Hyperelastic Osteogenic Bone Substitute Scaffolds Enabled Through 3D Printing

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Statement of Purpose: Although hard tissue engineering has come a long way with respect to materials utilization, there is still significant room for improvement particularly in the area of materials development and processing. Commonly used materials such as sintered hydroxyapatite (HA) ceramics, although bioactive, are costly to manufacture in custom shapes, brittle, and difficult to fit into irregular defect sites. Synthetic polymers, such as PLGA and PCL, although robust and abundant, lack inherent bioactivity and osteo–conductivity and –inductivity. Recent efforts have thus focused on creating structures from composites of these two material systems using 3D printing methods [1]. 3D printing not only permits the rapid fabrication of complex and reproducible structures, but it can also be used to enable the fabrication of new constructs with very unique properties, even if composed of well-established material systems such as HA and PLGA or PCL. In this work, we show how a room-temperature, solvent-based 3D printing technique can be used to quickly and scalably produce HA-polymer composites defined by ≥90wt% HA content with hyperelastic and highly bioactive properties.

Methods: HA-polymer 3D inks were synthesized via suspension, dissolution, and agitation of 90wt% HA and 10wt% PLGA or PCL in a graded solvent mixture. The inks were then printed into scaffold constructs using a 3D-Bioplotter (EnvisitonTec GmbH, Germany), a direct write assembly (extrusion)–based printing platform. The microstructure of printed constructs as well as single extruded strands were characterized using scanning electron microscopy (SEM). Quasi-static, cyclic, and dynamic mechanical testing were used to elucidate the material and resulting construct mechanical properties. Surface properties were quantified using dynamic contact angle, calcium staining, and optical profilometric techniques. Printed scaffolds were seeded with human mesenchymal stem cells (hMSCs) and cultured over the course of 4 weeks in standard growth medium, *without osteogenic inducing factors*. Cell viability was assessed using a live/dead stain (Invitrogen) and fluorescent confocal microscopy (FCM). SEM was used to investigate cell morphology, ECM deposition, and mineral synthesis, the latter of which was analyzed using energy dispersive X-ray spectroscopy (EDS). DNA content and normalized alkaline phosphatase (ALP) activity were determined using standard fluorescent and colorimetric kits, respectively. RT-qPCR was used to measure fold increase in osteogenic gene activity with respect to initially seeded hMSCs.

Results: The resulting as-printed structure (Fig. 1a), despite being 90wt% ceramic possessed hyperelastic properties, which permitted it to be significantly deformed (at least 40%) and recover to its original size and shape upon unloading (Fig. 2b). These properties are the result of the unique material microstructure (Fig. 2c), which is the direct result of the specific ink preparation and printing process. Furthermore, its microstructure has similar features to that of natural bone in terms of porosity, roughness, hydrophilicity, and exposed HA. *In vitro* evaluations with human hMSCs reveal that this material not only supports cell viability (Fig. 2d) and proliferation (Fig. 2e), but also strongly promotes osteogenic activity, including ALP production (Fig. 2e), upregulation of osteogenic genes (Fig. 2f), ECM deposition (Fig. 2g), and new HA synthesis having a calcium to phosphate ratio close to that of natural bone.

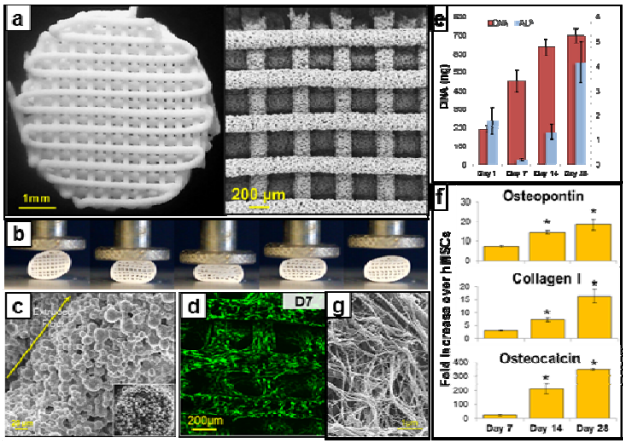

Figure 1. (a) Optical and SEM top-down views of 3D-printed scaffolds. (b) Printed construct under cyclic compression over time. (c) SEM image of microstructure. (d) FCM Live/dead staining of seeded MSCs (e) DNA content and ALP activity and (f) osteogenic gene expression. (g) Matrix synthesis observed by SEM.

Conclusions: This new 3D printed HA composite scaffold shows remarkable hyperelastic mechanical properties that has never been demonstrated before for a material having such a high wt% ceramic content. The ability to be physically handled and compressed, while still rebounding, makes them ideal implants for minimally invasive surgical procedures, as well as for press fitting into irregular-shaped defects. The microstructure and physical properties demonstrate high inherent porosity, exposed HA at the surface, and ability to readily absorb surrounding fluids. These properties directly contribute to the biological response of stem cells seeded within the material. Even *without the aid of osteogenic medium*, cells proliferate to fill the scaffold volume, undergo osteogenic differentiation, and deposit bone specific ECM and mineral, demonstrating the highly osteoinductive nature of the scaffold. Beyond the composite's unique properties, the fact that the comprising materials are already FDA approved, relatively cheap, and can be rapidly printed on demand and at ambient conditions into small or large complex functional implants, can potentially revolutionize current clinical strategies to treat hard tissue defects.

References: 1) (Shore L. Biomat. 2007;28:5921-5927.)