3D Printing of Bone-Templated Scaffolds

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Statement of Purpose: Biomimetic 3D tissue engineered systems have been proposed for investigating molecular mechanisms of disease progression and for screening drugs [1]. We have utilized 3D printing technology to investigate how the mechanical and topological properties of the bone microenvironment influence both cellular interactions and tumor progression in bone. Trabecular curvature, pore size, and pore shape have also been reported to affect the rate of new bone formation. Cells sense and respond to radii of curvature larger than themselves, and the rate of new bone formation increases with the curvature of the surface [2]. The Structure Model Index (SMI) parameter characterizes the structure of trabecular bone, with 0 representing plate-like (e.g., 2D) structures and 3 representing rod-like structures.[3] The trabecular structure largely varies between values of 0 and 3, rather than being predominantly plates or rods. Vertebral trabeculae have an SMI typically closer to a rod-like structure (SMI=3), whereas femoral trabeculae have structure closer to plates (SMI=0). We reason that 3D scaffolds replicating the mechanical and topological properties of bone at different anatomic sites will enable the development of 3D cell culture models for investigating the spatio-temporal dynamics of both bone cellular physiology and cancer progression [1]. We have developed a fabrication process in which wax templates of trabecular bone are prepared by a 3D inkjet printer and subsequently filled with reactive polyurethanes to create scaffolds with elastic modulus and SMI comparable to human bone.

Methods: Human cadaver samples from the proximal tibia, proximal femur, and lumbar vertebrae were obtained from the Program in Advanced Anatomy and Simulated Skills Program at Vanderbilt. Isolated tissue samples were imaged using uCT technology from Scanco Inc. Using Scanco software the scans were converted into STL images, a format representative of the surface of bone uCT images, which specifically captures the trabecular architecture. These images were inverted using the same software to create a representation of the trabecular spacing (Tb.Sp.) The STL images of Tb.SP were then uploaded into the Solidscape Studio 3Z Printer for fabrication. The resulting wax molds were filled with a reactive two-component polyurethane (PUR) composed of lysine diisocyanate (LDI), a poly(ε-caprolactone-co-glycolide) triol (M_w=300 or 3000 g/mol), and iron(III) acetylacetonate (FeAA) catalyst. The polyol (900 Da) was prepared from glycerol starter and backbone comprising 70wt% ε-caprolactone, 20wt% glycolide, and 10wt% D,L-lactide. The mixture was cured overnight under vacuum at 80° C. The cured polyurethane structure was extracted from the wax mold using acetone solution to dissolve the wax (sulphonamide derivatives, polyester resin, and benzoate derivatives,) and dried under vacuum overnight. The resulting structures were imaged by SEM and uCT. Nanoindentation was performed on 2D films and on the 3D biomimetic PUR scaffolds. Rat bone marrow-derived stromal cells and monocytes, as well as bone-metastatic MDA-MB-231 breast cancer cells were incubated with the scaffolds under standard cell culture conditions.

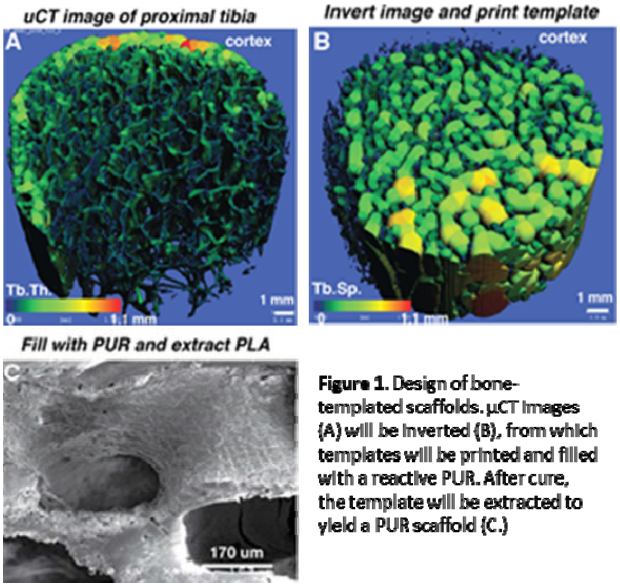

Figure 1. Design of bone-templated scaffolds. µCT images (A) will be inverted (B), from which templates will be printed and filled with a reactive PUR. After cure, the template will be extracted to yield a PUR scaffold (C.)

Results: µCT images of trabecular bone from the femur (Fig. 1A) and an SEM image of the femur-templated PUR scaffold (Fig. 1C) are shown in **Fig. 1**. The scaling of the trabecular meshwork was found to be comparable to that of the original bone. Furthermore, the elastic modulus (measured by nanoindentation) of rigid and compliant PUR networks in the 3D scaffold was comparable to that of 2D films prepared from the same polymers. Rat bone marrow-derived stromal cells and monocytes, as well as bone-metastatic MDA-MB-231 breast cancer cells, attached to and proliferated on the scaffolds.

Conclusions: Using a novel 3D inkjet printing approach, we have fabricated biomimetic scaffolds from synthetic polymers that replicate the mechanical and topological properties of bone. In future work, bone-templated scaffolds will be cultured with stromal cells, monocytes, and tumor cells to replicate the bone microenvironment in a bioreactor. These studies aim to investigate the effects of the bone microenvironment on the spatio-temporal dynamics of tumor progression in bone. Our long-term goal is to apply this approach to the development of patient-derived xenograft models for screening the efficacy of anti-tumor drugs in patients.

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