SiO2 and ZnO Dopants in 3D Printed TCP Scaffolds Enhances Osteogenesis and Angiogenesis *in vivo*

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Statement of Purpose: β-tricalcium phosphate (β-TCP) makes an ideal choice of material for orthopedic scaffolds due to its excellent biocompatibility, but the material itself is merely osteoconductive. The current trend in orthopedics technologies is to investigate methods to add osteoinductivity, or the ability to propagate active bone formation. There have been many studies demonstrating the importance of trace elements in bone formation, specifically zinc and silicon. Silicon has been noted to be an important trace element in osteogenesis, with research results indicating a strong stimulatory effect on cellular activities such as proliferation, differentiation, and mineralization of osteoblast cells as well as facilitating osteogenic differentiation of mesenchymal stem cells [1–3]. Zinc, also considered an essential trace element, is released during the skeletal breakdown process and has demonstrated the ability to inhibit osteoclastic bone resorption[4,5]. It also boosts osteogenic characteristics by inducing osteoblastogenesis as well as osteoblastic differentiation and mineralization [6,7]. The objective of this study is to understand the effect of dopant chemistry on *in vivo* osteogenesis and angiogenesis in tricalcium phosphate (TCP) 3-D printed scaffolds. Our working hypothesis is optimal presence of dopants and designed microstructure can produce bone scaffolds that can induce new bone growth by affecting both angiogenesis as well as osteogenesis. The rationale for this study is if we can optimize the amount and chemistry of dopants in TCP, along with microstructure, we will be able to produce scaffolds with desired properties.

Methods: Powders were made in batches of 100g and doped with 0.25 wt % ZnO and 0.5 wt % SiO_2 as a binary dopant system, ball milling in ethanol for 6 h and then dried. Cylindrical scaffold CAD files (diameter 7 mm and height 10.5 mm) were created with interconnected channels of 1000µm, 750µm, and 500µm. Scaffolds were fabricated using a 3-D printer (R-1 R&D printer by ProMetal, Irwin, Pa) and sintered at 1250 ˚C for 2 hr. Bicortical femoral defects were made in a rat model and samples were analyzed by various methods at 6, 8, 12 and 16 weeks.

Results: Samples stained with Goldner's Trichrome (**Figure 1**) showed evidence of increased bone growth in doped samples at early time points, with nearly complete infiltration in both samples by 12 weeks. Immunohistochemical staining, demonstrated increased Collagen I (**Figure 2**) formation as well as increased Osteocalcin (**Figure 3**) in samples containing SiO_2/ZnO dopants. von Willebrand factor (vWF) staining (**Figure 4**) also shows significant increase in new blood vessel formation in samples containing the dopants.

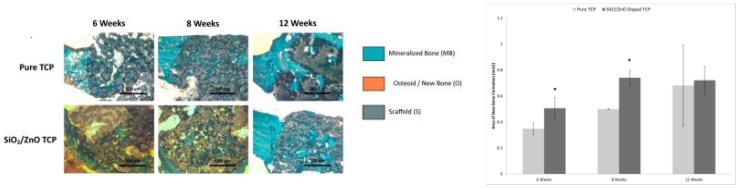

Figure 1. Left- Goldner's Trichrome staining of samples up to 12 weeks. Right – histomorphological analysis.

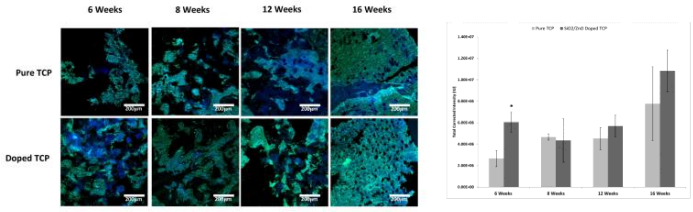

Figure 2. Collagen I formation up to 16 weeks. Green indicates collagen I, wile blue indicates cell nuclei

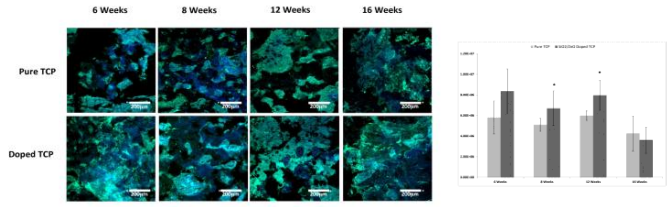

Figure 3. Osteocalcin formation up to 16 weeks. Green indicates Osteocalcin, wile blue indicates cell nuclei

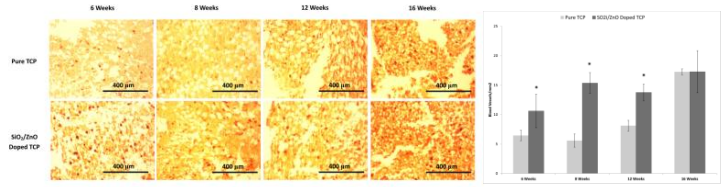

Figure 4. vWF staining up to 16 weeks. Red staining indicates presence of blood vessel.

Conclusions: The addition of SiO_2 and ZnO dopants into TCP scaffolds resulted in an increase in new bone growth and angiogenesis. This is likely to the modulation of collagen I formation at early time points, which can affect the downstream osteocalcin production in osteoblasts [8]. Enhanced angiogenesis is likely due to increased angiogenic factor production in osteoblasts, such as VEGF due to the incorporation of Zn^{2+} into the scaffolds [9]. Authors would like to acknowledge the financial support from the NIH (NIBIB grant # NIH-R01-EB-007351).

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