SrO and SiO_2 Doped Tricalcium Phosphate bone tissue engineering Scaffolds with improved mechanical and biological properties

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Statement of Purpose: Tricalcium phosphate (TCP) scaffolds are preferred for bone tissue reconstruction due to its compositional similarity with bone mineral. However reliable mechanical property of TCP scaffolds is a challenge for increasing needs of bone tissue therapies. Many different methods, such as porogen removal and 3D-printing, can be used to fabricate porous TCP scaffolds. We have used naphthalene as a porogen, to produce pores by sublimation. Strontium oxide (SrO) and Silica (SiO_2) as dopants, are also added to be beneficial to biological property and microstructure of scaffolds [1, 2]. **Objective** of this research is to understand the effects of porogen to TCP ratio, dopants addition and sintering cycle, on biological and mechanical properties of TCP scaffolds. Our **hypothesis** is that mechanical property will be improved by manipulating processing parameters and dopants while improving *in vitro* bone cell proliferation, differentiation and *in vivo* osteogenesis.

Methods: Scaffolds were made using TCP powders. 2wt.% PVA was added as binder to the slurry containing TCP. Dried powder was mixed with desired amount of naphthalene. The mixture was molded and sintered at two different temperatures: 1150 and 1250 ºC. Doped scaffolds were made by mixing 0.5 wt.% SiO_2 and 1 wt.% SrO with TCP powder. Phase composition was evaluated using X-ray diffraction (XRD) method. Instron machine was used to evaluate compressive strength of the scaffolds. Microstructure was observed under a scanning electron microscope (SEM). Pure and doped scaffolds were implanted into rabbit femoral condyle defect model for *in vivo* experiment. New bone formation was examined by histomorphology and immunohistochemistry after 16 and 20 weeks post implantation.

Results: Figure 1 shows the XRD patterns of pure and doped scaffolds sintered at 1150 and 1250 ºC. β to α phase transformation was observed at 1250 °C. Scaffolds sintered at 1150 ºC showed a compressive strength of 32.85 ± 1.41 and 22.40±2.70 MPa for pure and Si-Sr-doped TCP scaffolds, respectively. The decrease in compressive strength of the doped scaffolds is mainly caused by increased open porosity from 29.07% to 37.15% due to dopants addition.

Figure 2 and **Figure 3** show osteoid like new bone formation and osteocalcin expression. Doped samples showed higher bone formation and osteocalcin expression at both time points which is in line with reported literature results showing that Sr and Si addition can accelerate osteoblast cell proliferation and differentiation [3,4]. In addition, bone growth was enhanced better in both pure and doped samples after 20 weeks of implantation, compared to the 16 weeks.

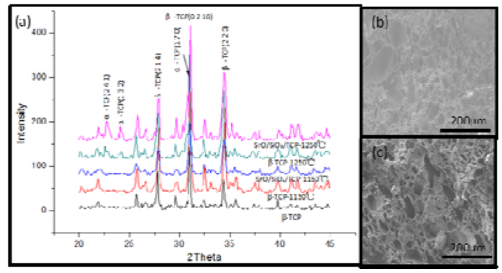

Figure 1. (a) XRD patterns of pure and doped TCP scaffolds sintered at 1150 and 1250 ºC, SEM micrographs of (b) doped and (c) pure scaffolds sintered at 1150 ºC.

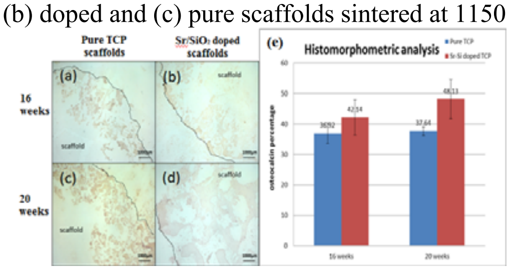

Figure 2. (a), (b), (c) and (d) are osteocalcin expression images. Scaffold's area, which is labeled, and host tissue are separated by a curved line and brown area indicatuing osteocalcin expression. (e) histomorphometric analysis of osteocalcin fraction in pure and doped scaffolds after 16 and 20 weeks implantation(n=4 p=0.05).

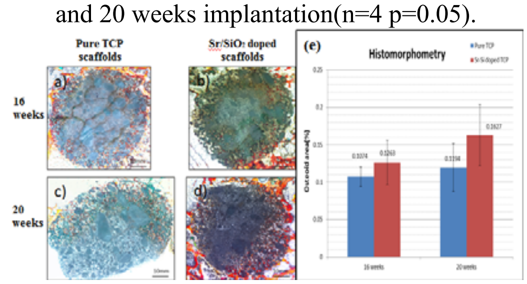

Figure 3. (a), (b), (c) and (d) are histomorphological graphs by trichrome staining. Orange area denotes osteoid. (e) histomorphometric analysis of osteoid fraction for pure and doped scaffolds after 16 and 20 weeks (n=4 p=0.05).

Conclusions: Porous TCP scaffolds were fabricated by naphthalene removal method with macroporosity around 100µm. Improved osteoid formation and osteocalcin expression in doped scaffolds showed that this kind of scaffolds is very promising to be used as bone fixation. Authors would like to acknowledge the financial support from National institute of Health (Grant # NIH-R01-EB-007351).

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