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Silver Doped Resorbable Tricalcium Phosphate Scaffolds for Bone Graft Applications

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Abstract

Bone graft procedures, in particular maxillofacial repair, account for half of the orthopedic procedures done in the US each year. Infection is a major issue in surgery, and should be of primary concern when engineering biomaterials. Silver is of renewed importance today, as it has the ability to potentiate antibiotics against resistant bacterial strains. In order to reduce long term infection risks, it is necessary for the scaffold to maintain a silver ion release for the length of the healing process. In this study, silver doped porous β -tricalcium phosphate (β -TCP) scaffolds were engineered using liquid porogen based method with the goal of meeting these requirements. Silver was added to the β -TCP at three different dopant levels: 0.5 wt% Ag_2O , 1 wt% Ag_2O and 2 wt% Ag_2O . Immersion in pH 5 acetate buffer over a 60 day period resulted in a total cumulative ion release between 32 and 54 μM for dense control scaffolds, and between 80 and 90 μM for porous scaffolds. Porosity increased the dissolution rate of the scaffolds by a factor of 2. Human Osteoblast cell lines were grown on the scaffolds to measure cytotoxicity and cell proliferation. Porosity increased osteoconduction by doubling the cell growth, and there was no significant cytotoxic effect even for the 2 wt% Ag_2O , as cells were observed on all the samples. Our results showed that silver can be released over a long period without compromising the biocompatibility of the scaffolds.

Keywords

tricalcium phosphate (TCP); porous scaffold; mechanical strength; silver release; cytotoxicity

1. Introduction

The skeleton plays a crucial role in the anatomy of all vertebrates as it is the load bearing structure that the rest of the body is built around. As a result of this, some of the most crippling ailments are afflictions of the musculoskeletal system, which are bone related diseases and traumas. Although very rarely life threatening today, musculoskeletal diseases including accident related trauma and arthritis, are a heavy burden on society. 50 % of adults

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over the age of 18 are affected by them only in the USA [1]. Musculoskeletal (MSK) conditions was found to affect more than 1.7 billion people worldwide according to a study conducted in 2012 [2]. The same study also found MSK conditions as the second greatest cause of disability. This burden is only set to increase, driven up by factors such as an ageing population, more active lifestyles, and increased life expectancy. Data from the center for disease control (CDC) shows that in the US in 2010, there were over 5 million surgical procedures on the musculoskeletal system [3]. Out of these 5 million procedures, the vast majority treated patients over the age of 30. Over 1 million procedures were total joint replacements, and a further 2 million were bone grafts. On top of these grafts and implants, countless screws, plates, and pins are used to stabilize fractures and breaks.

Luckily for us, bone is one of only two biological materials along with the liver that can repair itself when damaged. This does not however eliminate the need for bone grafts and implants, both permanent and temporary. Autografts, where the recipient and donor is the same person, are considered the gold standard for bone repair, however they are limited by donor site availability and morbidity, and can only be used for small defect repair [4]. Allografts, bone grafts from a different individual, and exografts, where the donor is from another species, are limited by recipient autoimmune response as well as disease transmission. The development of synthetic engineered biomaterials has therefore been crucial in satisfying the demands of what has become a \$2.5 billion industry [5]. Advances in biomaterials research have resulted in improvements in performance, durability, and cost of synthetic implants. Bone grafts used in maxillofacial repair constitute half of the orthopedic procedures done in the US each year. These grafts are usually made from synthesized calcium phosphate (CaP) bioceramics, which have the same mineral composition of bone.

This study focuses on tricalcium phosphate (TCP) bioceramics, which have the advantage of being bioresorbable meaning they will naturally dissolve within the body over time [6]. Because the chemistry is similar to that of natural bone [7–8], cells will adhere and proliferate easily on the material. This material property is called osteoconductivity. Osteoconductivity is also increased by the structure of the material, as open pores can provide a three dimensional structure for the bone to regrow as the scaffold dissolves. The advantage of bioresorbable osteoconductive bioceramics is that once the patient is healthy there is no need for removal surgery. However their poor mechanical properties preclude them from being used in load bearing applications. The two most critical material properties for orthopedic grafts are osteoconductivity and osteoinductivity, which describe the material's ability to further promote cell differentiation, proliferation, and formation of bone structures [7]. Two things determine osteoconductivity, both the morphology of the material, in particular porosity, as well as its chemical and bioactive properties. Osteoinductivity is provided by locally delivered growth factors and hormones.

With all these advances persists the oldest, most common, and costliest problem that orthopedic surgeons have to contend with: post-surgery periprosthetic infection [9–12]. The presence of a foreign body reduces the necessary number of bacteria to cause infection by a factor of a thousand [5]. The resulting infections are difficult to diagnose, and end up leading to extra surgeries, and further complications with the patients' health. With the development

of nanotechnology and nano-scale processing techniques, attempts have been made to combat infection in various ways, including nanotube surface coatings for drug delivery, encapsulated antibiotics, and other localized delivery methods [7, 13–14]. Long term infection risks complicate the issue further, as biofilms develop quickly and are very hard to eliminate. These surviving bacteria colonies greatly increase the risk of secondary infection, which can be generalized and life threatening [15]. This long term risk creates the need for a graft material which can retain antibacterial properties over a significant time period. Effective local antibiotic treatment became possible with the advent of nanotechnology, however controlled spatial and temporal delivery, in particular of antibiotics, remains one of the principal challenges for tissue engineers [16]. In addition to well-known antibacterial properties [17–19] silver has proven to be a potentiator for antibiotics, to the point of resensitizing resistant bacteria strains in recent *in vivo* studies [20]. Additionally, silver can easily be used as an elemental dopant [17, 21–22].

The aim of this study was to create a viable porous bone graft that will supply a local concentration of silver ions for the lifetime of the implant, thus diminishing the long term infection risk that exists for patients today. The effects of silver on material processing, physical properties, *in vitro* ion release through dissolution, and cytotoxicity of TCP scaffolds were examined. The role of porosity on the *in vitro* dissolution kinetics and osteoconduction of resorbable β -TCP scaffolds was also investigated.

2. Materials and methods

2.1. Silver doped bone graft fabrication

The TCP used in this study was prepared according to the equation (i) from precursors calcium phosphate and calcium carbonate (High purity from sigma Aldrich).

The precursors are mixed in stoichiometric amounts, ball milled then the mixture was heat treated at 1050 °C for 24 hours. The resulting TCP is then grinded and doped with the appropriate mass of silver oxide (sigma Aldrich 99.9% purity). The doping levels chosen are 0.5 wt%, 1 wt% and 2 wt% Ag₂O, keeping a batch of pure TCP as a control. These doping levels were chosen after careful analysis of previous research [23]. Previous work done on compact samples with silver indicated that the ideal loading level to avoid cytotoxicity and yet still observe antibacterial effects is between 2 and 4 wt% Ag₂O. Since this study is interested in porous samples, which have an increased surface area and a higher dissolution rate, the optimal loading was kept below 2 wt%. The TCP powders were then ball milled in ethanol with zirconia milling media for 6 hours in order to obtain a uniform grain size. For ball milling, the powder to ethanol ratio is 2 g for 3 mL, and the mass ratio of powder to milling media is 1:5. The batches of powder are then processed into all the different samples required to complete the study.

Two main types of samples, porous and compact, were made. The compact samples were prepared by uniaxial pressing. For mechanical testing, compact samples with 7.5 mm diameter and 10 mm height were made. The compact samples were further processed in a cold isostatic press (CIP). The samples are vacuum sealed into a double layer of latex gloves, then put in liquid for 2 minutes at 50 psi. Since the porosity of bone is in the range 30% to 75%, two porosities were chosen, one at each end. In order to engineer porosity into the TCP samples, an emulsion method was used. Kolliphor EL, a commercial product based on castor oil, was used as an emulsifying agent to stabilize porogen droplet size. The emulsifier was added to paraffin oil and TCP powder, and the whole mixture was then stirred for less than 45 sec with a stirring bar. The porosity of the samples is dictated by the volume fraction of powder to porogen in the initial mixture. To obtain highly porous samples (75% porosity), 20g of TCP were mixed with 20g of paraffin oil, to which 13.4 mL of emulsifier were added. The homogeneous slurry obtained is a mixture of powder and dispersed porogen droplets, with the emulsifier stabilizing the porogen. The slurry was poured into 1 inch tall cylindrical molds with a diameter of $\frac{3}{4}$ ". These molds were put into an oven at 80°C for several days to dry and harden before removing them from the mold for sintering. The slurry was found to be stable for pure TCP samples. However, once the dopant was added, the stability of the slurry was greatly reduced. The slurry was very difficult to obtain, and the oil droplets had a tendency to separate from the slurry.

The samples were sintered in a high temperature furnace (Zircar). The sintering procedure established by Perera et al. [24], which has been optimized by this research group is to sinter at 1250°C for 2 hours. The high temperature sintering is preceded by an hour dwell time at 300°C and 600°C to dry and burn out additives before sintering. The heating rate up to 600°C was 1°C per minute for the porous samples and 3°C per minute for the dense samples. From 600°C to 1250°C the heating rate for all samples was 1°C per minute.

2.2. Characterization: Phase, density and porosity

Phase analysis of the sintered scaffolds was carried out a diffractometer (PW 3040/00 Xpert MPD, Philips, Netherlands), using K_{α} radiation and a Ni filter. The intensity was measured over the range 20° to 60° in steps of 0.05° and 0.5 seconds. Phase percentage was determined by calculating the relative intensity of the three principal peaks for each phase using the equation (ii).

$$\text{Relative intensity} = \frac{\text{intensity of peak of phase analyzed}}{\text{intensity of all the peaks of all the phases}} \quad (\text{ii})$$

The bulk density, apparent density, and the open and closed porosity were recorded for all the samples. Bulk density (ρ_b) was calculated using the dimensions of each sample. By the Archimedes principle, the buoyancy force exerted on the sample is the weight of water displaced, thus the mass change is proportional to the volume of the sample. The apparent density was calculated according to the Archimedes principle using the equation (iii).

$$\text{Apparent density } (\rho_a) = \frac{\text{Dry weight}}{\text{Dry weight} - \text{Weight in water}} \quad (\text{iii})$$

Total, open and closed porosity were calculated using the following set of equations:

$$\text{Total porosity: } P_t = 1 - \rho_b \quad (\text{iv})$$

$$\text{Closed porosity: } P_c = 1 - \rho_a \quad (\text{v})$$

$$\text{Open porosity: } P_o = P_t - P_c \quad (\text{vi})$$

Data for each level of silver loading came from three dense samples and six porous samples, which were used for an average.

2.3. Microstructure and pore morphology

Microstructural analysis was done using a field emission scanning electron microscope (FESEM, FEI Inc., USA). Samples were dried, and sputtered with a thin gold film (10–20nm) (Technics Hummer V, USA), in order to be observed under the FESEM. The micrographs were taken in high vacuum using an acceleration voltage of 30 kV, with an equipped ETD (Everhart-Thornley Detector) for secondary electrons. Three samples were taken from each batch, and several batches were characterized to check for consistency. The average grain size of the ceramics was determined for each condition using the ASTM standard E-112-88 protocol. This consists of drawing a set of arbitrary lines on an image, and counting the number of grain boundaries intersected by that line. The mean lineal intercept length is given by the following equation (vi):

$$\bar{L}_L = \frac{1}{\bar{N}_L} = \frac{L_T}{PM} \quad (\text{vii})$$

Where $\bar{N}_L$ is the average number of is intercepts per line, L_T is the total length of the test lines, P is the total number of grain boundary intersections, and M is the magnification. Using image analysis software ImageJ, pore sizes were measured for each composition, and pore size distribution and ranges were calculated. Pore interconnectivity, and quality of manufacturing process were also evaluated. In order to estimate the pore size distribution, a stereological correction factor was applied. Since the pores were observed to be spherical, the simple correction is of the form:

$$D_{sphere} = \frac{D_{circle}}{0.785} \quad (\text{viii})$$

2.4. Mechanical properties

The mechanical performance of the scaffolds was evaluated by measuring the compressive strength. This was measured with a vertical screw driven universal testing machine (AG-IS, Shimadzu, Japan). The cross head speed was kept constant at 0.33 mm/min and the force was 50,000 N. Dimensions of the samples were taken, from which the stress required to break the sample was calculated. Compressive strength was reported in MPa for different porosities and silver loading levels. For each data point, a minimum of 8 samples were tested in order to have a statistically significant result. For ceramics, mechanical strength is dependent on sample volume, and the presence of defects and cracks. These initial conditions introduce a lot of variability in the mechanical testing results, therefore it is useful to plot the mechanical reliability as a failure probability P_f as a function of compressive stress. This was done by calculating the Weibull modulus, m , which represents the shape of the distribution function. The Weibull modulus is calculated with the following equation (ix):

$$P_f = 1 - \exp \left(- \left(\frac{\sigma}{\sigma_0} \right)^m \right) \quad (\text{ix})$$

The dataset is ranked, and by plotting $\ln \left\{ \ln \left(\frac{1}{1 - \text{median rank}} \right) \right\}$ against $\ln(\text{strength})$, a regression line with the Weibull coefficient m is obtained. This also yields a value for σ_0 , which is then substituted with m into the previous equation, and plotted for a range of stresses.

2.5. Ion release kinetics

Silver ion release from the TCP scaffolds was studied using both the compact and porous samples. The samples were submerged in 5 mL of buffer solution, one set at pH 5 acetate buffer. Each silver loading level, type of sample and pH level was tested in triplicate. The samples were kept in an incubator at 37°C under constant shaking at 150 RPM. Buffer solution was changed entirely for each time point, and 5 mL of fresh solution added to the vials. Time points were taken for 6 hours, 12 hours, 24 hours, 48 hours, and then at several days interval for a total time of 60 days. The concentration of silver and calcium ions was measured for each time point in order to characterize ion release kinetics. Ion concentration measurements were carried out using an atomic absorption spectrophotometer (AAS 6800 series, Shimadzu, Japan). Since the samples all had a different mass, it was necessary to normalize the measured concentrations. By dividing the concentration in ppb by the mass in mg, a concentration value in ppm corresponding to a sample mass of 1mg was considered. Unit conversion to μM is done by multiplying the concentration in ppb by the molar mass of silver. Cumulative concentration was calculated for all samples, and the data from pure TCP was used as a baseline to calculate adjusted concentration.

2.6. Cytotoxicity

The cytotoxicity of the scaffolds was investigated by studying the proliferation of human fetal osteoblast cells (hFOB). These cells are an immortalized osteoblastic line (hFOB 1.19, ATCC® CRL-11372) derived from human fetal bone tissue. Cultures were prepared on samples of each type and each silver loading level at incubation times of 3, 7 and 11 days. All of the samples were sterilized before the study by autoclaving at 121°C for 30 minutes. Samples were seeded with 100000 cells, then immersed in 1mL of Dulbecco's Modified Eagle Medium (DMEM; containing salts, amino acids, glucose, vitamins, iron and phenol red) enriched with 10% fetal bovine serum. Cell media was changed every two days, and the samples were kept in an incubator with an atmosphere regulated at 37°C and 5% CO₂ humidified air. Cell proliferation was assessed using MTT assay colorimetry. MTT is a yellow tetrazole dye which is reduced by cell enzymes to formazan, which is purple. The optical density of an MTT assay is proportional to the number of cells. The MTT solution was prepared by dissolving MTT in PBS at a concentration of 5mg/mL, then filter sterilizing. After removing samples from the cell media, 100 µL of MTT solution was added, followed by 900 µL of cell media. Samples were then incubated for 2 hours, then transferred to new well plates and submerged in 500 µL of solubilizer solution (made from 10% Triton X-100, 0.1N HCl, and isopropanol). The solubilizer dissolves crystallized formazan, and 100 µL of the supernatant is then analyzed with a plate reader at a wavelength of 570nm. Each condition had triplicate samples, and three measurements were made for each sample.

3. Results

3.1. Phase composition

X-ray diffraction (XRD) patterns of the sintered scaffolds are presented in Figure 1. XRD analysis showed that the sintered scaffolds are composed of both α -TCP and β -TCP. Major XRD peaks of scaffolds corresponds to the characteristic β -TCP (JCPDS # 09-0169) and α -TCP (JCPDS # 09-0348) peaks. The TCP scaffolds are 64–71% β -TCP and 29–36% α -TCP. The appearance of α -TCP in the ceramic is expected, as the sintering temperature was 1250°C, which is above the phase transition. The detailed peak analysis is shown in Table 1. The addition of silver dopant can be seen to have an effect on the phase composition, as phase percent α -TCP increases with the addition of dopant. In the XRD data, there is a slight peak shift (0.05° or less) in the silver doped samples.

3.2. Microstructure, density and porosity

Micrographs of the porous scaffolds are presented in the Figure 2. The average grain size was measured to be 3.51 µm for porous and dense scaffolds. This average grain size was consistent for both dense and porous samples, and all silver doping levels. Despite the overall porosity remaining constant for all silver doping levels, the pore morphology varies significantly. There is a clear increase in pore size with the addition of silver dopant, as illustrated in the Figure 2. Plotting the pore size distribution as shown in Figure 3 also gives a good idea about the effects of silver loading. The presence of Ag leads to a spread in the distribution, on top of the increase in pore size. Necks between pores were observed in approximately 50% of the pores in the cross section, indicating a good level of interconnectivity. Using image analysis, it was determined that the neck size varied between

20 and 100 μm . After measurements and calculations, it was found that the porosity did not show any significant dependence on the composition. The results for the dense and porous samples are shown in Tables 2 and Table 3, respectively. After accounting for the error range of the density calculations, the porosity values were found to be statistically similar. The dense samples have an average apparent density of 86.5%, and an average total porosity of 20.6%. The porous samples have an average apparent density of 73.6%, and an average total porosity of 78.6%.

3.3. Mechanical properties

The compressive strength of the highly porous samples was 1.38 ± 0.28 MPa, and the compressive strength of the dense samples is given in Table 4. These results are consistent with the literature [25]. Before finding the probability to failure of the entire dataset of dense samples, it was necessary to verify that the addition of silver had no significant effect on the strength. These values are the average of 6 to 8 different sample tests. Each composition was within the error range of the rest, apart from the 2 wt% Ag which had some consistent data points. This variance can be accounted for by the small number of samples for each composition, and the large variation in initial conditions. The preliminary conclusion based on this data is that the effects of silver loading were negligible. In order to corroborate this statement, all the data points were ranked and plotted as a Weibull regression. The Weibull modulus is clearly consistent for the entire dataset, and by combining all the data points into one plot the accuracy of the analysis is ensured. The probability to failure curve for compact TCP samples used in this study is plotted in Figure 4. A reliability of 0.9 (10% failure rate) is calculated to be 140.6 MPa, and 0.1 reliability (90% failure rate) is calculated to be 211.4 MPa. The Weibull modulus m is 7.55, and the characteristic strength σ_0 is 189.3 MPa.

3.4. Ion release kinetics

The cumulative release of silver ions from the samples at pH 5 buffer is plotted in Figure 5 for the dense and for the porous scaffolds. The porosity increase yields a near twofold increase in ion release. The final cumulative concentration lies within the optimal range of 30–100 μM .

3.5. Cytotoxicity

Cytotoxicity of these scaffolds was tested using cell proliferation assay. MTT cell proliferation assay can be used to test the biomaterials associated cytotoxicity. The idea is that cellular proliferation will go down significantly compared to control if the materials exerts any cytotoxicity. The MTT assay results are plotted for each time point in Figure 6. The first observation is that there is a marked increase in cell proliferation on the porous samples compared to the dense samples. The number of cells was more than doubled, which means the porous samples were more osteoconductive than the dense ones. In addition, cells proliferation results show that the silver loading was not cytotoxic for osteoblasts. The 2 wt % Ag doped samples showed a very slight decrease in cell proliferation, however this decrease is not significant enough to show any cytotoxic effects.

4. Discussion

The stated objective of this study was to engineer a bone graft material which is osteoconductive, osteoinductive, bioresorbable, maintains silver ion release, and non-toxic. Porosity, pore morphology, pH, silver doping, and mechanical properties are the focus of this study, and it is important to understand the role of each one. Obtaining an osteoconductive scaffold is dependent on two main factors: (I) the first one is the choice of material and (II) the second one is the material morphology. β -TCP has the appropriate chemical composition for osteoblast proliferation and mineralization of new tissue [6–7]. The micro porosity favors cell adhesion, and osteoblasts can easily absorb the minerals necessary for growth from the scaffold [26]. The material must promote cell differentiation and the formation of three dimensional cellular structures. An important factor influencing this is the macroporosity [27]. This is engineered by careful control of the processing parameters. There are many ways to introduce pores into a ceramic, and in this study a porogen-based method was used. This method consists of dispersing the porogen in the form of droplets which are stabilized by the addition of an emulsifier. In this case, the overall porosity of the scaffold depends on the volume ratio between ceramic powder and pore forming agent. The emulsifier stabilizes the droplets to a certain size, based on the surface energy of the porogen. Surface energy between the ceramic, the emulsifier and the porogen are critical in determining the pore size and pore morphology. The addition of silver oxide in the slurry created an additional set of interactions, which destabilized the porogen droplets. This resulted in the silver doped samples forming larger droplets, leading to an increase in pore diameter as well as pore size distribution. However, no significant difference was observed in microstructure and mechanical strength between pure and silver doped TCP scaffolds in this study. Liu et al. [28] also reported the similar trend in the silver doped TCP. The pores were interconnected, which is also a very important factor to obtain good osteoconductivity. The MTT assay results for 11 days with human fetal osteoblast cells showed no cytotoxicity caused by the addition of silver in these dense and porous TCP scaffolds. Moreover, the MTT assay showed a marked increase in cell proliferation on porous samples, which is proof of the osteoinductive properties caused by porosity in these scaffold.

In order for the scaffold to be bioresorbable, it must dissolve within the body. When a bioceramic is immersed in an aqueous environment, the dissolution/precipitation process which is undergoes is comprised of several mechanisms, which are all pH dependent. The results of this study show that in an acidic environment, the dissolution/precipitation process is dissolution dominated, and the scaffolds disappear. In a neutral environment, the process is precipitation dominated, which results in the material actually gaining mass. *In vivo*, the local pH can vary considerably according to many biological factors. In places where there is increased cell activity, inflammation or infection, the local pH levels drop. In the case of bone grafts, the damaged area where the scaffold is implanted will be acidic. Thus the acidic buffer is more representative of the conditions the scaffold would be subject to *in vivo*. Based on the *in vitro* dissolution results, the scaffolds are indeed bioresorbable.

Silver has an important role to play in tissue engineering, because in addition to having natural antibacterial properties, it can potentiate antibiotics against resistant bacterial strains

[29–31]. Thus the scaffold should supply a local silver ion concentration for the duration of the healing process. In this study, the addition of silver as a dopant into the scaffold was found to be an effective method of achieving this. Since silver is contained within the material lattice, ions are released throughout the lifetime of the scaffold, providing a sustained local ion concentration. It was demonstrated *in vitro* that the scaffolds showed no cytotoxic effects despite this silver ion release. Human osteoblast cell lines were able to proliferate on all of the samples in the study. Our results show silver doped TCP scaffolds have great potential to be used for infection prevention with long term sustained silver ion release.

Conclusions

The scaffolds made in this study provided valuable insight into the effects of porogen, emulsifier and dopants on the resulting material. The final overall porosity is proportional to the volume percent of ceramic in the mixture. The pore morphology is dictated by the stability of the porogen, which is controlled by varying the concentration of the emulsifier. This study showed that the addition of 0.5 wt% Ag₂O, 1 wt% Ag₂O and 2 wt% Ag₂O in TCP had a significant effect on the pore size and distribution, as they introduce additional interactions into the mixture, and destabilized the emulsion. The compressive strength of the highly porous scaffolds was 1.38 ± 0.28 MPa, while the dense scaffolds exhibited a range between 165 ± 21 and 198 ± 10 MPa. Although the porosity increase resulted in twofold increase in ion release, it was within the optimal range of 30–100 μ M over 60 days. *In vitro* studies on dissolution kinetics and cytotoxicity showed that porosity increases dissolution rates, and hence ion release. MTT assay for 11 days on human fetal osteoblast cells did not show any cytotoxicity caused by these silver doped TCP scaffolds.

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Figure 1.
XRD spectra of pure and silver doped TCP scaffolds sintered at 1250 °C for 2 hours.

Figure 2.
Pore morphology of sintered porous TCP samples: A: Pure TCP, B: 0.5 wt% Ag, C: 1 wt% Ag, D: 2 wt% Ag.

Figure 3.
Pore size distribution of Pure and 0.5 wt% Ag doped TCP.

Figure 4.
Probability of failure in compression for dense TCP scaffolds.

Figure 5. Cumulative Ag ion release in pH 5 buffer from dense TCP samples (a); Cumulative Ag ion release in pH 5 buffer from porous TCP samples (b).

Figure 6.
MTT assay of hFOB on the porous and dense scaffolds.

Table 1

XRD peak analysis and phase composition of silver doped TCP

Peak angle θ (degrees)	Pure TCP	0.5 wt% Ag-TCP	1 wt% Ag-TCP	2 wt% Ag-TCP
<u>α-TCP</u>				
22.72/22.90	7%	7%	7%	9%
24.10	5%	6%	5%	7%
30.75	17%	20%	22%	20%
α -TCP phase %	29%	34%	35%	36%
<u>β-TCP</u>				
27.77	17%	17%	15%	16%
31.05	30%	27%	28%	27%
34.37	23%	22%	23%	22%
β -TCP phase %	71%	66%	65%	64%

Table 2

Densities and porosities of dense TCP scaffolds

Composition	Bulk density	Apparent density	Total porosity	Closed porosity	Open porosity
Pure TCP	80.23% ± 0.43	87.51% ± 0.15	19.77%	12.49%	7.28% ± 0.57
0.5 wt.% Ag	81.44% ± 1.32	86.87% ± 0.21	18.56%	13.13%	5.43% ± 1.53
1 wt.% Ag	78.10% ± 0.44	84.03% ± 1.72	21.90%	15.97%	5.93% ± 2.16
2 wt.% Ag	77.86% ± 0.36	87.40% ± 0.63	22.14%	12.60%	9.54% ± 0.99

Table 3

Densities and porosities of porous TCP scaffolds

Composition	Bulk density	Apparent density	Total porosity	Closed porosity	Open porosity
Pure TCP	22.43% ± 1.28	76.51% ± 3.19	77.57%	23.49%	54.08% ± 4.47
0.5 wt.% Ag	20.98% ± 1.33	70.50% ± 4.35	79.02%	29.50%	49.52% ± 5.68
1 wt.% Ag	20.78% ± 0.62	76.62% ± 3.86	79.22%	23.38%	55.84% ± 4.48
2 wt.% Ag	21.45% ± 1.07	70.70% ± 3.70	78.55%	29.30%	49.25% ± 4.77

Table 4

Average compressive strength of dense TCP samples

Sample composition	Compressive strength
Pure TCP	165 MPa $\pm$ 21
0.5 wt% Ag	175 MPa $\pm$ 18
1 wt% Ag	162 MPa $\pm$ 24
2 wt% Ag	198 MPa $\pm$ 10