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Mechanical and biological properties of hydroxyapatite/tricalcium phosphate scaffolds coated with poly(lactic-co-glycolic acid)

Xigeng Miao*, Dawn Meifang Tan, Jian Li, Yin Xiao, Ross Crawford

Institute of Health and Biomedical Innovation and School of Engineering Systems, Queensland University of Technology, Corner of Blamey Street & Musk Avenue, Kelvin Grove, QLD 4059, Australia.

*Corresponding author. Tel.: +61 7 3138 6237.
E-mail address: x.miao@qut.edu.au (X. Miao).

Abstract

Regeneration of bone, cartilage, and osteochondral tissues by tissue engineering has attracted intense attention due to its potential advantages over the traditional replacement of tissues with synthetic implants. Nevertheless, there is still a dearth of ideal or suitable scaffolds based on porous biomaterials and the present study was to develop and evaluate a useful porous composite scaffold system. Here, hydroxyapatite (HA)/ tricalcium phosphate (TCP) scaffolds (average pore size: 500 μm; porosity: 87%) were prepared by a polyurethane (PU) foam replica method, followed by modification with infiltration and coating of poly(lactic-co-glycolic acid) (PLGA). The thermal shock resistance of the composite scaffolds was evaluated by measuring the compressive strength before and after quenching or freezing treatment. The porous structure (in terms of pore size, porosity and pore interconnectivity) of the composite scaffolds were examined. The penetration of the bone marrow stromal stem cells (BMSCs) into the scaffolds and the attachment of the cells onto the scaffolds were also investigated. It was shown that the PLGA incorporation in the HA/TCP scaffolds significantly increased the compressive strength up to 660 kPa and the residual compressive strength after the freezing treatment decreased to 160 kPa, which was however sufficient enough for the scaffolds to withstand subsequent cell culture procedures and a freezing drying process. On the other hand, the PLGA coating on the strut surfaces of the scaffolds was rather thin (< 5 μm) and apparently porous, maintaining the high open porosity of the HA/TCP scaffolds, resulting in desirable migration and attachment of the bone marrow stromal stem cells, although a thicker PLGA coating would have imparted a higher compressive strength of the PLGA-coated porous HA/TCP composite scaffolds.

Keywords: hydroxyapatite; poly(lactic-co-glycolic acid); porosity; quenching; cell attachment.

1. Introduction

Bone defects are conventionally treated by replacement with bone grafts and synthetic bone filling materials. However, tissue engineering approach, which stresses the tissue regeneration rather than tissue replacement has become popular recently. Porous biomaterials (also called scaffolds) used

in tissue engineering allow cells to attach, proliferate, differentiate, and eventually become specific tissue(s). While scaffolds are expected to disappear after implantation in vivo, a certain level of mechanical strength is required for the scaffolds to withstand a certain level of physiological loading. The open porosity of the porous scaffolds is also important for the tissue's development from cells, where cell culture medium and growth factors can be easily accessible though the open pores. On the basis of previous studies [1-8] on the preparation and characterisation of scaffolds for tissue engineering, open porosity, compressive strength, and feasibility for cell migration have been realized to be the main criteria for good scaffolds.
For bone tissue engineering, the design of scaffolds should mimic the structure and properties of the bone extra-cellular matrices. Because bone consists of a porous composite of interpenetrating phases of hydroxyapatite and collagen, the scaffolds for bone regeneration should be similarly porous composites with interpenetrating ceramic and polymer phases. Porous hydroxyapatite/ tricalcium phosphate (HA/TCP) composite was thought to be ideal for the ceramic phase, as it is known for its excellent osteoconductivity and in some cases even osteoinductivity [9]. On the other hand, poly(lactic-co-glycolic) acid (PLGA) polymer was selected for the polymer phase, as it is FDA approved biodegradable polymer with some degree of ductility and good biocompatibility [10, 11]. Thus the dual porous HA/TCP/PLGA composite scaffolds should minimize the problems confronted either with the porous sole PLGA polymer (low compressive strength) or with the porous sole HA/TCP ceramics (mechanically weak and brittle). In fact, porous calcium phosphate scaffolds [2, 3, 5] have been toughed by either polymer coating on the struts, or polymer infiltration into the struts (if with open micropores), or both.

Most studies on scaffolds have dealt with the mechanical properties and cell or tissue ingrowth properties. The thermal shock resistance of the ceramic-polymer composite scaffolds has been largely forgotten. The reason could be that tissue engineering scaffolds are less likely to be subjected to a temperature higher than that of the body temperature of 37 ^{o}C, unlike the case of conventional engineering ceramics and composites, where thermal shock resistance is an important thermomechanical property. However, it is justifiable to evaluate the thermal shock resistance of the HA/TCP/PLGA composite scaffolds. Firstly, the porous composite scaffolds are often subjected to a low temperature process with the lowest temperature being that of liquid nitrogen. For example, when cells are seeded onto the scaffolds and go through the cell culture, the engineered cells/ tissues may need to be stored in a freezer or even in a liquid nitrogen tank. Secondly, there is an active research area of bone-cartilage (osteochondral) tissue engineering, which requires the preparation of bilayered composite scaffolds, where a porous polymer scaffold will need to be attached onto a porous ceramic-polymer composite scaffold by a low temperature process, such as the thermally induced phase separation method [6]. Thus, the resistance of the scaffolds to a low temperature thermal shock has been identified as another requirement for tissue engineering scaffolds.

Successful tissue engineering also requires the uniform seeding of cells in scaffolds and cell seeding should be followed by cell attachment, proliferation and differentiation, and secretion of extracellular matrices. No matter whether a dynamic cell culture such as using a perfusion bioreactor or a conventional static cell culture is used, well-interconnected pores are prime requirements for the scaffolds. Tissues have been often observed to develop preferentially around the peripheral of the scaffolds both in vitro and in vivo, which can be due to the poor circulation of nutrients in the central part of the scaffold, or due to the poor pore interconnectivity across the scaffold. Thus, it is important to evaluate the cell penetration and the cell attachment on the scaffolds before other processes of the cell to tissue development are studied and it is regretted that previously such studies were not attempted with these calcium phosphate/ PLGA composite scaffolds.

2. Materials and methods

2.1. Preparation of composite scaffolds

2.1.1. Coating PU foams with a ceramic slurry

The ceramic slurry was prepared by mixing 160g of HA (average particle size 2 μm) with 40g of β-TCP (average particle size 2.5 μm) using a ball mill under a wet condition for 2 hours. The resulting paste was then dried at 100^{o}C for 24 hours, followed by heat treatment at 900^{o}C for 2.5 hours with cooling and heating rates set at 5^{o}C/min. The calcined ceramic pieces were milled again for an hour before 125ml of distilled water was added. The ceramic-water mixture was further milled for another 24 hours. Then 1ml of 25% ammonium salt of polymethacrylic acid (NH4PMAA) solution (Darvan C, R. T.Vanderbilt) was added to the ceramic paste, followed by mixing for about 30 minutes. Finally 1ml of 2wt% PVA (polyvinyl alcohol) solution was added to produce the final slurry. Then the polyurethane (PU) foams were dipped into the slurry, followed by gently squeezing the foams several times to allow the slurry to penetrate the foams and the excess slurry to be squeezed out. Compressed air through an air gun was used to avoid the blockage of pores. The ceramic slurry coated PU foams were left to dry at room temperature for at least 24 hours.

2.1.2. Sintering of the ceramic coated PU foams

The ceramic slurry coated PU foams were fired in an electric furnace (Modutemp Furnace) using a four stage schedule, including (1) heating from room temperature to 600^{o}C at a rate of 1^{o}C/minute to burn out the PU foam; (2) the temperature was raised from 600^{o}C to 1200^{o}C at a rate of 5^{o}C/minute; (3) the temperature was held at 1200^{o}C for 4 hours to sinter the ceramic; (4) cooling the furnace down to room temperature at a rate of 5^{o}C/minute. The HA/TCP scaffolds were taken out after the furnace had cooled down. Each sample was weighed and kept in a desiccator.

2.1.3. Coating the sintered HA/TCP scaffolds with poly-lactic-co-glycolic acid (PLGA)

PLGA pellets (Sigma-Aldrich; PLA:PGA = 75:25; molecular weight = 90,000 - 126,000) were dissolved in dichloromethane (CH_2Cl_2) solvent, such that every 4g of PLGA was dissolved in 10ml of dichloromethane. The sintered HA/TCP scaffolds with small dimensions of about 10x10x10 mm^3 were then immersed into the PLGA solution for more than 30 seconds each to allow for complete infiltration. The soaked scaffolds were then placed in a centrifuge running at 350 rpm for 1 minute in order to remove the excess PLGA solution away from the scaffolds. The scaffolds were then taken out of the centrifuge tubes and left to dry in a fume hood overnight, and were weighed individually before being stored in a desiccator.

2.1.4. Quenching the scaffolds for evaluating the thermal shock resistance

The HA/TCP scaffolds with and without PLGA coating were quenched by placing the scaffolds in a -80^{o}C freezer for 30 minutes and then placing them at room temperature for 30 minutes. This was repeated for 3 times. Compression testing was then performed on the quenched scaffolds as described in section 2.2.3.

2.2. Sample characterization

2.2.1. Total porosity of the HA/TCP scaffolds without the PLGA coating

When the HA/TCP scaffolds were not infiltrated and/ or coated with any PLGA, the pores responsible for the total porosity were the macropores between the struts and the micropores within the struts. The total porosity of the sintered HA/TCP scaffolds was determined by using the following equations: bulk density (ρ_B) = weight of the sample divided by volume of the sample; theoretical density of the HA/TCP composite (ρ_0) = 3.16g/cm^2; relative density (R.D.) = (ρ_B / ρ_0) x 100%; and total porosity = 100% - R.D. The dimensions and the weight of each sample were

measured and recorded using a vernier calliper and an electronic balance, respectively. Three identical specimens were used to determine the total porosity.

2.2.2. Structural observation

The scaffolds were sectioned with a knife edge along the sagittal and the transverse planes to give the best overview of the porous structure to confirm the pore interconnectivity and homogeneity. The scaffold samples were mounted onto aluminium stubs with a carbon tape and were coated with a gold film on a sputter coater (BioRad SC500). The porous structure of the scaffolds was then examined using a scanning electron microscope (FEI QUANTA 200) under the acceleration voltage of 10 kV.

2.2.3. Compressive testing for scaffolds with and without quenching

The compressive strength and the compressive modulus of each of the scaffolds were measured using a Hounsfield testing machine (Model: H10K/M527). The dimensions of each sample were about 10x10x10 mm^3. The scaffolds were reasonably homogeneous and thus the orientation of the cut surfaces was not specially recorded. Rubber pads were placed on the top and the bottom surfaces of each sample to ensure an evenly distributed load on the sample. The cross-head loading speed was set at 0.5mm/min. Seven to nine identical specimens for each sample group were used for the compressive testing.

2.2.4. Fracture surface analysis

Various types of scaffolds subjected to the compression testing were collected and the fracture surfaces were examined by scanning electron microscopy as described in Section 2.2.2.

2.3. In vitro evaluation by cell culture

2.3.1. Sample sterilization

The scaffolds with dimensions of 5x5x5 mm^3 were decontaminated by soaking them three times in 70% ethanol for 15 minutes each, then rinsed three times with potassium phosphate buffer solution (PBS) for 15 minutes, and finally left to dry overnight in a sterilized hood. The sterilized scaffolds were sealed in a 24-well plate and kept in a fridge at 4^{o}C for later use.

2.3.2. Cell seeding and culture

Bone marrow stromal stem cells (BMSCs) maintained in our labs were used for the study. In brief, expanded BMSCs pellets by subculture were resuspended in a growth medium of the Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and 1% antibiotic–antimycotic. The cell suspension had a final concentration of 2.4 x 10^6 cells/ml. Then 100 μl of the cell suspension was applied to each of the scaffolds. After 1 hour of incubation, the wells were filled with the culture medium and incubated at 37 ^{o}C for 1 day to observe the initial cell attachment.

2.3.3. Observation of cell penetration and attachment

The growth medium in each well was pipetted out, and immediately replaced with PBS. The rinsing was repeated three times for each sample, and then the scaffolds were fixed with a 3% glutaraldehyde solution. The scaffolds were then processed by soaking them in an osmium tetroxide solution for one hour, then dehydrated through a series of ethanol solutions with graded concentrations, and followed by two changes of 100% amyl acetate for 15 minutes each. The scaffolds were then dried using a supercritical point dryer (Denton Vacuum critical point dryer) before observation using scanning electron microscopy, as shown in Section 2.2.2.

3. Results and discussion

3.1. Porous structures of HA/TCP scaffolds with and without the PLGA coating

The HA/TCP composite porous scaffolds were fabricated by replicating the porous structure of the polyurethane (PU) foams. The scaffolds, like the PU foams, had a highly interconnected structure with open macropores (Fig. 1(B) and Fig. 1(C)) of the pore sizes ranging from 300μm to 700μm. The average pore size of the macropores of the scaffolds was about 500μm, estimated by the macropore sizes taken from the SEM micrographs. The total porosity of the HA/TCP scaffolds was determined to be approximately 87%. The macro-porosity of the scaffolds after infiltration and coating with the PLGA was slightly decreased due to the observed thin polymer coating present on the strut surfaces; the macropores were not made significantly smaller by the polymer coating. In a similar study [12], Chen et al. had shown about 2.2 % reduction of the macroporosity as a result of the polymer coating on the ceramic scaffolds. However, the micropores in the struts were infiltrated with the polymer; the weight gain after the PLGA infiltration and coating compared to the bare HA/TCP scaffolds indicated that the PLGA corresponded to ~23 wt% of the PLGA-coated HA/TCP scaffolds, which was a significant amount and would have a significant effect on the strength and the toughness of the composite struts.

It was observed that the HA/TCP scaffolds had more crack-like defects on and within the ceramic struts than those coated with PLGA. The crack-like defects (Fig. 1 (C)) of the HA/TCP scaffolds resulted from the burn-off of the PU struts that were initially coated with the HA/TCP ceramic particles. However, the crack-like defects in the HA/TCP struts could be filled with the polymer after the polymer solution dipping plus centrifugation. Our previous studies [2, 5] had demonstrated the removal of the crack-like defects due to the PLGA polymer phase filling into the open voids. The struts of the HA/TCP scaffolds showed a smooth and sintered surface that revealed ceramic grains with sizes between 0.5μm and 2μm. On the other hand, the strut surfaces of the PLGA coated HA/TCP scaffolds had a thin PLGA layer with a crater-like structure, which was left behind from the burst bubbles or caused by the evaporation of the chloroform solvent used to dissolve the PLGA. The thin and porous PLGA coating was advantageous as the bioactive HA/TCP struts were not fully covered or shielded by the non-bioactive PLGA.

3.2. Compressive strengths of HA/TCP scaffolds with and without the PLGA coating

Compression testing was performed on both the HA/TCP scaffolds and the HA/TCP scaffolds coated with the PLGA polymer. It was found that the HA/TCP scaffolds were able to withstand a maximum compressive stress between 0.05 MPa and 0.07 MPa (Fig. 2(A)), while those coated with PLGA showed a compressive strength in the range of 0.62 MPa and 0.79 MPa (Fig. 2(B)). Obviously the compressive strength of the scaffolds coated with PLGA was substantially higher (about 10 folds) than those without the PLGA polymer coating. As a general trend, the compressive strength of a scaffold depends on its macro-porosity, macro-pore size, macro-pore geometry, and the strength of the struts. In the present study, the ~ 2 % macroporosity reduction due to the polymer coating could not obviously influence the macro-porous structural parameters. However, the micropores of the HA/TCP scaffolds were filled with PLGA, resulting in a significant amount of PLGA (i.e. 23 wt%) in the whole PLGA-coated HA/TCP scaffolds. In other words, the open micro-pores and/or crack-like defects in the struts of the scaffolds were infiltrated with the PLGA phase, which made the original weak and brittle ceramic struts into stronger and tougher ceramic-polymer composite struts. The formation of the composite struts could explain the fact that the seemly thin and porous polymer coating could improve the compressive strength of the ceramic scaffolds by more than 10 folds. Similar results were also observed by Chen et al. in a study on the PDLLA-coated bioactive glass scaffolds [12]. In addition, the compressive modulus of the HA/TCP scaffolds ranged from 2.21MPa to 2.99MPa and those coated with PLGA were in the range of 14.08MPa and 19.29MPa.

Polymer infiltration into and coating onto the struts of the HA/TCP scaffolds could reduce the defect sizes of the ceramic struts and result in ceramic-polymer composite struts, leading to the increased compressive strengths of the scaffolds. In general, ceramics can be strengthened and toughened by the incorporation of a ductile polymer or a metallic phase. In the present study, the infiltration of the polymer into the open porous ceramic struts led to the formation of an interpenetrating ceramic-polymer composite. The strengthening and toughening mechanism in an interpenetrating composite was studied by Pezzotti et al. [13]; they proposed a micron-scale crack-bridging mechanism evident by the polymer ligaments that were stretched upon crack opening along the crack wake. Yet, the compressive strength and the compressive modulus of the HA/TCP scaffolds coated with the PLGA were still lower than those of human cancellous bone. This was due to the high porosities and the large pore sizes of the scaffolds. However, it is believed that the compressive strength of the scaffolds can be improved by applying a thicker PLGA coating, which could be realized by using repeated polymer solution dipping and centrifugation. A vacuum infiltration process as shown in Peroglio et al.'s study [14] could be additionally used to facilitate the filling of the crack-like defects of the HA/TCP ceramic struts. However, a thick PLGA coating has the problem of lack of bioactivity, which was why bioactive glass particles were loaded in the coating in our previous studies [2, 5]. An optimised centrifugation process may also be needed to maintain the high degree of the open macroporosity for the subsequent cell penetration in the macropores.

3.3. Compressive strengths of the quenched HA/TCP scaffolds with and without the PLGA coating

The resistance of the HA/TCP composite scaffolds with and without the PLGA coating to the quenching operation or process was also evaluated. It was found that the compressive strength and the compressive modulus of the HA/TCP scaffolds with and without the polymer coating were all badly affected by the quenching process. Specifically, the compressive strength of the HA/TCP scaffolds after quenching was in the range of 0.02 MPa and 0.03 MPa (Fig. 3(A)), and the compressive strength for the HA/TCP scaffolds coated with PLGA was in the range of 0.10 MPa and 0.21 MPa (Fig. 3(B)). These compressive strength results were much lower than those without the quenching (Fig. 2). In other words, the compressive strength of the HA/TCP scaffolds had halved after quenching, while those of the coated scaffolds had dropped by about four times (Table 1). Fortunately, the porous HA/TCP scaffolds infiltrated and partially coated with the PLGA had sufficient mechanical strength for scaffold shaping and handling, in spite of the high porosities of the scaffolds and the harsh treatment of quenching the scaffolds.

3.4. Fracture surface morphologies with and without quenching

Different fracture surface morphologies were observed for the PLGA-coated HA/TCP scaffolds with and without quenching. For the case of without quenching, the PLGA coating was observed to be stretched slightly before breaking off and the PLGA coating was featured with a ductile fracture surface due to some degree of ductility of PLGA. On the other hand, flat fracture surface of the ceramic component was observed and it indicated the brittle nature of the ceramic material. Most importantly, the remaining PLGA coating was still intact after fracture and attached closely to the ceramic struts (Fig. 4 (A)), which indicated the good interfacial bonding the PLGA coating. For the case of after quenching, the PLGA coating on the scaffolds was observed to detach from the ceramic struts (Fig. 4 (B)). The PLGA coating was also observed to have been stretched before breaking off and the ceramic struts were still rather brittle.

The interfacial debonding was believed to be caused by the different thermal shrinkage of the PLGA coating and the ceramic struts during quenching. PLGA should have much higher thermal expansion coefficient than that of the HA/TCP composite in the low temperature range (from room

temperature to liquid nitrogen temperature). Thus there must exist a high level of thermal stresses to cause the interfacial separation. However, accurate analysis of the debonding is not possible due to the unknown data of the thermal expansion coefficients and the complex structure of the PLGA-coated HA/TCP scaffolds. The debonding of the PLGA coating could also cause the decrease of the compressive strength of the PLGA-coated HA/TCP composite scaffolds, as it is well known that interfacial bonding plays an important role in the strengthening and toughening of the ceramic-polymer composite systems [15, 16].

3.5. Initial cell penetration into and attachment onto the PLGA-coated HA/TCP scaffolds

The bone marrow stromal stem cells were seeded into the scaffolds by dropping the cell suspension. As a result, the cells were not homogeneously seeded across the surface of the scaffolds. The penetration of the cells into the scaffolds was evaluated using the cross-sections of the scaffolds. SEM observation (Fig. 5) revealed that the cells could penetrate into a depth of 4.5 mm, yet the cell density along the depth direction was not homogeneous; the cells were most plentiful on the surface and the cell density gradually decreased with the depth. The significant cell penetration depth was due to the highly interconnected pores of the HA/TCP scaffolds coated with PLGA. The cell seeding homogeneity can be improved by using a dynamic cell seeding method such as the spinner flask stirring culture method.

The bone marrow stromal cells were observed to attach well onto the strut surfaces of the PLGA-coated (actually partially coated) HA/TCP scaffolds; the cells could attach well to the exposed ceramic HA/TCP surface and the PLGA coating surface (Fig. 6), indicating the biocompatibility of both the ceramic phases and the PLGA phase. While the PLGA-coated HA/TCP scaffolds showed adequate cell penetration and attachment, the stem cells' proliferation and differentiation was not examined. It is believed that the cells' proliferation and differentiation is controlled by the struts' surface chemistry, the surface morphology, and potentially the presence of a growth factor. Thus, the PLGA-coated HA/TCP scaffolds can be further modified to increase not only the mechanical properties but also the biological properties.

5. Conclusions

In the present study, the hydroxyapatite (HA)\ tricalcium phosphate (TCP) composite scaffolds were modified with poly(lactic-co-glycolic acid) (PLGA). The low temperature thermal shock resistance and the initial cell penetration and the attachment were evaluated. The present study led to the following conclusions:

1. The pore size (~500 μm) and the pore interconnectivity of the fabricated HA/TCP scaffolds closely mimicked those of the initial polyurethane foams used. The average total porosity of the scaffolds was about 87%. PLGA solution dipping followed by centrifugation resulted in a thin layer of porous PLGA coating, which did not impair the open porous structure but imparted the coated scaffolds with mechanical integrity.

2. The HA/TCP scaffolds modified with the PLGA coating had a compressive strength (0.66 MPa) and a compressive modulus (16.85 MPa), which were remarkably higher that those of the bare HA/TCP scaffolds (0.06 MPa and 2.58 MPa, respectively). After quenching testing, the compressive strength and the compressive modulus of the HA/TCP scaffolds modified with PLGA were decreased to 0.16 MPa and 6.65 MPa, respectively, but the quenched scaffolds could still tolerate the handling actions for cell culture procedure.

3. The highly porous and well interconnected scaffolds enabled the bone marrow stromal stem cells to penetrate through a depth of 5mm. The seeded bone marrow stromal stem cells showed good initial attachment onto both the HA/TCP biphasic ceramic and the PLGA polymer of the scaffolds.

The PLGA-coated HA/TCP scaffolds in their own right may be useful for bone tissue engineering involving low or no load bearing applications. The monolithic composite scaffolds could also be useful for integrating with a sole polymer scaffold layer, so that the formed bilayered scaffolds could be useful for osteochondral tissue engineering. Work is being done to modify the bilayered scaffolds and direct the bi-differentiation of bone marrow stromal stem cells for osteochondral tissue engineering.

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Tables

Table 1 Effects of the PLGA coating and the thermal shock treatment on the compressive properties of the HA/TCP scaffolds.

Samples	Thermal shock treatment	Mean compressive strength, kPa	Mean compressive modulus, kPa
HA/TCP scaffolds	No	60 ± 7	258 ± 24
	Yes	30 ± 4	108 ± 12
HA/TCP scaffolds coated with PLGA	No	66 ± 7	168 ± 15
	Yes	16 ± 2	665 ± 50

Figure Legends

Fig. 1 SEM micrographs of the polyurethane foam (A), the cut surface of the HA/TCP scaffold (B), and the polished cross-section of the HA/TCP scaffold showing the crack-like defects (C).

Fig. 2 Three randomly selected stress-strain curves of the HA/TCP scaffolds (A) and the HA/TCP scaffolds coated with PLGA.

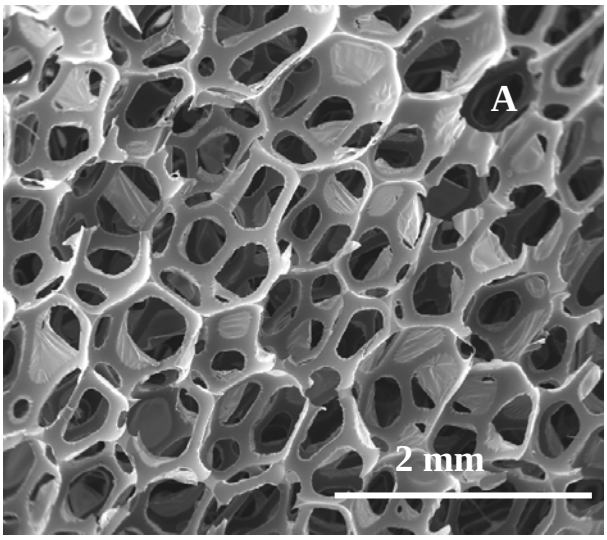
Fig. 3 Three randomly selected stress-strain curves of the HA/TCP scaffolds after thermal shock treatment (i.e. quenching) (A) and the HA/TCP scaffolds coated with PLGA after quenching.

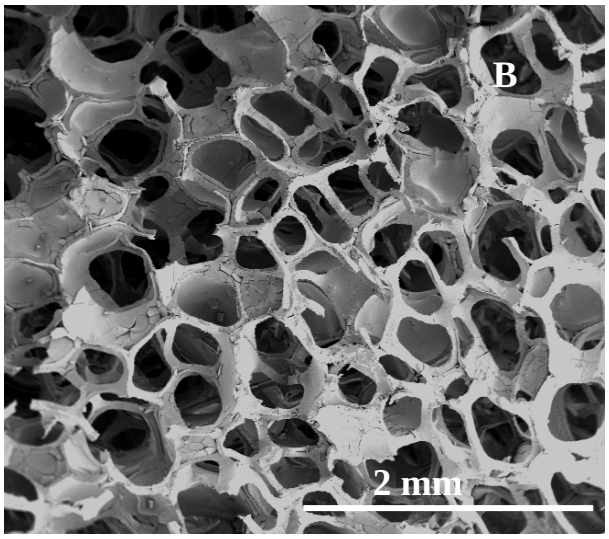
Fig. 4 SEM micrographs of the fracture surfaces of the PLGA-coated HA/TCP scaffolds: (A) without quenching showing a good interfacial bonding and (B) after quenching showing the debonding of an interface.

Fig. 5 SEM micrograph showing the attachment of the stem cells onto the strut located at a depth of approximately 4.5mm from the top surface of a PLGA-coated HA/TCP scaffold.

Fig. 6 SEM micrographs showing the attachment of the stem cells on both the exposed ceramic strut surface (A) and on the PLGA surface coated on the ceramic strut (B) of the PLGA-coated HA/TCP scaffold.

Figures





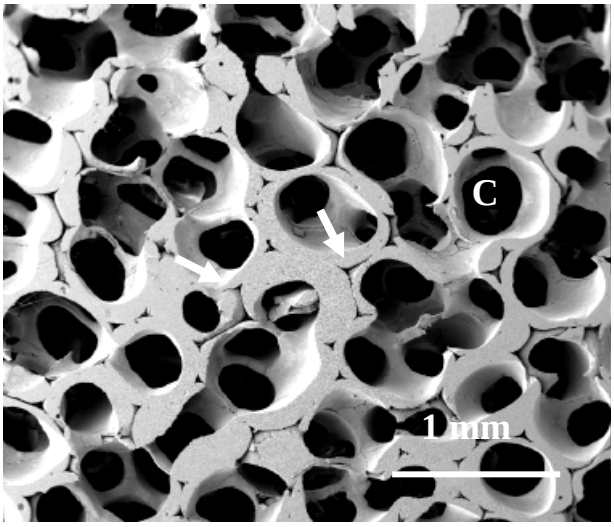

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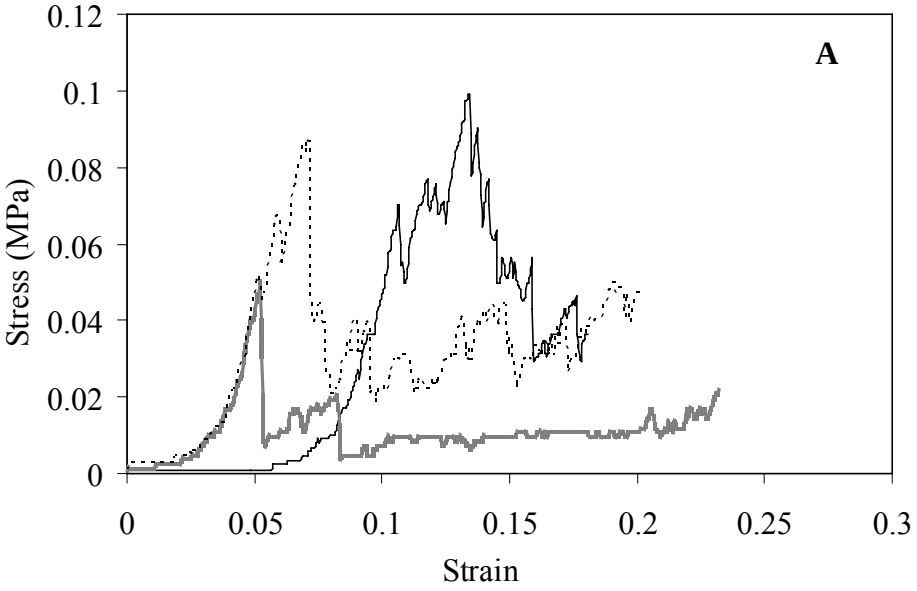
Fig. 1


A
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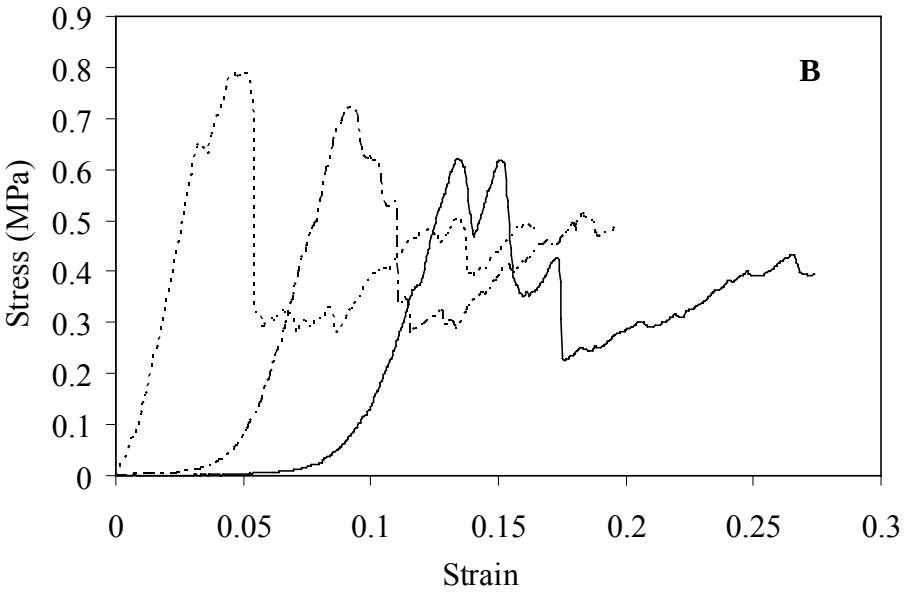
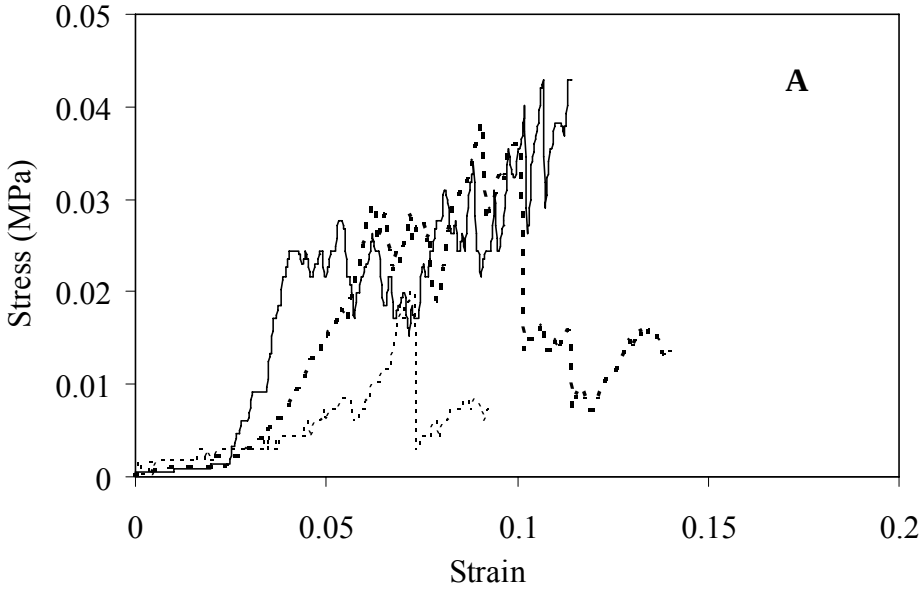

B
Stress (MPa)
Strain
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
0.05
0.15
0.25

Fig. 2


A
Stress (MPa)
Strain
0
0.01
0.02
0.03
0.04
0.05
0.1
0.15
0.2

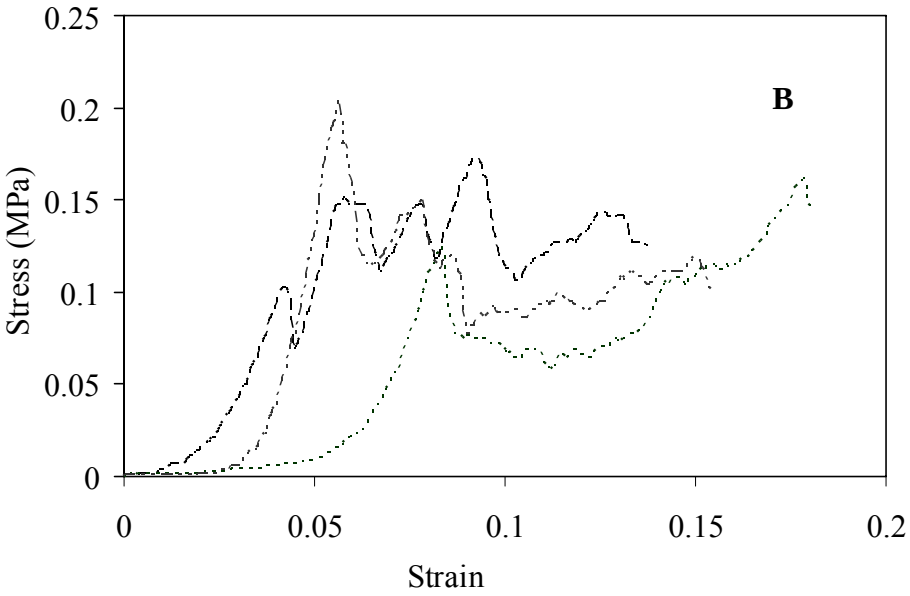
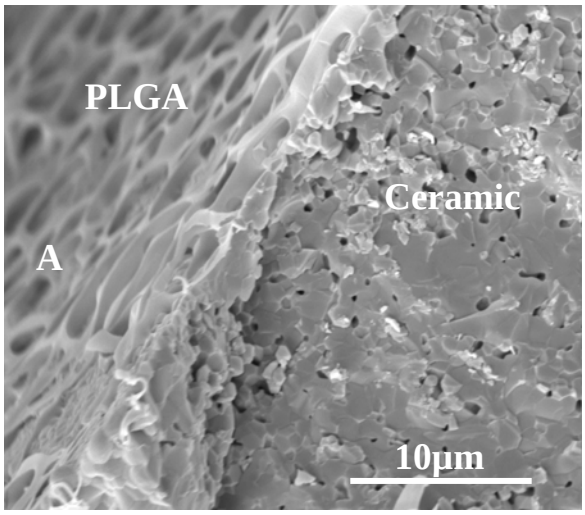

0.25
0.2
0.15
0.1
0.05
0
Stress (MPa)
B
0
0.05
0.1
0.15
0.2
Strain

Fig. 3


PLGA
Ceramic
A
10µm

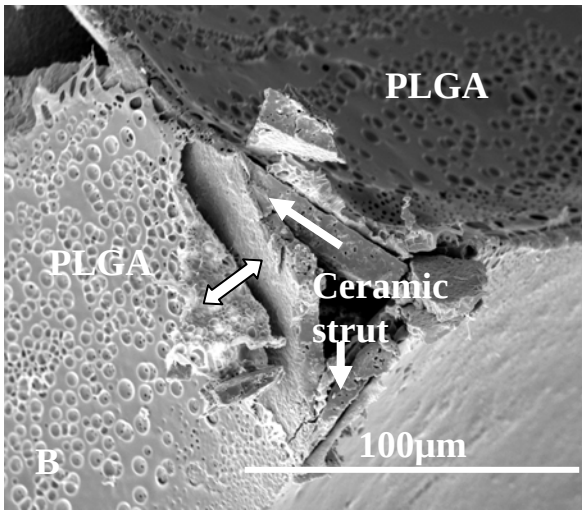


Fig. 4

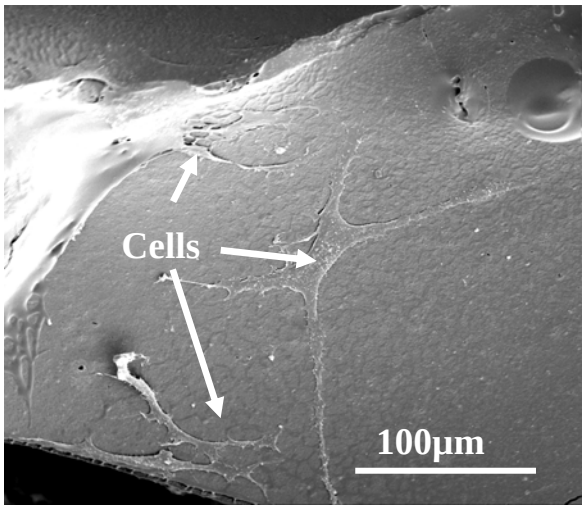


Fig. 5

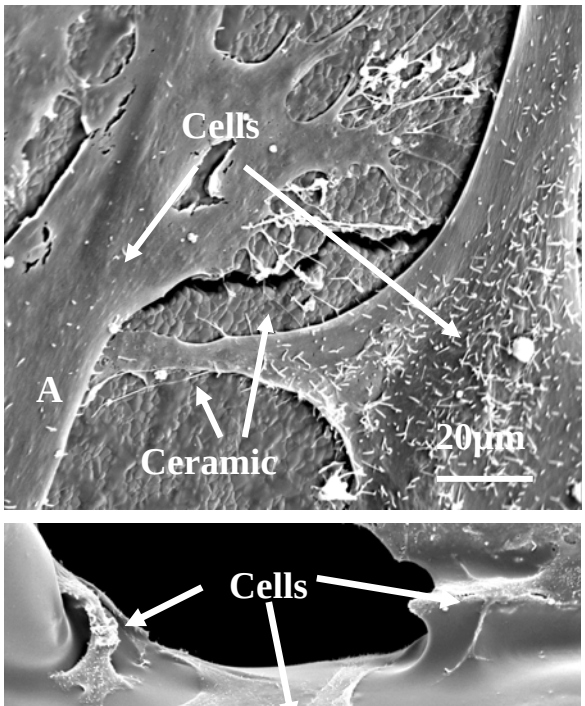


Fig. 6