due to the high porosity and due to the intrinsically low mechanical strengths of the calcium phosphates. One way to strengthen and toughen the porous CPC was to infiltrate the microporous struts with a bioactive polymer, and also coat the struts with a bioactive polymer. However, a bioactive polymer was not available for the project, but a biodegradable polymer of PLGA was used instead. Since PLGA is not bioactive, thus PLGA coating must be made bioactive by incorporating a bioactive second phase. Since bioactive glass is known to be more bioactive than hydroxyapatite, thus a PLGA solution containing dispersed bioactive glass particles was used to infiltrate and coat the sintered porous CPC, as shown in Fig. 5. Several factors affected the coating process, namely, the viscosity of the slurry, the content of the bioactive glass fine particles, and the volatility of the organic solvent. The coating quality was also controlled by the details involved in the dipping coating process.

Since the struts of the sintered porous CPC contained micropores that were open or interconnected, as shown in Fig. 3, the PLGA and some fine bioactive glass particles were able to penetrate into the open pores. As a result, a ceramic/ polymer composite of the interpenetrating phases was formed for the struts, as shown in Fig. 6. The significance of the interpenetrating composite can be seen from two aspects: Firstly, the composite was better than the pure porous sintered CPC due to the better mechanical integrity of the composite. In fact, several studies have indicated the strengthening and toughening effect in the interpenetrating composites. For example, Pezzotti et al. [11] prepared hydroxyapatite/ polymer interpenetrating composites and found that the high toughness of the composites was mainly due to a micron-scale crack-bridging mechanism operated by the polymer ligaments that were stretched upon crack opening along the crack wake. Secondly, a pure porous PLGA alone is mechanically weak and non-bioactive and subjects to change of shape and pH value in vivo. As to the interpenetrating composite, the pH change due to the biodegradation of PLGA would be mitigated and the distortion of the PLGA framework would be restricted by the relatively biostable porous hydroxyapatite framework.

The dipping of the sintered porous CPC into the PLGA-bioactive glass slurry also resulted in a layer containing a PLGA matrix and the bioactive glass particles on the surface of the struts of the sintered porous CPC. The thickness of the coating layer could be controlled by number of the repeated dipping cycles. Due to a filtering effect of the micropores of the struts, larger bioactive glass particles were blocked and deposited on the surface of the struts. On the other hand, the evaporation of the solvent in the PLGA slurry resulted in a mesh-like or honeycomb-like structure. Thus a large number of bioactive glass particles were found exposed (Fig. 7), which was advantageous as far as bioactivity was concerned. The bioactivity of the bioactive glass was indeed confirmed after the immersion test using the simulated body fluid. Fig. 8 shows an apatite layer

formed on the PLGA-bioactive glass coating on the sintered porous CPC. The cracks observed in the apatite layer were common and were due to the poor interfacial bonding between the polymer and the apatite coating and due to the plastic deformation of the polymer layer occurring during the sample preparation. It should be mentioned that the apatite layer was confirmed after analysis with EDS, which indicated a right intensity ratio of the Ca peak to the P peak.

The present study led to a new method of preparing porous calcium phosphate ceramics. The advantage of the method was the attainment of a high pore interconnectivity and a high porosity. The successful rate of sample preparation was high after the firing to remove the PU foams and sinter the remaining porous structures. However, the method resulted in large defects in the struts after the removal of the PU struts. This shortcoming could be overcome by repeated dipping in the CPC slurry, followed by sintering again. In addition, the applied PLGA–bioactive glass composite coating resulted in better mechanical properties and without the loss of bioactivity. Of course, other bioactive or biodegradable polymers could be used for the purpose of the current project. The porous and sintered CPC further coated with PLGA-bioactive glass could not be broken easily when thrown on the ground and could also tolerate the actions of cutting with a diamond grit-impregnated blade. In terms of mechanical properties (see Table 1) and bioactivity, the currently prepared porous bioactive/ biodegradable composites would be useful as bone tissue engineering scaffolds for non- or low loading bearing applications.

4. Conclusions

Porous hydroxyapatite-based calcium phosphate ceramics with macropores sizes of about 2.0mm - 0.7mm and micropore sizes of about 5 μ m were prepared by firing calcium phosphate cement coated on the struts of polyurethane foams at 1200°C for 2 hours. The calcium phosphate cement was prepared at room temperature by mixing tetracalcium phosphate ($\text{Ca}_4(\text{PO}_4)_2\text{O}$) and dicalcium phosphate anhydrous (CaHPO_4) powders with sodium phosphate (Na_2HPO_4) solution. The tetracalcium phosphate was prepared by firing the mixture of pyro-calcium phosphate ($\text{Ca}_2\text{P}_2\text{O}_7$) and calcium carbonate (CaCO_3) at 1350°C for 5 hours, followed by quenching in air. The prepared porous hydroxyapatite-based ceramics exhibited high porosities and high pore interconnectivities.

However, the mechanical strengths of the porous hydroxyapatite-based calcium phosphate samples were far too low ($\ll 1$ MPa). After impregnating and coating with the bioactive glass (33 wt%)-PLGA composite, the macropores of the sintered porous calcium phosphate (mainly hydroxyapatite) ceramics remained highly interconnected. The compressive strengths of the PLGA-bioactive glass coated porous sintered calcium phosphates were also increased up to 7.7MPa. Finally, the coated porous calcium phosphate scaffolds could be trimmed by cutting actions and the bioactivity of the composite scaffolds was confirmed by the apatite layer formation in the simulated body fluid.

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Figure captions

Fig. 1 Stero-optical micrograph showing the pores (~1 mm) and the struts of a polymer foam.

Fig. 2 Stero-optical micrograph showing the pores (~ 0.7mm) and the struts of the sintered porous calcium phosphate (mainly hydroxyapatite).

Fig. 3 SEM micrograph of a fracture surface of a strut of the sintered porous calcium phosphate (mainly hydroxyapatite).

Fig. 4 XRD pattern for a sintered porous calcium phosphate (mainly hydroxyapatite) sample.

Fig. 5 Bioactive glass-PLGA coating on the sintered porous calcium phosphate with a medium pore size of 1.3mm.

Fig. 6 SEM micrograph of a fractured surface of the microporous strut (mainly hydroxyapatite) filled with PLGA.

Fig. 7 SEM micrograph of the 58S bioactive glass-PLGA coating on the sintered porous calcium phosphate (mainly hydroxyapatite).

Fig. 8 SEM micrograph showing the apatite layer formed on the bioactive glass-PLGA coating after the immersion in SBF.

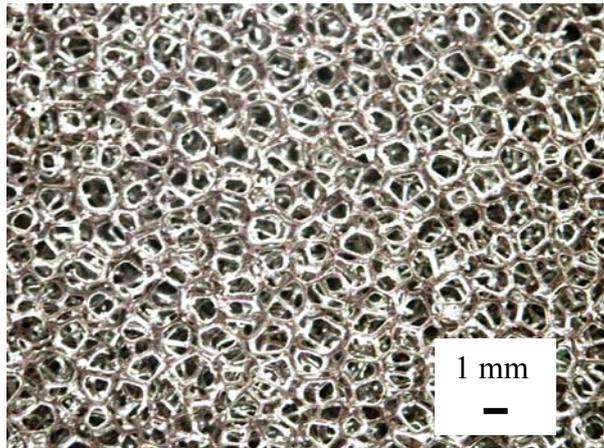


Fig. 1 Stereo-optical micrograph showing the pores (~ 1 mm) and the struts of a polymer foam.

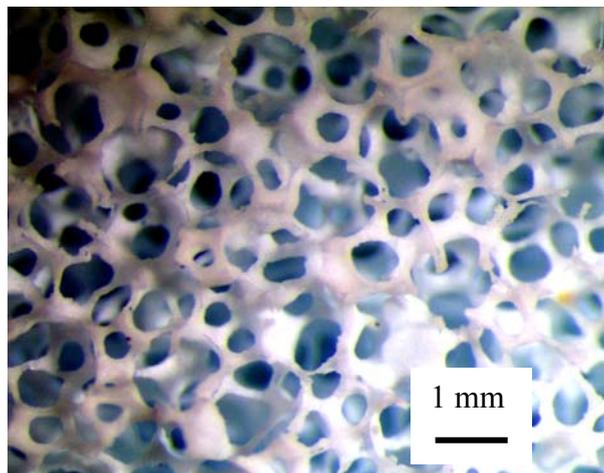


Fig. 2 Stereo-optical micrograph showing the pores (~ 0.7 mm) and the struts of the sintered porous calcium phosphate (mainly hydroxyapatite).

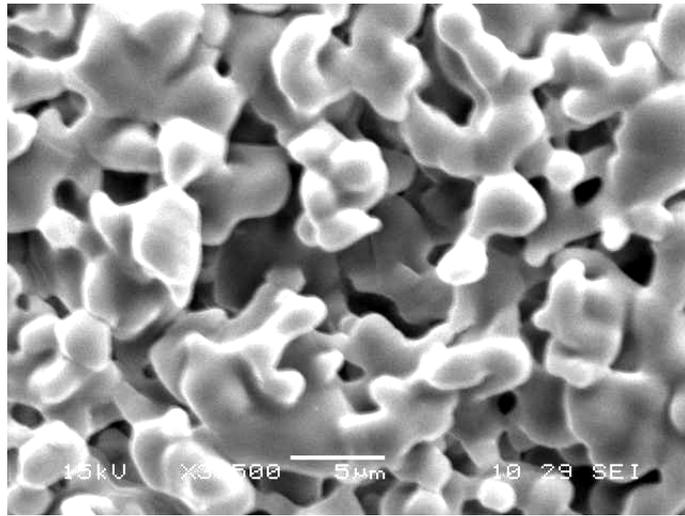


Fig. 3 SEM micrograph of a fracture surface of a strut of the sintered porous calcium phosphate (mainly hydroxyapatite).

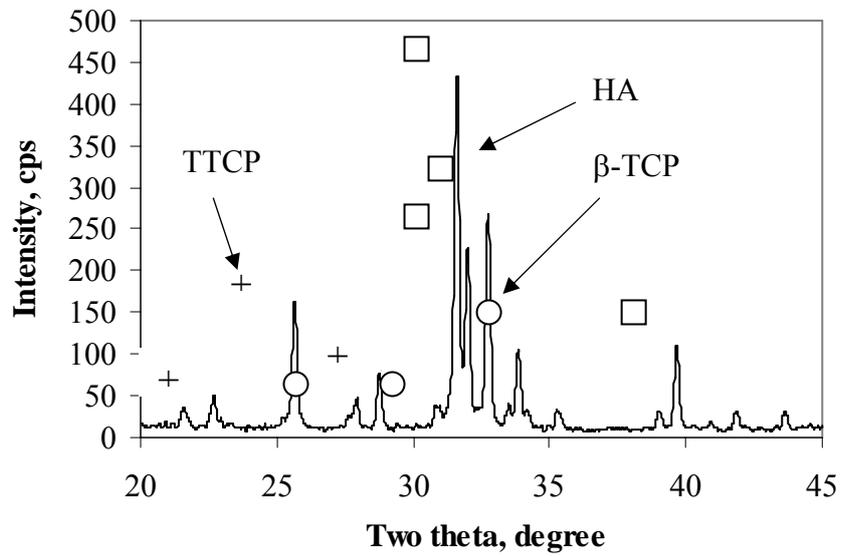


Fig. 4 XRD pattern for a sintered porous calcium phosphate (mainly hydroxyapatite) sample.

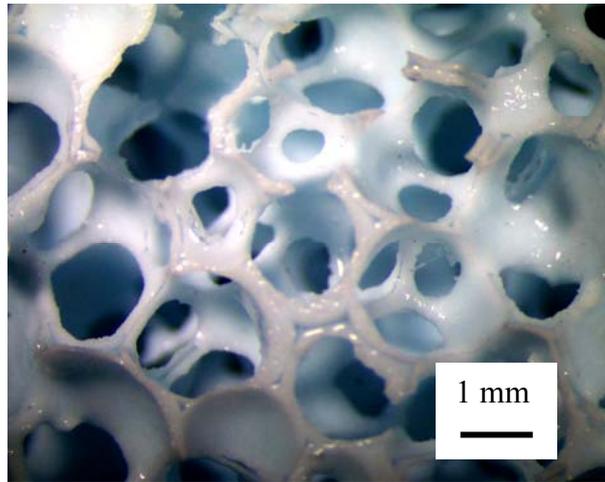


Fig. 5 Bioactive glass-PLGA coating on the sintered porous calcium phosphate with a medium pore size of 1.3mm.

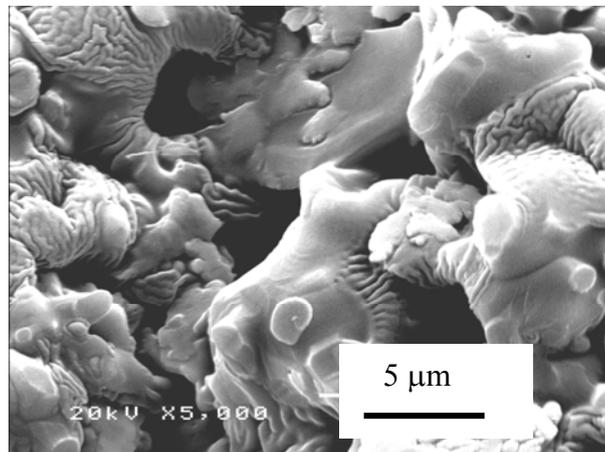


Fig. 6 SEM micrograph of a fractured surface of the microporous strut (mainly hydroxyapatite) filled with PLGA.

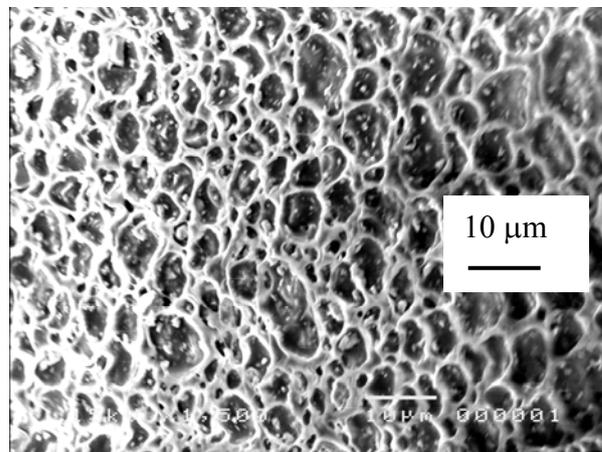


Fig. 7 SEM micrograph of the 58S bioactive glass-PLGA coating on the sintered porous calcium phosphate (mainly hydroxyapatite).

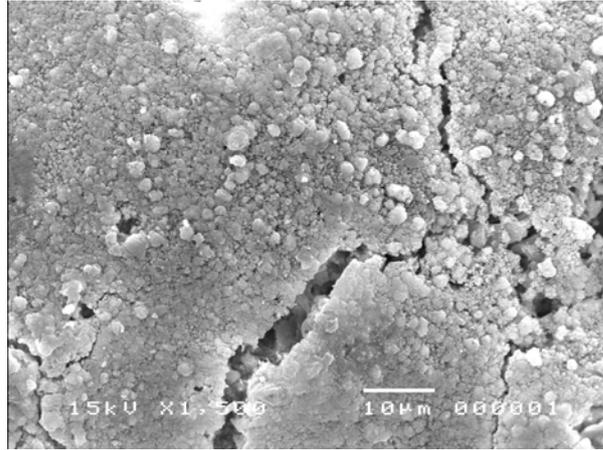


Fig. 8 SEM micrograph showing the apatite layer formed on the bioactive glass-PLGA coating after the immersion in SBF.

Table

Table 1. Comparison among the porous sintered CPC, the bioactive glass-PLGA coated porous sintered CPC, and cancellous (or spongy) bones.

Types of materials	Macropore size (mm)	Total porosity (%)	Compressive strength (MPa)	Compressive modulus (GPa)
Porous sintered CPC	2.0	91	0.03	1.55
	1.3	86	0.09	1.90
	0.7	75	0.20	2.22
Bioactive glass-PLGA coated porous sintered CPC	1.9		2.39	2.30
	1.2		2.56	2.47
	0.6		7.70	3.20
Cancellous bones	1-5	30-90	2-12	0.2-5