3D Printed Tricalcium Phosphate Scaffolds: MgO and SiO2 Doping for Enhanced Osteogenesis and Angiogenesis

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Introduction: Wide clinical applications of calcium phosphates (CaPs) bioceramics are due to their compositional similarities to bone mineral, excellent biocompatibility, bioactivity and non-immunogenicity [1]. Angiogenesis or new blood vessel formation is required for any tissue-engineered constructs to be functional. Survival of any tissue is dependent on the nutrient and oxygen supply from blood vessels [2]. Tissue engineering scaffolds with 3D interconnected porosity induce osteogenesis from surrounding cells and tissues through tissue ingrowth and nutrient transport into interconnected macro pores. Trace elements substitution in CaPs can influence the mechanical properties and improve both *in vitro* and *in vivo* biological responses. ***Objective*** of this study is to examine the influence of the magnesium (Mg^{2+}) and silicon (Si^{4+}) doping in 3D printed interconnected macro porous TCP scaffolds on the mechanical strength, and *in vivo* osteogenesis and angiogenesis. ***Our hypothesis*** *is that the presence of multiscale porosity along with MgO and SiO_2 will promote osteogenesis and angiogenesis.*

Methods: Pure and 0.5 wt. % MgO-0.5 wt. % SiO_2 doped TCP scaffolds were fabricated using three dimensional printing (3DP) technology. **Figure 1(a)** presents the schematic of 3D printing process, and sintered 3DP scaffolds (inset). Cylindrical scaffolds of 7 mm diameter and 10.5 mm height with different 3D interconnected square-shaped macropore sizes (500 µm, 750 µm and 1000 µm) were made for mechanical strength analysis. Implants of 3.4 mm in diameter and 5.2 mm in height having 350 µm interconnected designed macropores were made for the in vivo study. Scaffolds were tested for new bone and blood vessel formation in rat distal femoral defect model for 4, 8, 12, 16 and 20 weeks.

Results: Microstructural features showed the presence of multiscale porosity, i.e., designed macro and intrinsic micro pores, in the sintered 3DP scaffolds. The difference between designed porosity (between 27 % and 41 %) and sintered porosity (between 50 % and 56 %) was caused by the presence of intrinsic micro pores in the scaffold struts. Maximum compressive strength of 6.79 ± 1.14 MPa was achieved for 500 µm interconnected designed pore size of MgO-SiO_2 doped scaffolds. The presence of MgO and SiO_2 as dopants in TCP did not show any adverse effect on the mechanical strength as compared to our previously reported value for pure TCP (6.62 ± 0.67 MPa) [3]. Histomorphology and histomorphometric analysis showed that the presence of MgO-SiO_2 doping in TCP accelerated the wound healing process by inducing increased bone formation. The presence of multiscale porosity in the 3DP scaffolds facilitated new bone formation (**Figure 1(b)**). Histomorphometric analysis revealed increased early bone formation in doped TCP scaffolds. Histomorphometric analysis of TRAP positive stained tissue sections showed (**Figure 2 (b)**) a delayed TRAP activity in MgO-SiO_2 doped TCP compared to pure TCP scaffolds, which was probably caused by the presence of Mg^{2+} [4]. vWF staining showed increased new blood vessel formation inside the 3DP doped TCP scaffolds. Histomorphometric analysis of the vWF stained tissue sections confirmed significantly higher blood vessel area formation in MgO-SiO_2 doped 3DP TCP scaffolds compared to pure TCP (**Figure 2**).

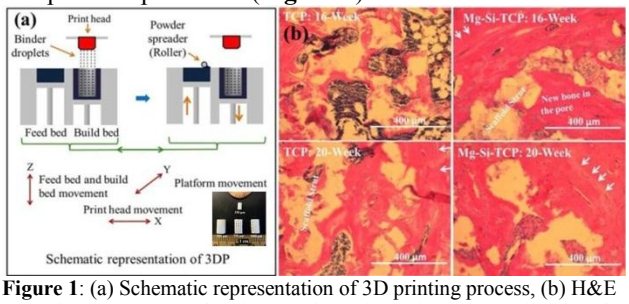

Figure 1: (a) Schematic representation of 3D printing process, (b) H&E stained tissue sections showing new bone formation. Arrows indicate the interface between scaffold and host bone; Color description: Black = Bone marrow; Pink/Reddish = New/old bone; Yellowish = acellular regions derive from scaffold.

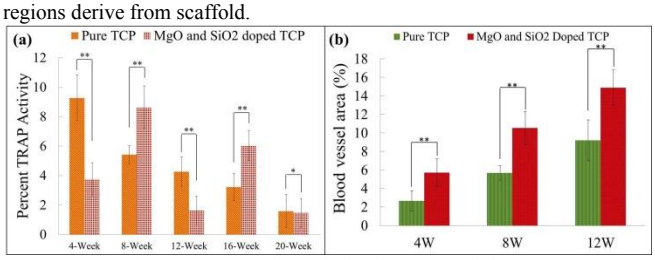

Figure 2: (a) Histomorphometric analysis of TRAP activity (TRAP positive area/total area, %) from 800 µm width and 800 µm height TRAP stained tissue sections (**p < 0.05, *p > 0.05, n=8); (b) Histomorphometric analysis of new blood vessel area comparisons between pure and doped TCP (vWF positive area/total area, %) from 200 µm width and 200 µm height vWF stained tissue sections (**p < 0.05, *p > 0.05, n=8).

Conclusions: The presence of MgO and SiO_2 in TCP showed beneficial for early wound healing, which was observed by increased osteogenesis through new bone formation, and enhanced angiogenesis through increased new blood vessel formation as compared to undoped TCP. Therefore, interconnected macroporous 3DP MgO and SiO_2 doped TCP scaffolds could be excellent candidates for effective early wound healing and tissue regeneration applications in bone tissue engineering.

References: (1) Bose and Tarafder. *Acta Biomaterialia* 2012; 8:1401-1421; (2) Rouwkema et al. Trends in Biotechnology, 2008; 26: 434–441; (3) Tarafder et al. *J Tissue Eng Regen Med* 2013;7:631-641; (4) Roy et al. *J Biomed Mater Res Part A* 2012; 100A: 2450–2461.

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