Three-Dimensional Printed Scaffolds for Segmental Defects in Long Bones

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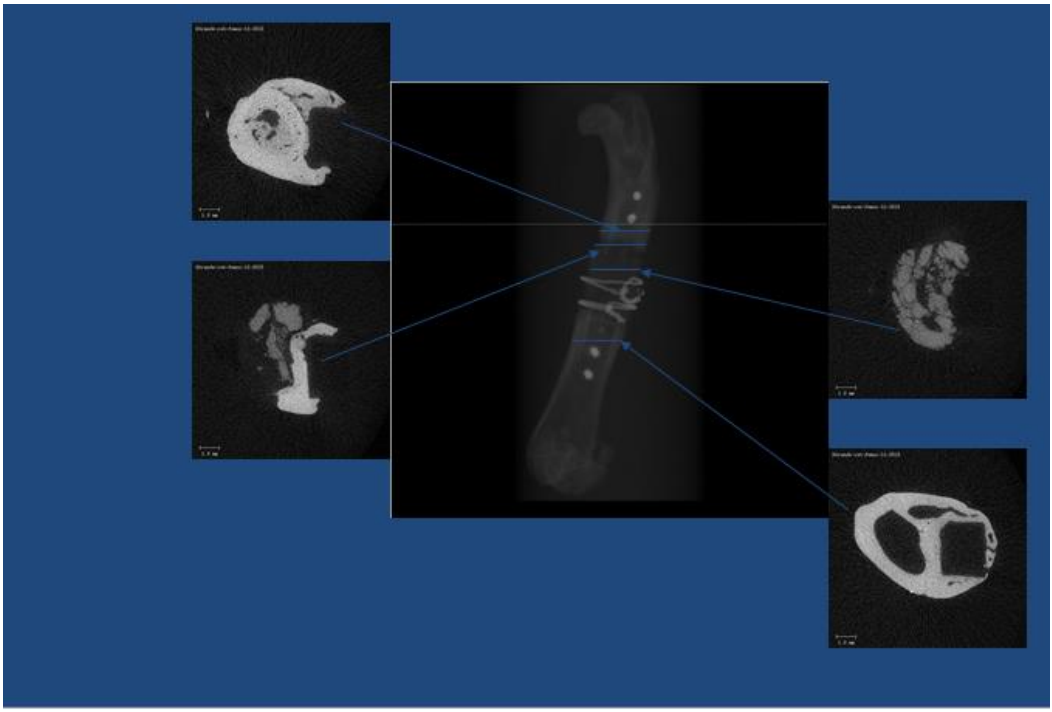
Background/Purpose: Segmental bone loss is a devastating injury that can result from trauma, malignancy, or infection with significant sequelae of disability, psychosocial stress, and financial burden especially in a young, active patient. While working with a multidisciplinary approach has been shown to improve outcomes, many advances have also been made within the orthopaedic discipline to improve time to union and functionality. Despite advances many techniques present challenging limitations, ubiquitous complications, and unpredictable results. The introduction of three-dimensional (3D) printing technology has created a new method of producing bone graft substitutes and has the potential to eliminate many of the problems associated with current management techniques for segmental bone defects. The purpose of the current study is to evaluate a novel 3D printed polylactic acid (PLLA)/calcium carbonate (CaCO3) scaffold as a viable substrate for bone regeneration in an in vivo model.

Methods: Design and printing: A 3D-CAD (computer-assisted design) model was created using the Rhino3d™ Wenatchee-OsX CAD designer. The segment was 8 mm in length and shaped to mimic the native anatomy of the rat femur. The graft was designed with multiple pores to allow for cellular infiltration, growth, and surgical fixation. Grafts were printed on a desktop printer extruding bioink and PLLA / CaCO3 filament concurrently. Bioink production: Type I bovine collagen was combined with 1 mL of 10X RPMI (Sigma-Aldrich). pH was neutralized. Low-viscosity sodium alginate was mixed into the solution at a 1:1 ratio and passed through a 0.22-μm filter. A CaSO4 solution was added to the collagen / alginate gel and set for 45 minutes. Previously harvested mesenchymal stem cells (MSC) are homogenously mixed into the gel and used. In vitro: Sprague Dawley rat bone marrow MSCs were isolated and cultured for 7 days at 1, 3, 5, and 7-day time points in 96-well plates on PLLA / CaCO3 disks. ~10,000 cells are seeded and grown in osteogenic media with 6 wells per time point per group. Three of the groups were analyzed for cell proliferation and histology and three for gene expression compared to a control group grown in monolayer. RT-qPCR (reverse transcription polymerase chain reaction) for genetic markers of osteoinduction and histology using Alizarin red / Alcian blue staining was completed. In vivo: Sprague Dawley rat femora were exposed by longitudinal incision and isolated. A PEEK (polyether ether ketone) fracture fixation plate was attached to the femur by four 0.70 x 5.70-mm screws. After rigid fixation of the plate, an 8-mm transverse middiaphyseal critically sized bone segment was removed by using a rotary osteotomy burr along with the adherent periosteum. The defect was either left empty as a control or a 3D-printed bone graft was inserted and fixed with cerclage wire. Following treatment the muscles, fascia, and skin were opposed in a routine manner with use of 4-0 Vicryl sutures. The animals were not immobilized postoperatively. At 16 and 24 weeks postsurgery, the animals were radiographed. At 24 weeks, the animals were euthanized and the femurs were harvested, formalin-fixed, and processed for histology and biomechanics.

See pages 49 - 106 for financial disclosure information.

Results: In vitro: Over the course of 7 days cells grown on PLLA / CaCO3 in vitro increased at a proliferation rate equivalent to unmanipulated controls, early calcification is present, and genetic markers of osteoinduction increase over time. In vivo: Postsurgery rats were ambulatory, no signs of infection or graft rejection were noted throughout the study. Varying levels of calcification were present. Fluoroscopy at 16 weeks and micro-CT at 24 weeks show bony ingrowth (Figure 1), and the graft did not lose significant strength over the course of the study.

Figure 1



Conclusion: In this in vitro model a novel 3D-printed PLLA / CaCO3 scaffold supports growth of rat bone marrow MSCs. The printed scaffold exhibited osteoinductive and osteoconductive properties. The use of this novel scaffold as a tool in the management of segmental bone defects shows promise. In vivo results suggest that a 3D-printed scaffold could prove to be a viable option for treatment of segmental defects for personalized medicine. Fluoroscopy taken at 16 weeks after implantation suggests bony ingrowth with cortical bridging. Gait analysis did not show any abnormalities, which suggests the implant was well tolerated. All of the above results suggest this concept could potentially be rapidly adopted as a print-on-demand solution in the operating room for various clinical situations.