$CaSiO_3$ microstructure modulating the *in vitro* and *in vivo* bioactivity of poly (lactide-co-glycolide) microspheres

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Abstract

Poly (lactide-co-glycolide) (PLGA) microspheres have been used for regenerative medicine due to their ability for drug delivery and generally good biocompatibility, but they lack adequate bioactivity for bone repair application. $CaSiO_3$ (CS) has been proposed as a new class of material suitable for bone tissue repair due to its excellent bioactivity. In this study, we set out to incorporate CS into PLGA microspheres to investigate how the phase structure (amorphous and crystal) of CS influences the *in vitro* and *in vivo* bioactivity of the composite microspheres, with a view to the application for bone regeneration. X-ray diffraction (XRD), N_2 adsorption-desorption analysis and scanning electron microscopy (SEM) were used to analyze the phase structure, surface area/pore volume, and microstructure of amorphous CS (aCS) and crystal CS (cCS), as well as their composite microspheres. The *in vitro* bioactivity of aCS and cCS – PLGA microspheres was evaluated by investigating their apatite-mineralization ability in simulated body fluids (SBF) and the viability of human bone mesenchymal stem cells (BMSCs). The *in vivo* bioactivity was investigated by measuring their *de novo* bone-formation ability. The results showed that the incorporation of both aCS and cCS enhanced the *in vitro* and *in vivo* bioactivity of PLGA microspheres. cCS/PLGA microspheres improved better *in vitro* BMSC viability and *de novo* bone-formation ability *in vivo*, compared to aCS/PLGA microspheres. Our study indicates that controlling the phase structure of CS is a promising method to modulate the bioactivity of polymer microsphere system for potential bone tissue regeneration.

Key words: $CaSiO_3$ phase structure; Amorphous; Crystal; Bioactivity

INTRODUCTION

Microspheres have received significant attention recently as an injectable material for bone tissue regeneration.[1,2] The main advantage of this approach, when compared with the traditional block scaffolds, is that minute microspheres can be combined with a drug vehicle and be administered by injection, opening up the possibility of filling defects of various shapes and sizes through minimally invasive surgery. When implanted, the microspheres system is expected to easily conform to an irregular implant site.[3] The ideal properties for a microsphere system for bone regeneration is to combine bioactivity with a capacity for controlled protein/drug-delivery.[4,5] To this end, a number of different materials, including inorganic Ca-P ceramics[3,6] and polymers[7,8] have been manufactured into microspheres for the purposes of drug delivery and bone tissue repair applications. Of these materials, ceramic microspheres, such as hydroxyaptite (HAp) ceramics are bioactive, but do not possess controllable protein/drug release and have less than optimal degradation kinetics.[3,6,9] Poly (lactide-co-glycolide) (PLGA)-based microsphere system is a potential drug carrier for bone tissue repair as PLGA is biodegradable with generally good biocompatibility.[10,11] As a true bone tissue engineering material, the bioactivity PLGA microspheres falls well short of being ideal.[12,13] A bioactive ceramic/PLGA composite material combines the bioactivity of the former with the drug/protein release properties of the latter, and presents one of best options for developing microsphere system for bone repair applications.[7,14]

Wollastonite ($CaSiO_3$; CS) ceramics combine high bioactivity with degradability,[15] and previous studies have shown that bioactive wollastonite coating on Ti alloys form an excellent bond with host bone.[16,17] Xu et al. showed that wollastonite ceramic scaffolds have improved degradation and bone-formation ability compared to traditional β-tricalcium phosphate ceramics when implanted in a rabbit calvarial defect model.[18] It has been demonstrated that incorporating crystal CS (cCS) into PHBV microspheres

creates a composite material with greatly enhanced the hydrophilicity and the proliferation and differentiation potential of bone cells.[19-21] According to these results, we hypothesized that incorporating CS into PLGA polymer microspheres would result in a material with significantly enhanced *in vitro* and *in vivo* bioactivity. Although there are earlier studies of cCS ceramics and their composites,[15,16,18-21] as far as we know, no previous studies have explored how the phase structure of amorphous CS (aCS) and crystal CS (Ccs) influences the *in vitro* and *in vivo* bioactivity of polymer microspheres. The basis for this hypothesis is the fact that the phase structure of bioactive ceramics greatly influences its bioactivity and rate of degradation. Therefore, the aims of this study were therefore to investigate how the phase structure of CS influences the *in vitro* and *in vivo* bioactivity of PLGA microspheres.

MATERIALS AND METHODS

Preparation and characterization of $CaSiO_3$ powders

Amorphous and crystal CS powders were synthesized by a chemical precipitation method.[22] Briefly, calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$, (Sigma-Aldrich, Castle Hill, NSW, Australia) was dissolved in distilled water (0.1 M), adjusted to pH 12 using $NH_3{\cdot}H_2O$, to obtain the Ca containing solutions. A 0.1 M sodium metasilicate (Na_2SiO_3) (Sigma-Aldrich) solution was added dropwise to the Ca containing solution while stirring, to produce a white precipitate. After stirring for 12 h, the precipitate was filtered, and washed three times with distilled water followed by one-time wash with ethanol. The remaining liquid was removed by vacuum filtration, and the precipitate was dried at 60ºC for 24 h, the resulting powders, amorphous $CaSiO_3$, are hereafter referred to aCS. Crystal $CaSiO_3$, (hereafter referred to as cCS), was obtained by subjecting aCS to 800ºC heat for 2 h; both the aCS and cCS powders were ground and sieved to 230 meshes. The size distribution of amorphous and crystal CS

particles synthesized by this method is around 0.1-0.3 μm and 0.3-5μm, respectively.

The phase and microstructure of aCS and cCS powders were characterized by X-ray diffraction (XRD) and scanning electron microscopy (SEM). Brunauer-Emmett-Teller and Barret-Joyner-Halenda were used to determine the surface area, the pore size distribution and the pore volume of aCS and cCS powders by N_2 adsorption-desorption analysis.

Preparation and characterization of CS/PLGA microspheres

PLGA, aCS/PLGA and cCS/PLGA microspheres were prepared by a double emulsion method. aCS/PLGA microspheres were typically prepared in a two step procedure: 2.4 g of PLGA (lactide:glycolide = 85:15) was first dissolved in 60 ml of 4% (w/v) chloroform, then 0.36 or 0.72 g of aCS powders was added to the PLGA/chloroform solution (concentrations of CS/PLGA: 15% or 30%) under constant stirring; the mixture was stirred for 30 min and then sonicated for 10 min, which produced a uniform emulsion. This mixture was then added to 300 ml of 0.5% polyvinyl alcohol (PVA) solution under a stirring rate of 1000 rpm for 1 h. The particles were collected by centrifugation at 5000 rpm for 5 min, washed 3 times in ddH_2O, then dried in a vacuum at 40ºC for 2 days to obtain aCS/PLGA microspheres.

cCS/PLGA and pure PLGA microspheres were prepared in a same way as the aCS/PLGA. The composition and morphology of the prepared microspheres were characterized by SEM and energy-dispersive spectrometer (EDS).

The effect of CS microstructure on the mineralization

The surface mineralization of the three microspheres was investigated using acellular simulated body fluids (SBF). The SBF was prepared according to previous publications [23] and 0.3 g of microspheres were immersed in 200 mL of SBF and kept at 37 ºC for various time points. The microspheres were soaked for 7 days and analyzed by SEM and EDS to determine their apatite-forming capacity. The pH of the SBF solution was tested after soaking the microspheres, without refreshing the solution. The concentration of Ca and Si ions released from the microspheres was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES). As we found that both crystal 15%CS-PLGA and amorphous 15%CS-PLGA microspheres had no apatite formation in SBF due to the low contents of CS in the microspheres, therefore, we selected 30%CS/PLGA microspheres for the further in vitro and in vivo testing.

The effect of CS microstructure on BMSCs viability

Human bone mesenchymal stem cells (BMSCs) were isolated from bone marrow samples ($n = 5$) following a protocol described previously.[24] All samples were obtained from patients at the Prince Charles Hospital, Brisbane, after informed consent was given; the project had the approval of the Ethics Committee of the Queensland University of Technology. The effect of microspheres on BMSCs viability was evaluated in a 6-well Transwells cell culture system (pore size for 0.4μm membrane). A schematic illustration of how the microspheres were loaded in this system is shown in Figure 1. With this culture system the released Ca and Si ions, as well as degradation products from the microspheres, will be dissolved into the cell culture medium, but the microspheres will not be in direct contact with the cells. PLGA, 30% amorphors CS/PLGA or 30% cCS/PLGA microspheres (15 or 25mg) were loaded onto the transwell membrane and incubated with $1x10^5$ BMSCs at 37ºC in 5% CO_2 for 1 or 7 days. Cell

morphology was studied by light microscopy and the cell viability were measured by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method: 500 μl of 0.5 mg/ml MTT solution was added in each well and incubated for 4 h at 37ºC. The reaction was terminated by the addition of dimethyl sulfoxide and formazan concentration was measured in a plate reader at 495 nm. The results were expressed as the absorbance reading from each well less the optical density value of a blank.

The effect of CS microstructure on the in vivo osteogenesis

The bone forming ability of the different microsphere types was assessed in a calvarial defect model in severe combined immunodeficient (SCID) mice, following a previous described method.[25] The surgeries were carried out according to the guidelines of the Animal Research and Care Committee of the Herston Medical Research Centre and the Queensland University of Technology. The surgical procedures were performed in aseptic conditions under general inhalation anesthesia. Briefly, a linear incision (1 cm long) was made in the left skull to reveal the bone surface. The periosteum was dissected from the bone surface and a full-thickness calvarial bone defect, 3 mm in diameter, was created with a trephine bur using a slow-speed dental drill. To avoid tissue damage due to overheating, 0.9% saline was dripped onto the contact point between the bur and bone and great care was taken to avoid injury to the dura mater. The microspheres were carefully placed into the defects and soft tissue above the defect was covered by the skin which was closed with skin staples.

The animals were euthanized 8 weeks after surgery and the defect areas were collected. The samples were fixed in 4% paraformaldehyde for 12 h at room temperature. All samples were scanned for bone formation within the defect site using a μCT40 imaging system (Scanco Medical, Bassersdorf,

Switzerland) with the following scan parameters: 20 mm field of view, 55 kVp X-ray energy setting, 1024 reconstruction matrix, slice thickness 0.02 mm, and a 250 ms integration time. Mineralized tissue was segmented from non-mineralized tissue using a global thresholding procedure with a value approximating 1.20 g/cm^3 (150 on micro-CT) (25% lower than 1.6 g/cm3) which is the mineral density of healthy human compact bone. Bone volume per defect (BV; mm3) was recorded as the measure of defect bone regeneration. After the μCT scanning, all tissue samples were decalcified in 10% EDTA, changed twice weekly, for 2 to 3 weeks, after which they were embedded in paraffin. Serial sections with the thickness of 5 μm, were cut and mounted on polylysine-coated microscope slides. All sections were stained with hematoxylin and eosin (H & E), and a general assessment of the tissue and wound healing was performed using a light microscope.

Statistical analysis

The data was expressed as means ± standard deviation (SD) for all experiments and were analyzed using One-Way ANOVA with a Post Hoc test. A p-value<0.05 was considered statistically significant.

RESULTS

Characterization of aCS and cCS powders

The phase structure of CS powders before and after 800ºC calcination is shown in Figure 2. aCS showed a weak and wide diffraction peak (Fig. 2a), whereas cCS showed a strong and sharp characteristic peak of β-CS after calcining (Fig. 2b). As would be expected, aCS has a different microstructure compared to cCS (Fig. 3). aCS has uniform micropartilces (Fig. 3a), the cCS has aggregated particles (Fig. 3b). N_2 adsorption-desorption isotherms and the corresponding pore distribution analysis for aCS and cCS

powders are listed in Figure 4. aCS shows an obvious isotherm pattern and the pore distribution mainly focuses on 40 nm (Fig. 4a); cCS has overlapping adsorption and desorption curves and a disordered pore distribution (Fig. 4b). The surface area and pore volume of aCS ($108.8 m^2/g$ and $0.61 cm^3/g$) are significantly higher than those of cCS powders ($4.4 cm^3/g$ and $0.02 cm^3/g$) (Table 1).

Characterization of CS/PLGA microspheres

SEM analysis shows, that the size distribution of the microspheres, has a size range of 5–10 μm (Figure 5). Pure PLGA microspheres have a smooth surface and no obvious Ca and Si peaks in their EDS pattern. By contrast, after incorporating 30% aCS and cCS into PLGA microspheres, some CS particles exist on the surface of PLGA microspheres and EDS analysis shows the obvious signal of Ca and Si (Fig. 5c and e). The ratio of Ca/Si in aCS/PLGA and cCS/PLGA microspheres is 0.96 and 0.99, respectively, confirming that CS has been incorporated into PLGA. However, there is no obvious CS particle distribution on the surface of 15%CS/PLGA microspheres due to the low contents of CS (Fig. 5b and d).

The effect of CS microstructure on the mineralization

SEM morphology and EDS analysis for the three microspheres types after soaking in SBF for 7 days are shown in Figure 6. The surface of PLGA microspheres has no obvious apatite mineralization deposition (Fig. 6a). A few apatite particles have formed on the 30%-cCS/PLGA microspheres and the P element signal is weak in EDS pattern (Fig. 6c). 30%-aCS/PLGA microspheres, however, show strong apatite-mineralization ability (Fig. 6b) and the formed apatite has a typical lathlike morphology with nano-size structure (Fig. 6d). EDS analysis shows the strong Ca and P peaks for the formed apatite on 30%-aCS/PLGA microspheres, and the ratio of Ca/P is 1.54 (Fig. 6b). However, there is no obvious

apatite formation on the surface of amorphous 15%-CS/PLGA and crystal 15%-CS/PLGA microspheres (Fig. 6e and f).

CS had an obvious effect of stabilizing the pH value of the SBF solution (Fig. 7). Pure PLGA microspheres induce a significant decrease in pH of the SBF. The pH is more stable when CS is incorporated into PLGA microspheres, with aCS having a greater ability to buffer the pH of SBF compared to cCS. The Ca and Si ions concentrations clearly show that aCS/PLGA microspheres have a faster Si ion release than cCS/PLGA microspheres (Table 2).

The effect of CS microstructure on BMSCs viability

The morphology of the BMSCs cultured in the Transwell culture system is shown in Figure 8. On day 1, there is no morphological difference of BMSCs cocultured with any of the microspheres, nor is there any apparent difference in the cell density (Fig. 8a). By day 7, the cells in all wells have clearly proliferated compared to day 1 (Fig. 8b). The MTS assay shows that incorporating CS powders into PLGA enhances BMSC viability after 7 days of culture, and that the rate of proliferation was greater when grown in a cCS/PLGA microspheres coculture compared to the PLGA and aCS/PLGA microsphere cocultures (Fig. 9).

The effect of CS microstructure on new bone formation

Histological analysis and μCT measurement showed that both aCS and cCS-incorporated PLGA microspheres enhanced *de novo* bone-formation ability compared to pure PLGA microspheres, and that the average amount of bone tissue formed by cCS/PLGA microspheres was significantly higher than that generated by aCS (Fig. 10 and 11). In the aCS/PLGA microspheres group, the defect was bridged by thin

trabeculae of woven bone contacting granules surrounded mainly by connective tissue. The centre of the defects contained only connective tissue and biomaterial. This contrasted with cCS/PLGA microsphere group, here large amounts of freshly deposited and immature bone tissue was found in areas where the cCS/PLGA microspheres had been resorbed (Fig. 10).

DISCUSSION

In this study we have explored the effect of the phase structure (amorphous and crystal) of CS on the *in vitro* and *in vivo* bioactivity of PLGA microspheres. We found that aCS had significantly higher surface area and nano-pore volume than cCS. Despite the significantly different physical properties of aCS and cCS, the incorporation of aCS and cCS both significantly enhanced the *in vitr*o and *in vivo* bioactivity of PLGA microspheres. aCS had greater apatite-mineralization ability in SBF environment compared to cCS; however, cCS/PLGA had greater BMSC viability *in vitro* and new bone-formation ability *in vivo* compared to the aCS/PLGA microsphere system. This study indicates that altering the phase structure of CS is a promising method to control the bioactivity of polymer microsphere system for potential bone tissue regeneration.

A double emulsion method was used in this study to prepare CS/PLGA composite microspheres. Compared to other methods, such as electrostatic spraying,[26] the advantage of a double emulsion method is that it is easy both to prepare and control the size of the microspheres. Our study showed that CS/PLGA microspheres had a relatively uniform size distribution (5-10μm). The small size and uniformity of these composite microspheres will significantly improve the injectability compared to the larger sized microspheres which typically result from electrostatic spraying.[26,27]

Although aCS and cCS have identical chemical composition, their physical properties are very different,

in particular crystanillinity, surface areas, and nanopore structure and size. The principal reason for the different physical properties is that cCS has been treated at high temperature, leading to a particle aggregation (Fig. 3) which results in decreased surface area and pore volume (Table 1). The greater surface area and pore volume of aCS lead to a faster dissolution rate than cCS (Table 2). Bioactivity is an important issue when considering the chemical interactions between an implant material and the bone tissue. The chief disadvantages of PLGA are that its bioactivity is less than optimal and the material's degradation products lead to an acidic environment which may elicit an inflammatory response.[28-30] The incorporation of CS into PLGA microspheres improves the apatite-mineralization ability of PLGA microspheres and buffers the pH environment of SBF. Interestingly, aCS/PLGA microspheres have better apatite-mineralization ability and pH-stability than cCS/PLGA and pure PLGA microspheres. According to the mechanism of apatite formation on CS based ceramics,[31-33] a plausible explanation for this is most likely that aCS releases more Ca and Si ions into the SBF solution and has a higher surface area than cCS. This results in the formation of more Si-OH groups on the surface of aCS, compared to that formed on cCS, which may contribute to inducing more apatite deposition on the surface of the aCS/PLGA microspheres. Similarly, the higher release of Ca and Si ions from aCS/PLGA microspheres more effectively buffers the decrease in pH than that of cCS/PLGA microspheres, by neutralizing the acidic degradation products of the PLGA microspheres.

A Transwell cell culture system was used in this study to evaluate how the degradation products of microspheres, including Ca, Si ions and by-products from PLGA, affected BMSC viability (see Fig. 1). The results indicate that the incorporation of CS into PLGA microspheres enhanced cell viability. The most likely explanation for this is that CS buffered the pH of cell culture medium, which was beneficial for BMSC growth.[34,35] Interestingly, cCS/PLGA microspheres seemed to have a more beneficial effect

on cell viability compared to aCS/PLGA microspheres. Similarly, both aCS and cCS enhanced the *in vivo* bone formation of PLGA microspheres, and in a similar fashion, cCS/PLGA microspheres increased bone formation capacity more than did aCS/PLGA microspheres. Previous studies have shown that, at a certain concentration range, Ca and Si ions actually stimulate cell proliferation.[32,36] cCS/PLGA microspheres may possibly provide an ionic environment which is more suitable for BMSCs and *in vivo* bone formation than does the aCS/PLGA system. It is, however, intriguing that cCS/PLGA microspheres have lower apatite-mineralization ability than the aCS/PLGA microspheres in SBF; these data seem to be contradictory to the *in vivo* results, which suggest that the SBF method has limitations when used to evaluate the *in vitro* bioactivity of biomaterials.[37,38] The *in vivo* bone formation ability is not only modulated by the apatite mineralization, but also by their ionic environment, for example, ion concentrations. To optimize the apatite-mineralization ability and ionic environment and further improve the bone-formation ability of CS/PLGA microspheres, two potential ways will be applied in our future study. One is to prepare amorphous and crystal CS composite particles firstly, and then the CS composite particles are incorporated into PLGA microspheres; the other way is to directly mix amorphous CS/PLGA microspheres and crystal CS/PLGA microspheres. The common aim of two potential ways is to harness the difference between amorphous CS and crystal CS in apatite mineralization and dissolution (or ion release), which is to optimize the *in vivo* bone-formation ability of CS/PLGA microspheres.

CONCLUSIONS

CS powders with amorphous or crystal phase structure were successfully incorporated into PLGA microspheres. The phase structure of CS is one of important factors to modulate the bioactivity of PLGA microspheres. Amorphous CS has induced an improved apatite mineralization on the surface of PLGA

microspheres, compared to crystal CS, but cCS/PLGA microspheres improved *in vitro* BMSC viability and *in vivo* new bone-formation in mouse, compared to aCS/PLGA microspheres. Our study indicates that controlling the phase structure of CS is a promising method to control the bioactivity of PLGA microsphere as injectable materials for potential bone tissue regeneration application.

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Figure Captions

Figure 1. Schematic illustrations of microspheres loaded in transwells system for cell culture. The released Ca, Si ions and degradation byproducts from microspheres will be dissolved in culture medium and response with cells; however, the microspheres will not directly contact with cells.

Figure 2. XRD pattern for $CaSiO_3$ powders before (a) and after (b) calcining at 800^oC.

Figure 3. SEM morphology for $CaSiO_3$ powders before (a) and after (b) calcining at 800 ^oC. Arrow points to aggregated particles.

Figure 4. N_2 adsorption-desorption isotherms and the corresponding pore distribution analysis for (a) amorphous and (b) crystal $CaSiO_3$ powders. The surface area and pore volume of amorphous and crystal $CaSiO_3$ powders are listed in Table 1.

Figure 5. SEM and EDS analysis for PLGA (a), amorphous 15%-CS/PLGA (b), amorphous 30%-CS/PLGA (c), crystal 15%-CS/PLGA (d), and crystal 30%-CS/PLGA (e) microspheres. Arrows point to $CaSiO_3$ particles in microspheres. The ratio of Ca/Si in amorphous 30%-CS/PLGA and crystal 30%-CS/PLGA microspheres is 0.96 and 0.99, respectively.

Figure 6. SEM and EDS analysis for PLGA (a), amorphous 30%-CS/PLGA (b, d), crystal 30%-CS/PLGA (c), amorphous 15%-CS/PLGA (e), and crystal 15%-CS/PLGA (f) microspheres after soaking in SBF for 7 days. (d) is higher magnification picture for apatite formation on amorphous 30%-CS/PLGA microspheres. PLGA microspheres did not show obvious P element peak (a), crystal 30%-CS/PLGA microspheres showed a weak peak of P (c) and amorphous 30%-CS/PLGA microspheres showed more obvious peak of P elements (b). The ratio of Ca/P for apatite on amorphous 30%-CS/PLGA and crystal 30%-$CaSiO_3$/PLGA microspheres is 1.54 and 1.97, respectively. However, there is no obvious apatite formation on the surface of amorphous 15%-CS/PLGA (e) and crystal 15%-$CaSiO_3$/PLGA (f) microspheres.

Figure 7. pH value change for SBF solution with soaking PLGA, amorphous $CaSiO_3$/PLGA and crystal $CaSiO_3$/PLGA (c) microspheres. The SBF solution with amorphous $CaSiO_3$/PLGA microspheres has a more stable pH value than pure PLGA and crystal $CaSiO_3$/PLGA microspheres.

Figure 8. BMSCs growth in the transwell culture system loaded with different weight (15mg and 25mg) of PLGA, amorphous $CaSiO_3$/PLGA and crystal $CaSiO_3$/PLGA microspheres for 1 (a) and 7 (b) days. “15” and “25” stand for 15 and 25mg (the weight) of the corresponding microspheres. “Blank” stands for blank control without adding any microspheres.

Figure 9. The viability of BMSCs in the transwell culture system loaded with PLGA, amorphous $CaSiO_3$/PLGA and crystal $CaSiO_3$/PLGA microspheres for 7 days. The incorporation of $CaSiO_3$ powders into PLGA enhanced BMSC viability. BMSCs viability in crystal $CaSiO_3$/PLGA microspheres is higher than that in PLGA and amorphous $CaSiO_3$/PLGA microspheres. "15" and "25" stand for 15 and 25mg (the weight) of the corresponding microspheres. "Blank" stands for blank control without adding any microspheres.

Figure 10. The *in vivo* bone formation was assessed by H&E. (a) and (b) for PLGA microspheres; (c) and (d) for amorphous $CaSiO_3$/PLGA microspheres; (e) and (f) for crystal $CaSiO_3$/PLGA microspheres. (a), (c) and (e) are low magnification images by 4 times (×4); (b), (d) and (f) are higher magnification images by 40 times (×40). Crystal $CaSiO_3$/PLGA microspheres have more new bone formation than PLGA and amorphous $CaSiO_3$/PLGA microspheres.

Figure 11. The new bone volume in the defects implanting three microspheres by Micro-CT analysis. Both amorphous $CaSiO_3$ and crystal $CaSiO_3$ enhance the bone formation ability of PLGA microspheres. Crystal $CaSiO_3$/PLGA microspheres have an improved bone formation ability than amorphous $CaSiO_3$/PLGA microspheres.

Table 1. The surface area and pore volume of amorphous and crystal $CaSiO_3$ powders.

$CaSiO_3$ powders	Surface area (m^2/g)	Pore volume (cm^3/g)	Pore size (nm)
Amorphous	108.8	0.61	40
Crystal	4.4	0.02	--

Table 2. The concentrations of Ca and Si ions for PLGA, amorphous $CaSiO_3$/PLGA and crystal $CaSiO_3$/PLGA microspheres.

microspheres	Si (ppm)	Ca (ppm)
30% aCS/PLGA	20.0	90.1
30% cCS/PLGA	8.8	92.1
PLGA	0	101.3