Load-Bearing Biomimetic Scaffolds for Vascularized Bone Regeneration

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INTRODUCTION: Significant bone loss can occur due to trauma or disease, resulting in large bone defects. Typically, autografts and allografts are used as bone grafts, but these are associated with disadvantages like limited availability, and the risk of disease transmission with allografts. In recent times, synthetic bone graft substitutes have come up as alternatives, but they fall short in one or more aspects as well. Tissue engineered bone grafts are viable alternatives. In order for these to be successful, we must first address two important issues - lack of cell infiltration throughout their structure and a lack of vasculature formation. We have developed 3D printed polylactic acid (PLA) scaffolds with distinct cortical and trabecular geometries. These are highly porous structures promoting capillary inflow of blood and bone marrow. They are also mineralized and prevascularized to promote regeneration of both bone and its vasculature. These scaffolds were evaluated in a load-bearing, rabbit critical-sized radial defect model and show promise as bone graft alternatives suitable for load-bearing scenarios.

METHODS: Computer Assisted Design (CAD) software was used to design the Scaffolds. The Ultimaker 2+/S3 3D printers were used to fabricate the scaffolds in transparent PLA. Scaffolds were tested under compression and their mechanical properties were compared to those of native bone. Scaffold's ability to promote capillary action was evaluated using fluids mimicking the viscosities of blood and bone marrow. Scaffolds were mineralized in concentrated simulated body fluid to incorporate a calcium phosphate coating which has been shown to promote osteogenesis. They were prevascularized by allowing vascular endothelial cells to grow within the cortical structure for 2 weeks followed by decellularization to leave behind a pro-angiogenic matrix. The osteogenic and angiogenic potential of the scaffolds was evaluated in vitro using human bone marrow mesenchymal stem cells (hMSCs). With approval from the Rutgers University Institutional Animal Care and Use Committee (IACUC), scaffolds with and without autologous bone marrow were surgically implanted in adult female New Zealand white rabbits after creating a 15 mm critical-sized radial defect. Allografts served as controls. The regeneration of bone tissue was studied over the course of 20 weeks using X-Ray images acquired every 2 weeks post-surgery until 10 weeks followed by live CT imaging at 12, 16 and 20 weeks.

RESULTS SECTION: The trabecular design displayed Ultimate Compressive Stress (UCS) and Compressive Modulus (CM) values of $9.55 \pm 0.45\ MPa$ and $325.13 \pm 21.89\ MPa$ respectively. The complete scaffold comprising both an inner trabecular and outer cortical section displayed a UCS of $39.83 \pm 2.07\ MPa$ and a CM of $603.37 \pm 24.79\ MPa$. Scaffold's ability to promote capillary action was observed with a range of viscosities matching those of blood and bone marrow. In our vivo evaluation, X-ray images showed progressive bone regeneration from 2 to 10 weeks, originating first from the ulna ridge just below where the radius was removed and from the two ends where the radius meets the implant (Figure 1A,B). The CT image taken at 12 weeks post-op showed significant bone regeneration (Figure 1C).

DISCUSSION: The UCS and CM values of our trabecular design fall within the limits for trabecular bone (0.2-10MPa and 7-200MPa). The complete scaffold displayed UCS and CM values closer to those of native whole bone i.e., 200 MPa and 1 GPa respectively. Scaffold's ability to promote infiltration of bone marrow into and throughout the scaffold reduces the need to add stem cells prior to implantation in vivo. In vitro evaluation has shown that the presence of mineral promotes osteogenic differentiation of stems cells. The decellularized lumens created within our cortical geometry are crucial to promoting the growth of vasculature upon implantation. The in vivo evaluation has shown that these scaffolds can maintain their structural integrity throughout the entire duration of the study while also promoting bone regeneration. Histological and immunohistochemical analysis will be done to study both bone and vascular regeneration in detail.

SIGNIFICANCE/CLINICAL RELEVANCE: The developed scaffolds show great promise as viable alternative bone grafts for vascularized bone regeneration and suitable for load-bearing applications. Distinct trabecular and cortical architecture combined with 3D printing technology allows for the fabrication of scaffolds with shapes, sizes, and mechanical properties customizable to the needs of different large bone defects.

IMAGES AND TABLES:

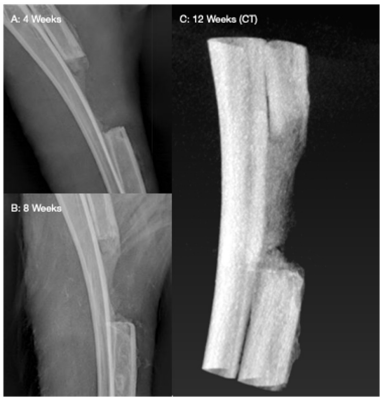

Figure 1: X-ray images taken 4 weeks (A) and 8 weeks (B) post-surgery. CT image showing significant bone regeneration at 12 weeks (C) post-surgery.