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Preparation and characterization of interpenetrating phased TCP/HA/PLGA composites

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Abstract

The purpose of this study was to fabricate composites consisting of three interpenetrating networks: tricalcium phosphate (TCP), hydroxyapatite (HA), and poly(DL-lactide-co-glycolide) (PLGA). The porous TCP network was first produced by coating a polyurethane (PU) foam with hydrolysable alpha-TCP slurry. The HA network was derived from a calcium phosphate cement (CPC) filled in the porous TCP network. The remaining open pore network in the HA/TCP composite was further infiltrated with a PLGA network. The three sets of spatially continuous networks would have different biodegradation rates and thus bone tissue would grow towards the fastest biodegrading network while the remaining networks still maintaining their geometrical shape and carrying the physiological load for the tissue ingrowth.

Keywords: Interpenetrating composite; Calcium phosphate cement; Tricalcium phosphate; Poly(DL-lactide-co-glycolide)

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1. Introduction

Tricalcium phosphate (TCP) is an important biodegradable ceramic material and has been subjected to intensive studies. TCP can form three polymorphs: β -TCP below 1180°C, α -TCP between 1180°C and 1430°C, and α' -TCP above 1430°C [1]. Several methods have been used for producing porous TCP ceramics. For example, Koc et al. [2] prepared porous TCP by slip casting of aqueous slurry of TCP powder and PMMA (polymethylmethacrylate) beads, followed by sintering. Aoki et al. [3] prepared porous β -TCP with about 100 μm of average pore diameter by a ceramic foaming method. However, all the porous TCP ceramics have low mechanical properties.

Similarly, several methods have been used to produce biodegradable porous CPCs (calcium phosphate cements), but they are also very weak mechanically. For example, in Barralet et al. [4] study, when the macroporosity varied from 22% to 31%, the compressive strength of the macroporous CPCs changed from 2.93 MPa to 0.6 MPa. According to Almirall et al. [5], macroporous hydroxyapatite originated from the hydrolysis of α -TCP had compressive strengths of 1.4 MPa to 2.7 MPa in the macroporosity range of 36% to 27%.

Methodologies should be available to produce biodegradable ceramics or composites with improved mechanical properties and with the ability of bone tissue ingrowth. Producing interpenetrating composites of biodegradable phases could be one of the methods. For example, Frayssinet et al. [6] improved the strength of macroporous calcium phosphate ceramics of interconnected porosity by filling the pores with highly soluble, self-setting CPC made of TCP and DCPD (dicalcium phosphate dihydrate). Miao et al. [7] also investigated the interpenetrating alumina composite with CPC as a filler. The compressive strength for the porous alumina with the CPC filler doubled compared with that of the parent porous alumina.

On the other hand, Miyazaki et al. [8] attempted to reduce the dissolution rate of a porous α -TCP by coating with organic polymers including hydroxypropylcellulose (HPC) and sericin, a kind of silk protein. The results on dissolution of α -TCP in buffer solutions indicated that α -TCP coated with sericin showed the highest chemical durability. The compressive strength also increased after coating with the HPC. Li et al. [9] prepared macroporous hydroxyapatite (HA) with interconnected channels on the nanometer scale. Then a polymer phase, Polyactive™, was incorporated into those micro-channels of the inorganic skeleton by vacuum impregnation. The mechanical properties of the composites with interpenetrating organic/inorganic phases were found to improve significantly in terms of impact strength compared with each phase alone.

Natural bone can be regarded as an interpenetrating ceramic (i.e. biological apatite)/ polymer (i.e. collagen) composite. There is a research interest in producing hybrid composites that mimic the natural bone structures. Pezzotti et al. [10] found that the fracture energy of hybrid nylon-infiltrated hydroxyapatite composites was increased by a crack bridging mechanism with plastic deformation of nylon ligaments. Yang et al. [11] reported that adding ionic groups into polymer resin chains appeared to improve the chemical bonding between the polymer and the calcium polyphosphate (CPP), leading to a higher bending strength for the newly formed CPP/ polycarbonate resin system.

The purpose of this paper is to report on the preparation of complex TCP/HA/PLGA composites that had interpenetrating phases with different biodegradable rates. The advantage of the complex biodegradable composites would lie in the improved mechanical properties or structural integrity and also the possibility of tailored biodegradation rates for bone tissue ingrowth. The preparation of the complex composites involved several steps. Initially, porous TCP (mainly β -TCP) was prepared via sintering α -TCP slurry coated polyurethane foams. This was followed by infiltrating the porous TCP with a CPC filler suitable for drug delivery. Lastly, biodegradable PLGA as a third interpenetrating phase was incorporated to improve the compressive strength.

2. Experimental

2.1 Raw materials

The main starting materials were beta-tricalcium phosphate (β -Ca₃(PO₄)₂; β -TCP) powder (Aldrich Chem. Co.), polyurethane (PU) foams (Foamex Asia), poly(DL-lactide-co-glycolide) with an inherent viscosity of 0.69 dL/g (PLGA (75:25); Birmingham Polymers, Inc.), tetracalcium phosphate (Ca₄(PO₄)₂O; TTCP), dicalcium phosphate anhydrous (CaHPO₄; DCPA), and sodium hydrogen phosphate (Na₂HPO₄). The TTCP powder was custom-made from pyro-calcium phosphate (Ca₂P₂O₇) and calcium carbonate (CaCO₃) [12]. Other chemicals and agents included dispersant DARVAN C - an aqueous solution of 25% ammonia polymethacrylate acid (NH₄ PMAA) (R. T. Vanderbilt Co. Inc.), polyvinyl alcohol (PVA) as a binder, and dichloromethane (CH₂Cl₂) as an organic solvent.

2.2 Preparation of α -TCP powder

Alpha TCP powder was prepared from β -TCP powder via high temperature phase transformation and subsequent quenching. Specifically, β -TCP powder was packed in a platinum crucible and placed in an electric furnace. The firing schedule was as follows: heating at a rate of 5°C/min from the room temperature to 1400°C, dwelling at 1400 °C for 2 hours, cooling down to 1150°C at a rate of 5°C/min, and finally air-quenching to the room temperature. By milling with a planetary ball mill, α -TCP powder was obtained and kept in a drying cabinet to prevent possible hydrolysis of the α -TCP powder.

2.3 Coating PU foams with α -TCP slurry

Pieces of PU foams (30 x30 x 20 mm³) with a pore size about 700 μ m were cut from a large PU foam sheet. The PU foams were then cleaned with a NaOH solution, followed by rinsing with distilled water before drying in air. On the other hand, an α -TCP slurry was prepared based on preliminary trials. The used α -TCP slurry contained 55 wt% α -TCP powder (relative to the amount of water), 1 wt% binder (PVA) (relative to the amount of α -TCP), 1 wt% NH₄PMAA (relative to the amount of α -TCP), and the rest distilled water.

The PU foams were then immersed into the slurry, followed by repeated actions of squeezing and releasing. Then the slurry-containing PU foams were taken out of the slurry, followed by shaking, gentle squeezing, and blowing with pressure air. Homogeneous coating of the α -TCP slurry on the PU foams was possible with practice. Then the coated PU foams were allowed to dry slowly in air at the room temperature.

2.4 Preparation of sintered porous TCP

In order to remove the PU foams (maybe toxic) and sinter the TCP particles on the struts of the PU foams, a slow heating rate was used during firing in air. Specifically speaking, the sintering schedule involved heating from the room temperature to 500 °C at a heating rate of 1 °C /min., dwelling at 500 °C for 1 hour, then heating to 1300 °C at a heating rate of 7 °C/min., dwelling at 1300 °C for 2 hours, and finally cooling down to the room temperature at 5 °C/ min.

2.5 Filling sintered porous TCP with CPC

The dry TTCP powder was mixed with dry DCPA powder in a vibration mill (SPEX 8000) using a jar with a ball inside. The molar ratio of TTCP to DCPA was 1:1 or the weight ratio was 72.9:27.1. The mixed powder was then made into a slurry based on the powder to water ratio of 1: 1 (weight). Again, 1 wt% dispersant (NH₄PMAA) was used to reduce the viscosity of the slurry.

The mixed powder slurry was poured into the sintered porous TCP body placed in an aluminum tray. Vibration and evacuation in a vacuum chamber were carried out to facilitate the infiltration of the mixed powder slurry. When the sintered porous TCP was fully infiltrated with the mixed powder slurry, drying in air was conducted to remove the water.

In order to produce a CPC filled porous TCP body, the mixed powder filled in the sintered porous TCP must be exposed to an aqueous solution of Na₂HPO₄ with the concentration of 1 mol/liter. This solution was able to initiate the setting reactions so that poorly crystallized hydroxyapatite could be produced.

2.6 Sintering of the CPC filled porous TCP

To alter the microstructure of the set CPC filled in the sintered porous TCP, firing in air at 1200 °C for 2 hours was also conducted. This step would change the porosity, pore size, and crystal shape of the set CPC, leading to a porous HA (CPC-derived)/TCP composite.

2.7 Infiltration with PLGA

To improve the mechanical properties of the sintered porous HA/TCP composite, PLGA was introduced into the remaining open pores to form a HA/TCP/PLGA composite. For the infiltration of PLGA (75:25), the PLGA was dissolved in dichloromethane (CH₂Cl₂) to form a viscous but flowable solution. The porous HA/TCP composite was then immersed into the PLGA solution, followed by vacuum infiltration. The process of immersion and drying was done repeatedly to increase the degree of infiltration.

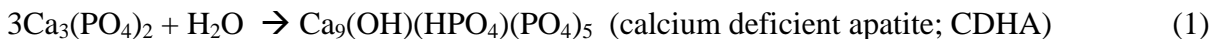
2.8 Characterization

The macroporous structures were observed using a stereozoom optical microscope (Leica MZ6). The phases present were analyzed by an X-ray diffractometer (XRD, Shimadzu 6000). The microstructure was observed by a scanning electron microscope (SEM, JEOL 5310). The compressive strength and the Young's modulus of the specimens were measured on an Instron universal tester (Model 5567) at the cross-head speed of 0.5 mm/min.

3. Results and Discussion

Polyurethane (PU) foams of different pore sizes (large, medium, and small) were tried for the study. A foam of the medium pore size (700 μm) is shown in Fig. 1, where the webs of the PU foam are connected or continuous with macropores (defined by $> 100 \mu\text{m}$) among the webs. The foams of the medium pore size could be evenly coated with α -TCP slurry and thus were chosen for the preparation of porous TCP ceramics and for the infiltration of CPC filler. On the contrary, the foams of the small pore size had most of their pores blocked by the TCP slurry or powder, and thus were not used for the subsequent infiltration of CPC. As to the foams of the large pore size, they led to a very low compressive strength of the sintered porous TCP, although they were easily coated with the TCP slurry.

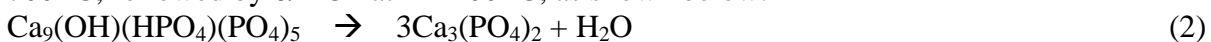
The interest in using α -TCP rather than β -TCP was due to the ability of hydrolysis of α -TCP. In fact, α -TCP has been used as a component for calcium phosphate cements. The hydrolysis of α -TCP can be described as:



It means that when α -TCP particles are mixed with water, a new phase of calcium deficient apatite will form. As shown by Almirall et al. [5], the CDHA phase would have the appearance of wool-like crystals. These crystals would be mechanically interlocked or entangled, resulting in a certain level of mechanical strength even without a high temperature sintering.

In the present study, α -TCP slurry was coated onto the PU foams and the hydrolysis of α -TCP would occur due to the water in the slurry and the moisture in air, resulting in some level of mechanical strength of the α -TCP green body coated on or supported by the PU foams. Indeed, α -TCP slurry-coated foams led to better mechanical integrity after drying and after firing to burn off the PU foams. Similarly, the α -TCP-coated PU foams could tolerate fast heating rate without collapse of the porous sintered TCP structures. Similar situation was found in the preparation of porous calcium phosphate ceramics from a calcium phosphate cement that was coated on PU foams [12].

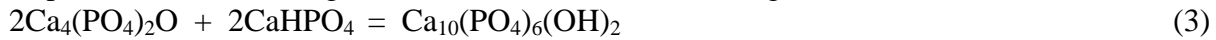
Fig. 2 shows a sintered porous TCP body. What happened was that after the removal of the PU foam below 500 $^\circ\text{C}$, the CDHA due to hydrolysis of α -TCP would be converted into β -TCP above 700 $^\circ\text{C}$, followed by α -TCP at $\sim 1200 \text{ }^\circ\text{C}$, as shown below:



The unhydrated α -TCP would also be partially converted into β -TCP and then α -TCP again, depending on the time and temperature during the heating and sintering process. During the slow cooling down process, some β -TCP phase was formed and some α -TCP phase was retained due to the elastic strain constraint from the surrounding matrix - an energy barrier for the α - to β -TCP phase transformation. Thus, the phases present in the sample as shown in Fig. 2 should contain both α -TCP and β -TCP phases. This was actually confirmed by the XRD analysis (Fig. 3) of an identical sample.

Porous β -TCP (+ α -TCP) ceramics prepared from the PU foams were rather brittle and weak mechanically. To improve the mechanical strength of the porous bodies and minimize the risk of

fast dissolution of the TCP phases in vivo, the pores of the porous TCP bodies were filled with the calcium phosphate cement (CPC), resulting from the mixed TTCP and DCPA powders packed in the pores and immersed in the Na₂HPO₄ solution. The TTCP (Ca₄(PO₄)₂O) and DCPA (CaHPO₄) phases would dissolve in the Na₂HPO₄ solution and whisker-like hydroxyapatite crystals would precipitate when the ions in the solution were saturated. The dissolution and the precipitation were responsible for the setting of the CPC and the overall setting reaction is shown as follows:



Although the Na₂HPO₄ is not shown in the above setting reaction, without Na₂HPO₄, i.e. if only pure water was used, the setting reaction would be very slow. Fig. 4 shows a fractured surface of the CPC filled in the pores of porous TCP; the micropores, unreacted particles, and HA whiskers are visible.

When the set CPC in a porous TCP body was sintered at 1200 °C, some microstructural change was observed. Firstly, the grain size of the TCP (mainly β-TCP) phase became larger than before due to the grain growth at the high sintering temperature. Secondly, the original whisker-like fine HA crystals as shown in Fig. 4 became large and equiaxed grains (Fig. 5). In addition, our previous XRD results indicated an increased crystallinity of the HA phase when the set CPC specimens were sintered at 1200 °C. Thirdly, the pore size of the sintered CPC was larger than that of the set CPC, as can be seen by comparing Fig. 4 with Fig. 5 and Fig. 6. The growth of the pores was accompanied by the growth of the HA grains. This microstructural development could be similar to the intermediate stage of the conventional solid sintering process, resulting in interconnected pores among the HA grains subjected to the Oswald ripening. Finally, the preferential sintering shrinkage of the CPC network as compared with the pre-sintered TCP network (without further sintering shrinkage), resulted in wide gaps formed between the TCP network and the sintered CPC network (Fig. 6). Nevertheless, the microstructure of the large open pores was suitable for impregnation with a ductile polymer phase. Indeed, PLGA (75/25) phase was able to fill the open pores to generate the TCP/HA/PLGA interpenetrating composites (Fig. 7).

In the present study, open or interconnected micropores were observed among the set CPC loaded in the sintered porous TCP. Larger open micropores were also present in the sintered CPC network, which was interlocked with the porous sintered TCP network. In the above two cases, the open micropores might be useful for drug delivery, as a drug dissolved in a solution could be loaded into the micropores through capillary suction and/ or vacuum infiltration. After the evaporation of the solvent of the drug solution, the drug would be deposited or adsorbed onto the internal surfaces of the micropores. Once the drug would be loaded into the porous ceramic body, subsequent firing should be avoided, but infiltration or encapsulation with a biodegradable polymer could be done using a polymer solution dipping method. In addition, some drugs could also be mixed into the polymer solution before the infiltration of the polymer would be carried out.

Fig. 8 indicates the low compressive strength (< 1MPa) of the as-sintered porous TCP ceramic body. The curve shows a brittle fracture of the porous structure, followed by compaction of the debris. Fig. 9 shows a stress-strain curve of the sintered porous TCP/HA interpenetrating composite. A compressive strength of 8.4 MPa and a Young's modulus of 0.5 GPa could be extracted from the curve. On the other hand, Fig. 10 shows a stress-strain curve of the sintered porous TCP/HA interpenetrating composite that was infiltrated with the PLGA. An increased compressive strength (32MPa) and a higher Young's modulus (0.84 GPa) were obtained in this case and the mechanical data are comparable to those of spongy bones, which show a compressive strength of 2-12 MPa and a Young's modulus of 0.05-0.6 GPa, respectively. While the mechanical properties of the complex composites were still not sufficient for load-bearing applications, the infiltration of the biodegradable PLGA phase did make the complex composites tougher, as evidenced by the fact that the composites could tolerate cutting with a diamond wheel saw and did not shatter when thrown on

the ground. This mechanical integrity would provide convenience for the fabrication and implantation of implants of the composites.

Since the porous TCP network and the porous HA network were loosely integrated due to the gaps or pores present, the interpenetrating TCP/HA composite would have a compressive strength that can be regarded as a combination of the compressive strength of the porous TCP network and the compressive strength of the porous HA network. Additional experiment indicated that the porous TCP bodies identical to the porous TCP networks in the interpenetrating composites had a compressive strength value of about 1 MPa. Thus the remaining compressive strength value of about 7 MPa must be due to the porous HA networks. This level of compressive strength seems reasonable according to various studies on various macroporous/ macroporous calcium phosphate ceramics [4, 5]. When the PLGA was infiltrated into the porous interpenetrating TCP/HA composite, the mechanical properties were much improved due to the reduced porosity and the enhanced interfacial bonding between the porous TCP network and the porous CPC network due to the interlocking ductile PLGA phase.

3. Conclusions

The present study has led to the following conclusions:

1. Porous TCP bodies of the beta and alpha-TCP phases could be produced by coating the 700 μm pore-sized PU foams with the hydrolysable alpha-TCP slurry, followed by sintering at 1300 °C.
2. The obtained porous TCP bodies had low compressive strengths of \sim 1MPa, which could be improved by using CPC as a filler, leading to \sim 8 MPa in the compressive strength.
3. The complex TCP/HA/PLGA interpenetrating composites showed even higher compressive strengths (\sim 30 MPa), indicating the importance of the ductile PLGA phase in the composites.
4. Although the interpenetrating composites were still insufficient for high load-bearing applications, they would lead to different biodegradation rates in vivo and could maintain their shape for bone tissue ingrowth.

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Figure captions

Fig. 1 Stereo-optical micrograph showing the pores and the struts of the polyurethane foam.

Fig. 2 Stereo-optical micrograph showing the pores and the struts of the sintered porous TCP sample.

Fig. 3 XRD pattern showing the presence of both α -TCP and β -TCP phases in the sintered porous TCP sample.

Fig. 4 SEM micrograph of a fracture surface of the set CPC in the porous TCP showing the presence of micropores, residual particles, and hydroxyapatite whiskers.

Fig. 5 SEM micrograph of a fracture surface of the sintered CPC filler in the sintered porous TCP sample.

Fig. 6 SEM micrograph showing the triangular voids in the TCP struts, the gaps between the TCP network and the sintered CPC network, and micropores in the sintered CPC network.

Fig. 7 SEM micrograph showing the PLGA phase (arrowed) infiltrated in the sintered porous CPC.

Fig. 8 Compressive stress-strain curve of the sintered porous TCP sample.

Fig. 9 Compressive stress-strain curve of the sintered porous TCP/HA interpenetrating composite.

Fig. 10 Compressive stress-strain curve of the sintered porous TCP/HA interpenetrating composite infiltrated with PLGA.

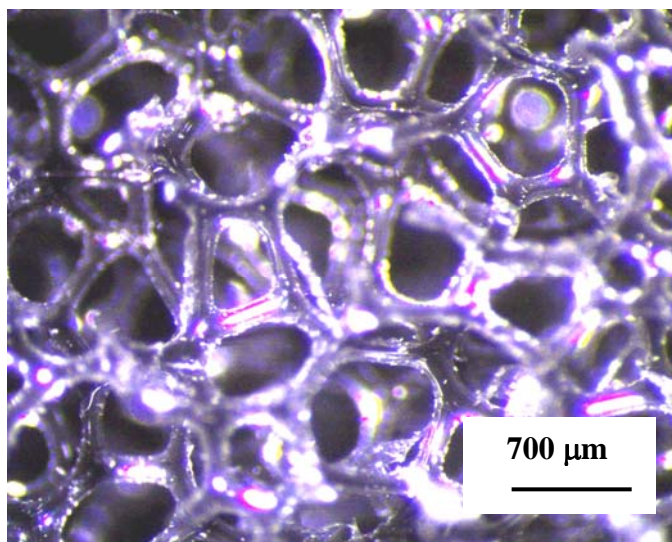


Fig. 1 Stereo-optical micrograph showing the pores and the struts of the polyurethane foam.

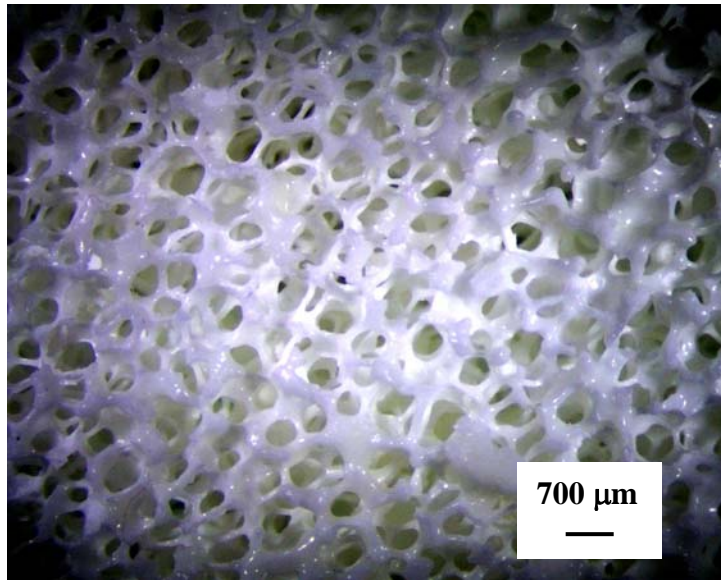


Fig. 2 Stereo-optical micrograph showing the pores and the struts of the sintered porous TCP sample.

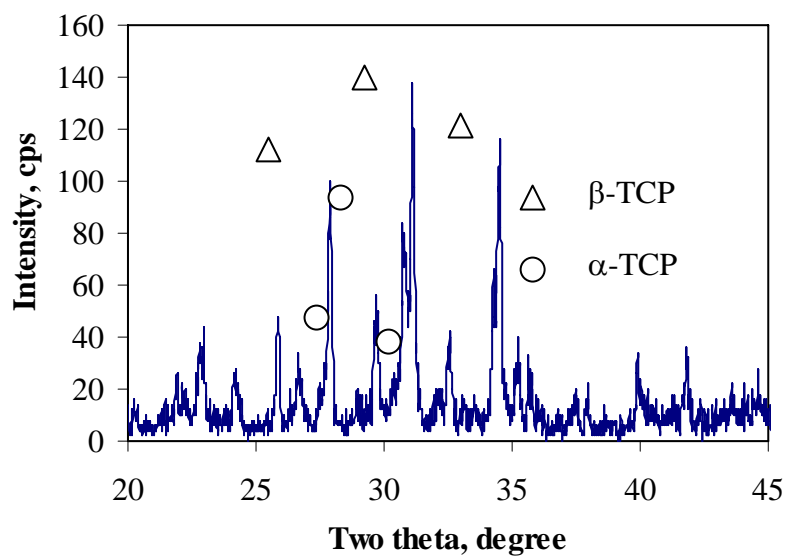


Fig. 3 XRD pattern showing the presence of both α -TCP and β -TCP phases in the sintered porous TCP sample.

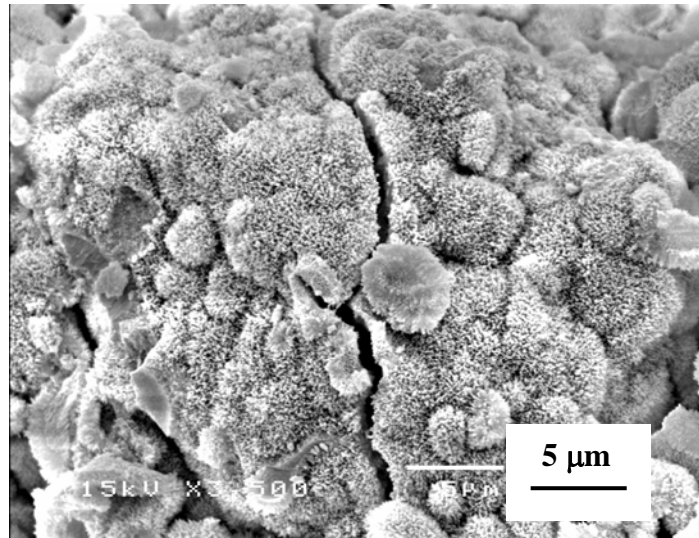


Fig. 4 SEM micrograph of a fracture surface of the set CPC in the porous TCP showing the presence of micropores, residual particles, and hydroxyapatite whiskers.

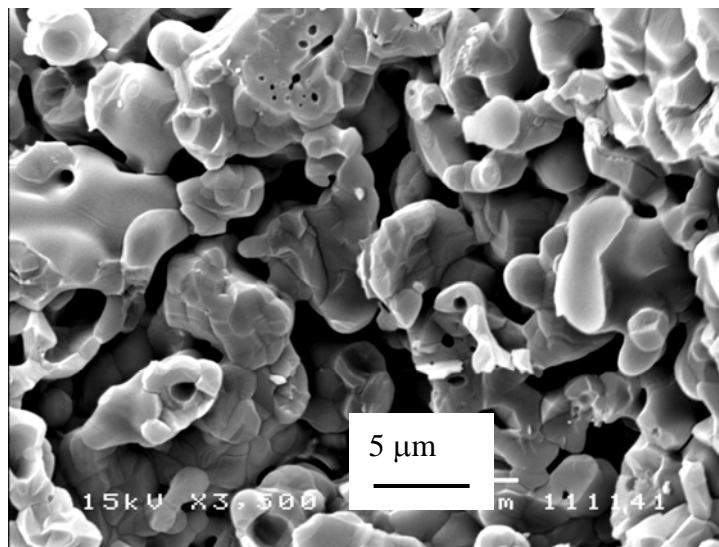


Fig. 5 SEM micrograph of a fracture surface of the sintered CPC filler in the sintered porous TCP sample.

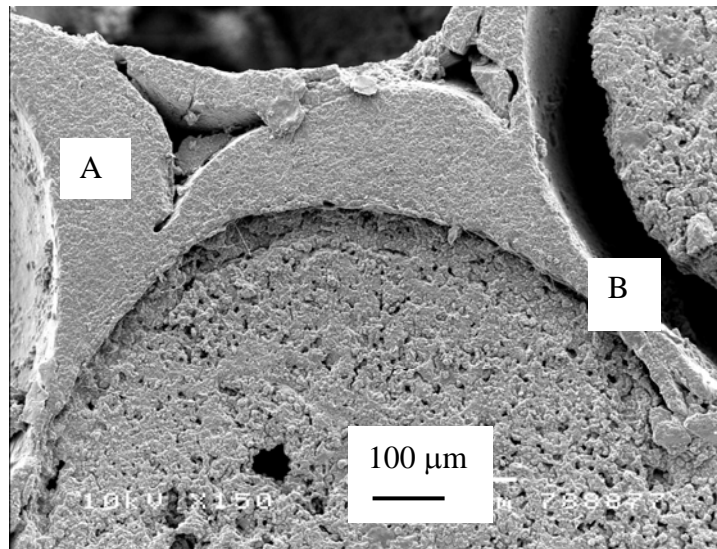


Fig. 6 SEM micrograph showing the triangular voids (A) in the TCP struts, the gaps (B) between the TCP network and the sintered CPC network, and micropores in the sintered CPC network.

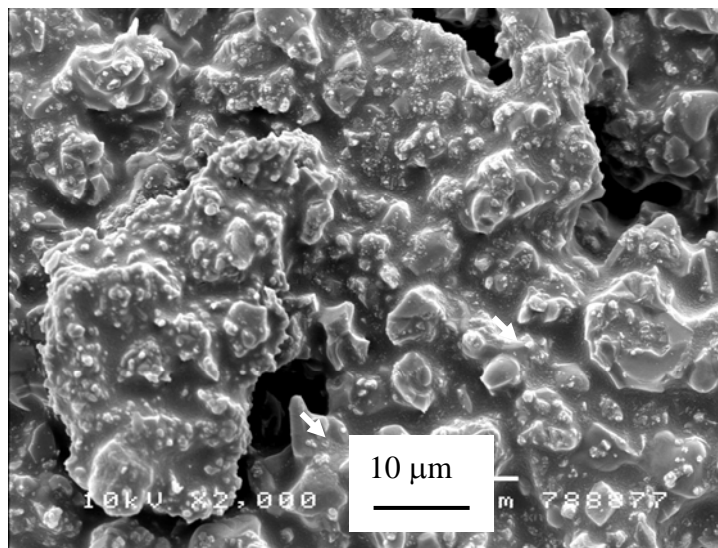


Fig. 7 SEM micrograph showing the PLGA phase (arrowed) infiltrated in the sintered porous CPC.

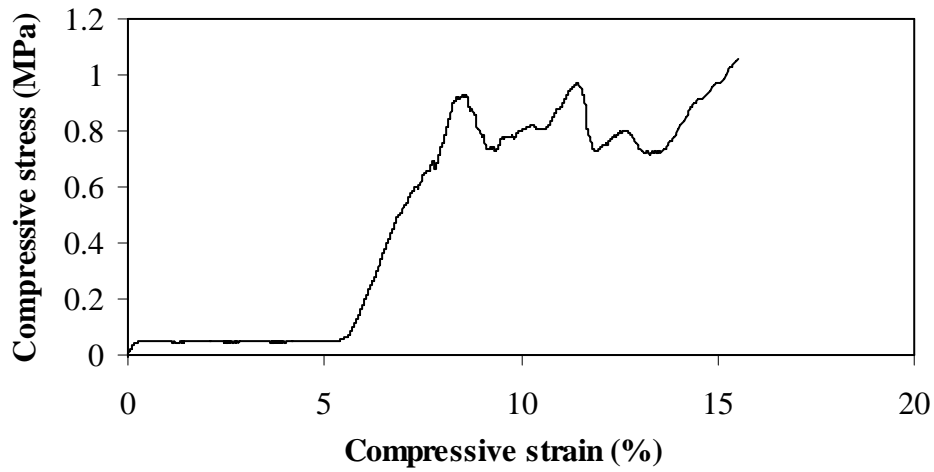


Fig. 8 Compressive stress-strain curve of the sintered porous TCP sample.

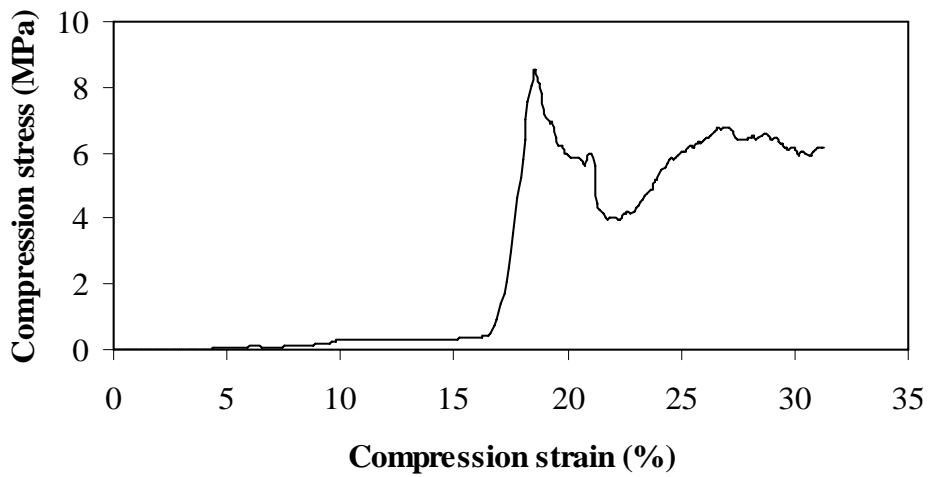


Fig. 9 Compressive stress-strain curve of the sintered porous TCP/HA interpenetrating composite.

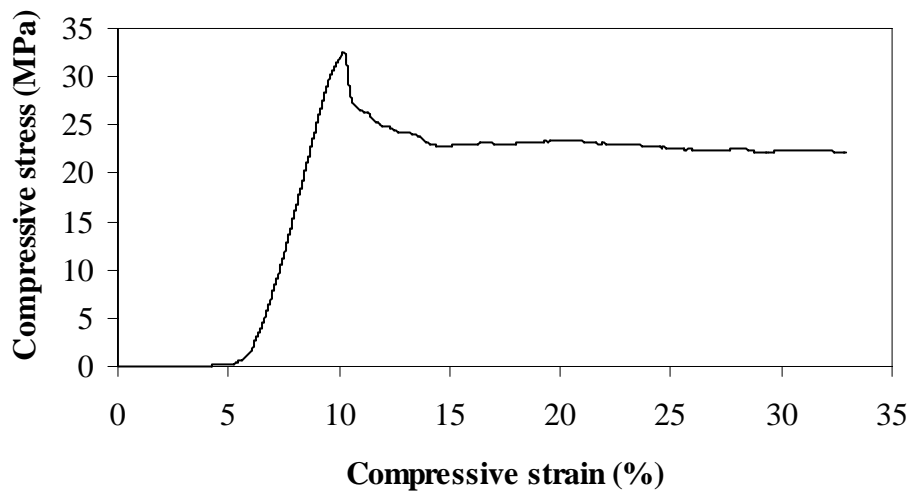


Fig. 10 Compressive stress-strain curve of the sintered porous TCP/HA interpenetrating composite infiltrated with PLGA.