In Vitro Evaluation of Inkjet 3D-Printed (3DP) Fe-Mn Biodegradable Metallic Scaffolds

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Statement of Purpose: Interest in degradable metals has rapidly increased in recent years. Iron has been investigated for biodegradable stents (Peuster M, Hesse C, Schloo T, et al. Biomaterials, 2006;27;4955-4962) and bone applications (Wegener B, Sievers B, Utzschneider S, et al. Mat Sci Eng B. 2011;176;1789-1796). However, the degradation rate is too slow for implantable devices, thus Fe has been alloyed with Mn to enhance the galvanic corrosion (Hermawan H, Dubé D, Mantovani D. Adv Mat Res. 2007;15-17;107-112). In this study, inkjet 3D printing (3DP) is used to fabricate Fe-Mn scaffolds by layer by layer printing the powder directly to form constructs (Fig. 1). The process can be used to manufacture customizable parts with controlled architecture, and thus has the potential to produce custom bone implants and void fillers. There is no reported literature to the best of our knowledge on inkjet 3D printed biodegradable metals. The present work provides an assessment of 3D printed Fe-Mn biodegradable scaffolds as a potential bone replacement material for craniofacial/dental and orthopedic defects and injuries.

Methods: Elemental powders of Fe and Mn (Alfa Aesar, Ward Hill, MA) corresponding to Fe-30Mn (wt%) were mixed to form a homogenous powder and 3D printed using the Ex-Lab (The ExOne Company, LLC, North Huntingdon, PA) machine with a water-based organic binder. The parts were cured at 200°C for 4h to remove binder and unbound powder, and then sintered at 1200°C for 3 h followed by a tumble finish treatment. Phase analysis of the Fe-30Mn alloy parts was conducted using X-ray diffraction (XRD, PANalytical, Almelo, the Netherlands). Tensile tests were conducted using MTS11 (MTS, Eden Prairie, MN). A tensile stress-strain curve was generated from each sample, from which tensile properties were calculated. Tafel plots were generated from potentiodynamic polarization tests of 3DP Fe-30Mn alloy and pure Iron (Alfa Aesar, 99.97+%) conducted in Hanks' Balanced Salts solution at 37.4 °C and were used to calculate corrosion rate according to ASTM G102-89. Tests were carried out in an electrochemical workstation (CH-604A, CH Instruments, Inc., Austin, TX) employing a three electrode cell with platinum as the counter electrode, Ag/AgCl as the reference electrode, and the sample as the working electrode. MC3T3 cells were cultured directly on 3DP Fe-30Mn at a density of 4×10^4 cells/mL. Viability of the seeded cells was evaluated after 1 and 3 days using the live/dead viability/cytotoxicity kit. Cells on the scaffolds were imaged using fluorescence microscopy and scanning electron microscopy.

Results: XRD showed the 3D printed Fe-30Mn powder formed ε and γ phases. Fig. 1 shows examples of printed and sintered porous and bulk structures, demonstrating the ability of 3D printing to fabricate complex parts with customizable open porosity. The 3DP Fe-30Mn exhibited an average ultimate tensile strength of 115.53 MPa, elongation of 0.73%, and Young's Modulus of 32.47 GPa, similar to values for human natural bone. The corrosion rate of the 3DP Fe-30Mn was 0.12 mm/year, twice that measured for pure Fe (0.06 mm/year). Fig. 2 shows comparable live cell attachment on the surface of sintered 3DP Fe-30Mn compared to tissue culture plastic. The high live cell coverage suggests good *in vitro* biocompatibility of the sintered 3DP Fe-30Mn. SEM images of MC3T3 cells attached on the porous 3DP Fe-30Mn scaffold (Fig. 3) showed cell-cell junctions (white arrow) and cellular extensions to pore walls (black arrows).

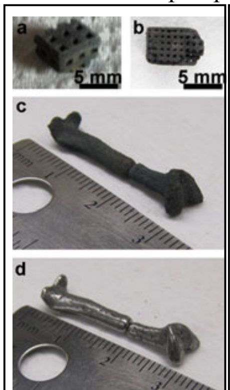

Figure 1. 3D printed Fe-30Mn after sintering: part with pore sizes of lengths a) 1 mm and b) 500 µm; 3DP miniature human femur c) before tumble finishing and d) after tumble finishing.

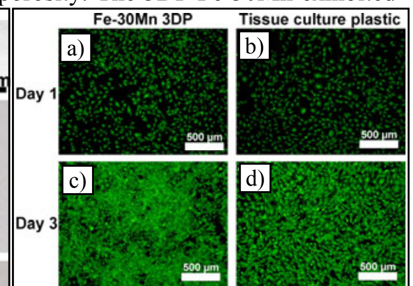

Figure 2. Fluorescent images from the live/dead assay of live (green) and dead (red) MC3T3-E1 cells attached after 1 and 3 days culture on a & c) 3D printed Fe-30Mn; b & d) tissue culture plastic.

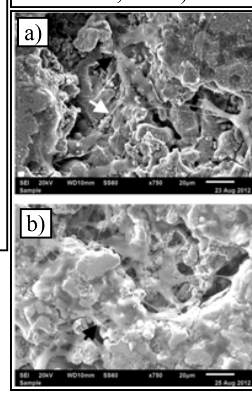

Figure 3. SEM images of MC3T3-E1 cells on the surface of 3DP Fe-30Mn after a) 1 and b) 3 days of culturing at 750x magnification.

Conclusions: 3D printed Fe-30Mn was sintered to form ε and γ phases. The printed alloy demonstrate favorable mechanical properties similar to human natural bone and an accelerated corrosion rate more appropriate for biomaterial applications compared to pure Fe. *In vitro* pre-osteoblast cell attachment results suggest excellent biocompatibility of the Fe-30Mn 3DP parts. The results of this study demonstrate for the first time the potential for inkjet 3D printed Fe-Mn scaffolds to be used as craniofacial/dental and orthopedic biomaterials.