Doped Tricalcium Phosphate Scaffolds by Thermal Decomposition of Naphthalene: Mechanical Properties and *In vivo* Osteogenesis in a Rabbit Femur Model

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Abstract

Tricalcium phosphate (TCP) is a bioceramic that is widely used in orthopedic and dental applications. TCP structures show excellent biocompatibility as well as biodegradability. In this study, porous β -TCP scaffolds were prepared by thermal decomposition of naphthalene. Scaffolds with 57.64 ± 3.54 % density and a maximum pore size around 100 μm were fabricated via removing 30% naphthalene at 1150°C. The compressive strength for these scaffolds was 32.85 ± 1.41 MPa. Furthermore, by mixing 1 wt % SrO and 0.5 wt % SiO₂, pore interconnectivity improved, but the compressive strength decreased to 22.40 ± 2.70 MPa. However, after addition of polycaprolactone (PCL) coating layers, the compressive strength of doped scaffolds increased to 29.57 ± 3.77 MPa. Porous scaffolds were implanted in rabbit femur defects to evaluate their biological property. The addition of dopants triggered osteoinduction by enhancing osteoid formation, osteocalcin expression and bone regeneration, especially at the interface of the scaffold and host bone. This study showed processing flexibility to make interconnected porous scaffolds with different pore size and volume fraction porosity with high compressive mechanical strength and better bioactivity. Results show that SrO/SiO₂ doped porous TCP scaffolds have excellent potential to be used in bone tissue engineering applications.

Keywords

bone tissue engineering; TCP scaffolds; *in vivo* response; SrO and SiO₂ dopants; PCL coating

1. Introduction

β -tricalcium phosphate (β -TCP) has long been considered as a key biomaterial for orthopedic and dental applications because of its outstanding biocompatibility as well as biodegradability. The compositional similarity between TCP and bone makes TCP an ideal candidate for orthopedic and dental applications. Biodegradability of TCP comes from the chemical dissolution *in vivo*. Inherent biodegradability [solubility product ($K_{\text{sp}} = 1.25 \times 10^{-29}$)] allows TCP scaffolds to degrade slowly, which is neither too long such as hydroxyapatite (HA) ($K_{\text{sp}} = 2.35 \times 10^{-59}$), nor too short like α -TCP ($K_{\text{sp}} = 3.16 \times 10^{-26}$).^{1,2}

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Tailored degradation in β -TCP can help with healing bone defects by slowly transferring the mechanical load to the newly formed tissue and maintaining the support structure.^{1,3-5}

Aside from biocompatibility and biodegradability, porosity and mechanical property of scaffolds are also important factors that can affect bone regeneration. Interconnected pores can not only interlock with host tissues, allowing cells to grow and substitute the implant, but can also be a pathway for nutrient supply during healing. It is reported that only interconnected macropores larger than 300 μm are beneficial for osteogenesis and angiogenesis,⁶⁻⁸ while the minimum effective recommended pore diameter is 100 μm for bone tissue ingrowth and nutrient supply.⁹⁻¹¹ Besides pore size, pore volume is also crucial in scaffold performance *in vivo*. Even though higher pore volume provides more space for bone tissue in-growth, such a design also reduces mechanical strength of the scaffolds substantially.¹² Overall, scaffolds used in bone tissue engineering usually have pore volume in the range of 30 to 70%.¹³ Thus the mechanical property improvement along with desired pore size of porous β -TCP is essential for accelerated osteogenesis and angiogenesis *in vivo*.

Polymer infiltration is an effective approach to enhancing mechanical property of brittle ceramics due to their excellent flexibility and toughness. Polycaprolactone (PCL) is one of the widely used polymers in bone tissue engineering. It has been shown that the infiltration of PCL can potentially enhance mechanical properties including compressive strength of porous ceramic scaffolds.^{14,15} In addition, as a commonly used biomaterial, PCL is well known for its biocompatibility and sustained biodegradability.¹⁵⁻¹⁹ Previous research has employed PCL in combination with calcium phosphate to fabricate scaffolds for bone tissue reconstruction.²⁰ Another application of PCL is to encapsulate bone growth factor such as rhBMP-2 for drug delivery.²¹ Many devices with PCL have already been approved by the US Food and Drug Administration.^{22,23}

Various approaches such as fuse deposition,^{24,25} 3D-printing,²⁶ electrospinning,²⁷ freeze-casting²⁸ and thermal decomposition²⁹ have been applied to the fabrication of porous ceramic scaffolds. Thermal decomposition is thought to be a cost-effective way to fabricate porous scaffolds because it creates pores simply by burning off the porogen during heat treatment without the need of any expensive equipment. As for the selection of porogen, a category of porogens such as PEG,³⁰ sucrose³¹ and naphthalene^{32,33} have already been exploited for preparing porous scaffolds. Naphthalene is the porogen that is easy to grind from the large chunk to powder of the required size. One concern of naphthalene porogen is its toxicity. Since naphthalene sublimates even at room temperature, the complete removal is entirely achievable during high temperature sintering.

Numerous studies have used dopants to address concern related to the lack of osteoinduction for calcium phosphate scaffolds during bone regeneration. It has been demonstrated that SrO can enhance the replication of preosteoblastic cells, stimulate bone formation and suppress bone resorption.³⁴⁻³⁷ Previous research also indicated that Si can stimulate cellular activities such as proliferation and differentiation of osteoblast-like cells, mineralization of human osteoblasts and osteogenic differentiation of mesenchymal stem cells.^{1,26,38-41} In addition, substitution of dopants, such as SrO and SiO₂, into TCP structure causes crystal structure change and is believed to be the reason for improved stability and bioactivity in TCP.^{38,42,43}

Since our preliminary mechanical and *in vitro* work with SrO and SiO₂ doped dense TCP ceramic compacts showed exciting results⁴³, therefore, we incorporate interconnected porosity to improve biological properties of scaffolds and follow up with detail *in vivo* studies in this present work. We hypothesize that porous TCP scaffolds with SrO/SiO₂ dopants prepared by naphthalene porogen will offer better osteogenic property than undoped TCP. We also hypothesize that infiltration of PCL can further improve mechanical property of these scaffolds. To validate our hypothesis, porous scaffolds with or without dopants were processed. Scaffolds were also coated with different molecular weight of PCL to measure improvement in mechanical property of TCP scaffolds. Apart from physical, mechanical and *in vitro* biological characterization, samples were implanted in rabbit distal femur for 16 and 20 weeks to evaluate their biological response *in vivo*.

2. Materials and methods

2.1 Materials

High purity (99.99%) β -TCP powder was purchased from Berkeley Advanced Biomaterials Inc. (Berkeley, CA) with an average particle size of 550 nm. The mothball (99% naphthalene) was purchased from Walmart (Pullman, WA). Polyvinyl alcohol powder (PVA), which was 87–89% hydrolyzed, and silicon dioxide (SiO₂, Puratronic, 99.999% purity) were procured from Alfa Aesar (MA, USA). Strontium oxide (SrO, 99.9% purity) was purchased from Aldrich (St Louis, MO, USA). Two different kinds of polycaprolactone (PCL) (Mw = 14000 and Mw = 50000) were procured from SIGMA-ALDRICA Co. (St Louis, MO, USA). All other chemicals were of analytical grade and used without further purification.

2.2 Processing of naphthalene/ β -TCP scaffolds

For porogen preparation, first, grinding procedures were done by mortar and pestle to break moth balls into smaller pieces. Those pieces were ball milled with zirconia balls for 1h to get homogeneous naphthalene powder particles. Then naphthalene was sieved by two different sieves, 180 μ m and 212 μ m. The powder passed through the sieve with 212 μ m mesh, but got stuck in the sieve with 180 μ m mesh, i.e., -212 μ m but +180 μ m, was used as porogen.

For pure β -TCP scaffolds, 2 wt % PVA, as binder, was mixed with β -TCP powder in water for 2h followed by overnight drying. As for the preparation of doped TCP scaffolds, dopants, 1 wt % SiO₂ and 0.5 wt % SrO, were mixed with β -TCP in ethanol via zirconia milling media for 6h. After that, powders were dried in an oven for one day at 40 °C and another day at 90 °C, following the same procedure with PVA binder. Finally, both pure and doped β -TCP powders were ball milled separately for another 1h to break the agglomeration.

Pure and doped β -TCP powders were mixed with porogen via ball milling for 2h. The porogen ratio of 30 wt % and 40 wt % was utilized to process scaffolds. Green scaffolds were fabricated in a cylinder shaped mold (6mm in diameter) by uniaxial pressing at 1 MPa for 30 sec. Scaffolds were then placed in an oven at 80 °C for 1d to remove the naphthalene via sublimation. Final scaffolds were prepared by sintering at 1150 °C and 1250 °C in a muffle furnace for 2h.

2.3 PCL infiltration for the naphthalene/ β -TCP scaffolds

PCL, $M_w = 14000$ and $M_w = 50000$, was utilized to improve mechanical property of scaffolds. First PCL solution was prepared using acetone as the solvent to acquire 10% PCL solutions of both molecular weights. PCL infiltration procedures were completed using a food saver machine (food saver V2244, Walmart, Pullman, WA, 99163). The infiltration force provided by the vacuum in the food saver machine helped PCL infiltration and coating on inside surfaces of scaffolds. All samples were treated with four vacuum cycles for PCL infiltration before characterization. All processing steps are illustrated in Fig. 1(a).

2.4 Physical and mechanical property evaluation

Phase analysis of sintered β -TCP scaffolds was conducted using X-ray diffraction (XRD) with a Philips PW 3040/00 Xpert MPD system (Philips, Eindhoven, The Netherlands) with CuK_α radiation and a Ni filter. Samples were scanned over a range of 20 and 60 degrees at a step size of 0.02 degree and count time of 0.5s per step. Phase percentage of α -TCP in the sintered scaffolds was determined from the relative intensity ratio of the corresponding major phases using the following relationship:^{44, 11}

Percent of the phase to be determined = Relative intensity ratio of the phase $\times 100$

$$\text{Relative intensity ratio} = \frac{\text{Intensity of the major peak of the phase to be determined}}{\sum \text{Intensity of major peaks of all phases present}}$$

Microstructures of sintered samples were taken by using a field-emission scanning electron microscope (FESEM) (FEI Inc., Hillsboro, OR, USA). Pore size was estimated from those images. Grain size was calculated via mean lineal intercept length method per ASTM Standard E 112–88. Back scattered SEM images were used for PCL coating on β -TCP. Apparent density of scaffolds was measured using Archimedes' principle. Compressive strength was measured using a screw-driven universal testing machine (AG-IS, Shimadzu, Japan) with a constant crosshead speed of 0.33 mm/min and a load cell of 50 KN. Mechanical data was presented as mean $\pm$ standard deviation based on five samples.

2.5 Biological characterizations

2.5.1 *In vitro* cell-material attachment—*In vitro* bone cell-material interactions on sintered scaffolds were investigated using human fetal osteoblast cells (hFOB) at three days of incubation. Cells were from the immortalized osteoblastic cell line derived from human bone tissue. Samples were sterilized by autoclaving at 121 °C for 3 min prior to cell culture. Aliquots of 150 μl cell suspension containing 2×10^5 cells were seeded by unidirectional seeding directly onto each sample in the wells of 24-well plates. After cell seeding, a 1 ml aliquot of DMEM enriched with 10% fetal bovine serum was added to the surrounding of each sample. Cultures were maintained at 37 °C in a 5% CO_2 /95% humidified air atmosphere. The culture media was changed every alternate day for the duration of the experiment. Then samples for testing were removed from the culture after 3 days of incubation. Samples for SEM observation were fixed with 2% paraformaldehyde/2%

glutaraldehyde in 0.1M cacodylate buffer overnight at 4 °C. Post-fixation was performed with 2% osmium tetroxide (OsO₄) for 2 h at room temperature. The fixed samples were dehydrated in an ethanol series (30%, 50%, 70%, 95% and 100% three times), followed by a hexamethyldisilane (HMDS) drying procedure. After gold coating, samples were observed under field emission scanning electron microscope (FESEM) (FEI 200F, FEI Inc., OR, USA).

2.5.2 *In vivo* study

Rabbit implantation procedure: To study initial *in vivo* bioactivity of these scaffolds, six female New Zealand white rabbits (Western Oregon Rabbit Company, Philomath, OR, USA) were used in this study. Rabbit surgery began with sterilizing instruments, supplies and wound closure material. Subcutaneous injection of buprenorphine (0.03 mg kg⁻¹ body weight) as premedication followed 1 h later by a mixture of ketamine (15 mg kg⁻¹ body weight) and medetomidine (0.25 mg kg⁻¹ body weight) was performed for anaesthetic induction. A mixture of isoflurane and oxygen gas anesthesia (by mask) was used as needed to maintain a surgical plane of anesthesia. After shaving and disinfection, defects with 5 mm width and 6 mm depth were drilled into the right and left distal femur. The defect was created in the lateral condyle by means of a 4 mm drill and is subsequently enlarged with 5 mm drills. The cavity was rinsed with physiological saline and any bone fragments washed out. The size of the defect was checked using a 6 by 10 mm deep standard cylinder. This defect did not create any lameness and signs of analgesia were minimal. Porous scaffolds, pure and SrO/SiO₂ doped conventionally sintered at 1150 °C, adopting 30% naphthalene as porogen, were pressed into femur defects of the same rabbit. At 16 and 20 weeks post-surgery, intramuscular injection of telazol®-ketamine-xylazine (TKX) was completed for sedating 3 rabbits at each time point. Finally, rabbits were euthanized by an intracardiac injection of sodium pentobarbital. All experimental procedures were performed based on the protocol approved by the Institutional Animal Care and Use Committee (IACUC) of Washington State University (Pullman, WA).

2.5.3 Histomorphology—Bone–implant specimens were fixed in 10% neutral buffered formalin solution for two days and dehydrated in graduated ethanol and acetone (70% ethanol, 95% ethanol 100% ethanol, ethanol: acetone (1:1) and 100% acetone) series. After embedding samples with Spurr's resin, each undecalcified implant block was sectioned perpendicular to the cylinder outside surface of the scaffold using a low-speed diamond saw. After polishing, the sections were stained by Goldner–Masson trichrome (GMT) stain and observed under a light microscope (Olympus BH-2, Olympus America Inc., USA).

2.5.4 Immunohistochemistry—Osteocalcin immunostaining was performed on formalin-fixed paraffin embedded samples using the ImmunoCruz™ staining kit (sc-2050, Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Samples were cut to 10–20 µm thin sections using a microtome (Leica microsystems, Heidelberg, Germany), deparaffinized in xylene and rehydrated in ethanol series and water. Then antigen retrieval was achieved by keeping sections in citrate buffer at 95 °C for 20 min. Endogenous peroxidase activity was quenched by peroxidase block solution for 5 min in an incubator. Subsequently, unspecific binding of the secondary antibody was blocked by incubating in serum block solution for 20

min. Sections were then stored at room temperature with diluted primary antibody against osteocalcin (ab13420, Abcam, MA, USA) followed by 30 min incubation in biotinylated secondary antibody. Peroxidase activities in sections were visualized by diaminobenzidine (DAB). Finally, sections were counterstained by hematoxylin and visualized under a light microscope (Olympus BH-2, Olympus America Inc., USA).

3.0 Results

3.1 Microstructure and phase analysis

Fig. 2 shows the XRD plot of pure and 1 wt % SrO/0.5 wt % SiO₂ doped β -TCP after sintering at 1150 °C and 1250 °C. The intensity data was plotted against two theta values. XRD spectra for scaffolds correspond well with the characteristic peaks of β -TCP (JCPDS # 09-0169) and α -TCP (JCPDS # 09-0348). Major β -TCP peaks were identified and labeled. Featured α -TCP peaks were also marked. β -TCP (0 2 10) and α -TCP (1 7 0) peaks are used, respectively, to estimate the percentage of α phase after the sintering, which is also shown in Table 1. There is no α -TCP at 1150 °C sintered samples. However, for samples sintered at 1250 °C, presence of α -TCP was detected.

Fig. 3 and Fig. 4 show microstructures of scaffolds for different composition and manufacture processes. All prepared scaffolds, no matter what the composition was and how they were processed, had a maximum pore size around 100 μ m. The difference in microstructure was observed in their pore distribution. For 40% porogen ratio and 1 wt % SrO/0.5 wt % SiO₂ composition, more pores were distributed on transverse surface of scaffolds compared with the scaffold using 30% porogen ratio, as shown in Fig. 3. Especially for scaffolds with dopants, larger pore size and higher amount of porosity were found under scanning electron microscope. Figs. 4 (a) and (b) show high magnification images of scaffolds showing increase in average grain size from $0.97 \pm 0.22 \mu$ m to $1.30 \pm 0.24 \mu$ m with the addition of dopants.

The morphology of PCL (Mw = 14000) after infiltration is like layers of coating on top of transverse surfaces of scaffolds under back-scattered imaging in SEM, as shown in Figs. 5 and 6 (a). (b) and (c). However the infiltrated PCL (Mw = 50000) is fiber like inside scaffolds, which can be seen from Figs. 5 (d), (e) and (f).

3.2 Density and compressive strength

Table 1 shows results for density, compressive strength and alpha phase percentage of the TCP. Four processing parameters are changed to optimize scaffold preparation: porogen ratio, sintering temperature, dopants addition and PCL infiltration. Scaffolds with 30% naphthalene had a total porosity of $42.36 \pm 3.54 \%$ (29.07% from open porosity). Compressive strengths of those samples were 32.85 ± 1.41 MPa. With increasing the porogen ratio from 30% to 40%, the total porosity increased to $53.16 \pm 0.74 \%$ (38.83% from open porosity), but higher porosity decreased the compressive strength to 16.45 ± 1.87 MPa, as expected. Through increasing sintering temperature from 1150°C to 1250°C for 40% naphthalene β -TCP scaffolds, total porosity decreased from $53.16 \pm 0.74\%$ (38.83% from open porosity) to $50.72 \pm 0.45\%$ (45.6% from open porosity). However higher open porosity and 20% α -TCP decreased the compressive strength to 14.65 ± 0.74 MPa. Adding

dopants (1 wt % SrO and 0.5 wt % SiO₂) to 30% porogen samples maintained the same total porosity, but open porosity increased to 37.15%, which caused the compressive strength decrease to 22.40 ± 2.70 MPa. Finally, by incorporation of PCL (Mw = 14000 and Mw = 50000) to TCP, the mechanical property for pure β -TCP scaffolds remained nearly the same. However, compressive strength of 1 wt % SrO/0.5 wt % SiO₂ doped samples improved from 22.40 ± 2.70 MPa to 29.57 ± 3.77 MPa and 26.20 ± 0.78 MPa via infiltrating 1.97 ± 0.28 % (Mw = 14000) and 1.35 ± 0.19 % (Mw = 50000) of PCL into the scaffold, respectively.

3.3 *In vitro* cell material interaction study, *in vivo* histomorphology analysis and immunohistochemistry

In vitro bone cell-material interactions on sintered scaffolds using human fetal osteoblast cells (hFOB) at three days of incubation showed excellent biocompatibility of these scaffolds, as shown in Fig. 7. *In vivo* studies showed new bone formation on the outer surface of scaffolds, as shown in Figs. 8 (a), (b), (c) and (d). Scaffolds implanted as long as 20 weeks in rabbit distal femur model showed more new bone formation than 16 weeks' time point. Significant increase of osteoid area indicated that bone regeneration was effectively enhanced by SrO/SiO₂ scaffolds compared to pure β -TCP control samples after 16 and 20 weeks' implantation.

Osteocalcin expression, as shown in Figs. 9 (a), (b), (c) and (d), was predominantly found in the area close to edges of scaffolds, but it started to penetrate into center of scaffolds after 20 weeks, especially for doped samples which was shown in Fig. 9 (d). Higher osteocalcin expression of doped scaffolds was detected at 20 weeks than at 16 weeks, but for pure samples the expression standard was not substantially affected. And at both time points, significant increase of osteocalcin expression was observed via adding dopants, which demonstrated SrO/SiO₂ effectively induced osteocalcin expression.

4.0 Discussion

Scaffolds for bone tissue engineering require interconnected porosity for effective bone healing. The decrease in compressive strength due to increasing porogen ratio from 30% to 40% is a result of increased porosity, especially open porosity.^{12,45} Even though more open porosity is beneficial for bone ingrowth, *in vivo* osteointegration and infiltration of polymer, as well as mechanical property of scaffolds are also critical.⁴⁶ Only when scaffolds can withstand *in vivo* biomechanical stresses at the defect site, scaffolds are effective in repair and reconstruction of bone disorders.^{11,47}

We have previously reported that 3D-printed porous β -TCP scaffolds improved their mechanical property by increasing the sintering temperature to 1250°C.¹¹ Similar results have also been reported by others showing that sintering above the transformation temperature of β - to α -TCP improves mechanical property.⁴⁸ However in this work, compressive strength of scaffolds using 40% porogen decreased from 16.45 ± 1.87 MPa to 14.65 ± 0.74 MPa due to increasing the sintering temperature from 1150°C to 1250°C. At 1250 °C, phase transformation of β - to α -TCP decreased the density from 3.07 g/cm³ to 2.86 g/cm³, respectively.⁴⁹ Development of microcracking due to expansion with the presence of the new phase can cause early mechanical failure.^{48,49} Finally, both the total porosity

(closed and open) and open porosity for sintering at 1250 °C is higher compared to 1150 °C sintered samples, a factor that will also contribute to lowering the compressive strength further.

Addition of dopants decreased compressive strength from 32.85 ± 1.41 MPa to 22.40 ± 2.70 MPa without any presence of α -TCP phase. Among other factors, this decrease is attributed to an increase in grain size for doped samples. Similar results were also reported for the grain size increase during the sintering for SrO-doped compacts, which also decreased mechanical property of scaffolds.⁴³ Pore size for doped samples is also bigger than pure samples, which contributes further to the drop in compressive strength. PCL was used for reinforcements of pure and SrO/SiO₂ doped TCP scaffolds. Either coating or fiber like PCL was further stretched by vacuum force in vertical direction to improve mechanical property.⁵⁰ A 10% PCL of Mw = 14000 solution was found easier to infiltrate inside porous scaffolds than the same concentration solution of PCL of Mw = 50000. The relatively low molecular weight made the solution less viscous. The mechanical property improvement for PCL with Mw = 14000 is found to be more effective than PCL of Mw = 50000. Meanwhile, the increase in open porosity produced by dopants addition is beneficial for mechanical property enhancement via PCL infiltration, as larger fraction of interconnected pores provide more room for PCL to attach and support the struts. This observation is clear from data presented in Table 2.

In earlier studies we have shown that TCP with 1 wt % SrO/0.5 wt % SiO₂ is non-toxic.⁴³ The porogen used in the present study, naphthalene, can be removed completely during sintering. Thus only a short period of cytotoxicity test for 3d was performed to validate our hypothesis that the use of dopants and porogen do not adversely affect cells and scaffold-materials interaction. Healthy cells can be clearly seen in Fig. 7, which justifies the feasibility for *in vivo* study.

Osteoid is the unmineralized bone matrix that forms prior to the maturation of bone tissue. Presence of osteoid shows early bone formation at the interface of the scaffold and the host bone.^{51,52} Osteocalcin, the most abundant noncollagenous protein, is a bone maturation marker.^{53,54} These two markers are commonly used to detect early bone formation and bone mineralization. Interface enhancement /pore characteristics such as pore size and interconnection also play a critical role in osseointegration. Both osteoid area and osteocalcin expression, as shown in Fig. 8 and Fig. 9, are observed near edges of scaffolds showing higher interfacial interactions between the scaffolds and the host tissue. However as implants gradually degrade in host environment, there is clear evidence of increased osseointegration into the scaffolds. This is evident from increased osteoid formation and osteocalcin expression at 20 weeks compared to the 16 weeks data, where both start to penetrate to the center of scaffolds.

Compared to pure TCP scaffolds, both osteoid and osteocalcin expression increase in doped scaffolds. Scaffolds with dopants have higher porosity, not only total porosity, but also open porosity, which is easier for cells to penetrate inside and grow. Also it has been reported that SrO and SiO₂ can regulate and induce bone regeneration. SrO has been reported to be beneficial for inducing osteogenesis through increased β -catenin formation which increases

osteogenic differentiation of mesenchymal stem cells.⁵⁵ SiO₂ is also a key factor for osteoblastic cell differentiation^{56–58} as well as increasing bone mineralization.⁵⁹ Hence, significant increase in osteoid and osteocalcin formation is found in doped scaffolds after 16 and 20 weeks' implantation, as shown in Fig. 8 and Fig. 9. Osteocalcin expression images show bone maturation initiation appears as early as 16 weeks for both pure and SrO/SiO₂ doped scaffolds, however, for SrO/SiO₂ doped scaffolds, it accelerates faster with time. Therefore, SrO and SiO₂ presence effectively enhance osteogenesis in both early bone formation and bone maturation. This study shows processing flexibility to make interconnected porous scaffolds with different pore size and volume fraction porosity with high compressive mechanical strength and better *in vivo* bioactivity in rabbit distal femur model.

5.0 Conclusions

Porous TCP and SrO /SiO₂ doped TCP scaffolds with both high compressive strength and excellent biocompatibility and osteogenesis were processed by naphthalene removal method. A compressive strength of 29.57 ± 3.77 MPa was achieved for doped scaffolds after 10% PCL of Mw = 14000 infiltration. Both pure and doped scaffolds were inserted to rabbit femur defects to test *in vivo* biological response of these scaffolds. Results showed that SrO and SiO₂ can effectively enhance osteoblast cells differentiation and maturation. Such results validate our hypothesis that addition of dopants can enhance biological response compared to pure TCP ceramic scaffolds. Porous scaffolds processed by this method show high compressive strength and excellent *in vitro* and *in vivo* biological response, which is promising for further studies in bone tissue engineering.

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Fig. 1.

(a) Processing illustration of porous TCP scaffolds by thermal decomposition of naphthalene and following PCL infiltration. (b) Full image of scaffolds

Fig. 2.

XRD plot of porous β -TCP and 1 wt % SrO/0.5 wt % SiO₂ doped β -TCP scaffolds after sintering. Pure β -TCP peaks are identified by their [h k l] lattice values, α symbol denotes α -TCP peaks. The characteristic peaks of β -TCP and α -TCP are based on (JCPDS # 09-0169) and (JCPDS # 09-0348).

Fig. 3.

Microstructure of scaffolds prepared by naphthalene, 2% PVA and pure β -TCP with or without SrO/SiO₂ dopant sintered at 1150 °C or 1250 °C for 2h. Both periphery and transverse surfaces are observed by SEM.

Fig. 4.

High magnification images for transverse surface of (a) pure β -TCP (grain size around $0.97 \pm 0.22 \mu\text{m}$) and (b) 1 wt % SrO/0.5 wt % SiO_2/β -TCP (grain size around $1.30 \pm 0.24 \mu\text{m}$) scaffolds sintered at 1150 °C for the grain size analysis.

Fig. 5. Microstructure of 1 wt % SrO/0.5 wt % SiO₂ doped β -TCP scaffolds after 10% PCL (Mw = 14000 and Mw = 50000) infiltration by vacuum for four cycles through the food saver machine. (a), (b) and (c) are infiltrated with PCL (Mw = 14000). (d), (e) and (f) are infiltrated with PCL (Mw = 50000).

Fig. 6. SEM and BSE images of 1 wt % SrO/0.5 wt % SiO₂ doped β -TCP scaffolds after 10% PCL (Mw = 14000) infiltration for four cycles by the food saver machine. (a) and (b) are SEM images. (c) and (d) are BSE images.

Fig. 7. SEM images of cytotoxicity test after incubation at 37 °C for three days for both pure β -TCP scaffolds and 1 wt % SrO/0.5 wt % SiO₂ doped TCP scaffolds. The surface of samples is covered with cells after incubation because all grain boundaries are unrecognizable.

Fig. 8.

(a), (b), (c) and (d) are panorama images of implant sections stained with Goldner's trichrome. The CaP implants are identified by gray/green/black color and osteoid formation by orange. (e) is histomorphometry analysis of osteoid fraction on trichrome sections. Osteoid fraction is calculated from osteoid area divided by a partial ring area which is close to the edge of scaffolds before panorama stitching (*P < 0.05, where n = 3).

Fig. 9.

(a), (b), (c) and (d) are expression of osteocalcin in pure TCP implants and SrO/SiO₂ doped TCP implants after 16 and 20 weeks in a rabbit distal femur model. Brown area indicates osteocalcin expression. Black lines illustrate the interface between implant and host tissue. The side labeled with “scaffold” is the transverse surface of scaffolds. (e) is immunohistochemical analysis of osteocalcin expression. For (a), (b) and (c), osteocalcin percentage is calculated from osteocalcin area divided by a partial ring area between the black and red line. For (d), it is calculated from osteocalcin area divided by the whole scaffold area on right of the black line (*P < 0.05, where n = 3).

Table 1

The density, compressive strength and phase analysis of different porogen ratio samples after conventional sintering at 1150 °C or 1250 °C (n = 5). All density data is normalized to theoretical density of β -TCP i.e., 3.14 g/cm³.

Composition	Sintering Condition	Relative Bulk Density (%)	Apparent density (%)	Total porosity (%)	Open porosity (%)	Compressive Strength (Mpa)	Alpha phase (%)
30% naphthalene pure TCP	1150 °C 2h	57.64±3.54	86.71±3.05	42.36±3.54	29.07	32.85±1.41	0
30% naphthalene 1% SrO/0.5% SiO ₂ doped pure TCP	1150 °C 2h	57.94±0.75	95.09±2.51	42.06±0.75	37.15	22.40±2.70	0
40% naphthalene pure TCP	1150 °C 2h	46.84±0.74	85.67±2.31	53.16±0.74	38.83	16.45±1.87	0
	1250 °C 2h	49.28±0.45	94.88±3.41	50.72±0.45	45.6	14.65±0.74	20

Table 2

The density, compressive strength and percentage of PCL infiltrated for scaffolds sintered at 1150 °C after PCL infiltration by 10% PCL (Mw = 14000 and Mw = 50000) solution. Percentage of PCL after infiltration is calculated from weight of PCL divided by weight of scaffolds (n = 5). All density data is normalized to theoretical density of β -TCP i.e., 3.14 g/cm³.

Composition	10% w/v PCL (Mw=14000)			10% w/v PCL (Mw=50000)		
	Bulk Density (%) before the infiltration	Percent of PCL after the infiltration	Compressive Strength (MPa)	Bulk Density (%) before the infiltration	Percent of PCL after the infiltration	Compressive Strength (MPa)
30% naphthalene pure β -TCP	57.73 $\pm$ 1.94	1.91 $\pm$ 0.18	32.00 $\pm$ 5.29	58.54 $\pm$ 1.04	1.22 $\pm$ 0.03	30.98 $\pm$ 3.79
30% naphthalene doped β -TCP	56.81 $\pm$ 3.21	1.97 $\pm$ 0.28	29.57 $\pm$ 3.77	57.43 $\pm$ 1.49	1.35 $\pm$ 0.19	26.20 $\pm$ 0.78