Mechanical Properties of Load Bearing Bone Regenerated across Large Critical Size Defects in Sheep using 3D Printed Scaffolds and Autologous Stem Cells

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INTRODUCTION: There are currently no reliable treatments to heal large critical size bone defects. Previous studies in our lab have demonstrated that 3D printed polybutylene terephthalate (PBT) scaffolds with a biomimetic trabecular porous pattern increase bone regeneration up to 600% compared to 3D printed scaffolds with simple geometric pores[1]. Additionally, our lab has demonstrated that biomimetic PBT scaffolds coated with β-tricalcium phosphate particles, and infused with autologous adipose derived stem cells, can bridge a 4.2 cm defect in sheep within 6 months[2]. The purpose of this study was to compare the mechanical properties of regenerated bones when the implanted scaffolds are supported with a non-dynamized intramedullary nail, a dynamized intramedullary nail, and following removal of the supporting hardware.

METHODS: This study was approved by the University of Arizona IACUC. PBT scaffolds were 3D printed using a biomimetic pattern obtained from sheep femoral heads. Scaffolds measured 4.2 cm in height and 2.2 cm in diameter. Two weeks prior to surgery, autologous adipose derived stem cells were harvested from the tail fat pad of the sheep and maintained in cell culture. The cells were seeded onto the scaffold 1 day before surgical implantation of the scaffold. A 4.2 mid-diaphyseal femoral defect was created in 19 sheep using a published procedure[3]. A scaffold was placed within the defect of 16 sheep, while three control sheep did not receive a scaffold. An intramedullary nail with interlocking screws was used for initial fracture stabilization and to secure the scaffold. At 6 months, 10 sheep underwent dynamization of the intramedullary nail by removal of the proximal interlocking screw. At 9 months, four sheep in the dynamization group underwent removal of the remaining hardware. Control (n=3), non-dynamized (n=6), and dynamized (n=6) sheep groups were euthanized at 9 months, while sheep that underwent complete hardware removal (n=4) were maintained for 12 months. Femora were explanted and cleaned of soft tissue before being radiographed and imaged on a Siemens Inveon micro-CT scanner at 100 um resolution. CT images were analyzed using 3D Slicer to measure total bone volume within the defect site. After imaging, bones were mechanically tested in axial compression and cantilever bending. Each mechanical test was repeated six times to a maximum load of 44 kg in compression and 26 kg in cantilever bending. In cantilever bend testing, the bone was loaded in the anterior-posterior plane with the anterior surface in contact with the loading pin first, following which the bone was rotated 180 degrees where testing was repeated with the loading pin in contact with the posterior surface of the bone.

RESULTS: Following 9 months *in vivo*, all but one dynamized sheep demonstrated bone union across the defect. One control sheep and one non-dynamized sheep had a union across the defect. Representative radiographs of the explanted bones are shown in Figure 1 (top). Mechanical testing results for stiffness in axial compression are shown in Figure 1 (Bottom Left). Mechanical testing demonstrated an increased stiffness in regenerated bone following hardware removal (1932±346 N/mm) compared to bone from control (458±513 N/mm) and non-dynamized (558±500 N/mm) sheep ($p<0.05$). Regenerated bone from the dynamized sheep (1339±870 N/mm) showed a trend in toward increased stiffness compared to control and non-dynamized sheep ($p>0.05$). MicroCT imaging results are shown in Figure 1 (Bottom Right). The regenerated bone volume in the control (10.04±3.39 cm^3), non-dynamized (9.89±3.69 cm^3), dynamized (14.69±4.59 cm^3) and hardware removal (12.70±1.35 cm^3) groups were similar ($p>0.05$) despite the difference in bone union between the groups.

DISCUSSION: Radiographs and microCT imaging demonstrate that stem cell seeded biomimetic PBT scaffolds induce bone regeneration across the 4.2 cm defect. Dynamization increased the union rate compared to the non-dynamized group, where a nonunion was observed at one of the ends of the defect site. Axial compressive stiffness was significantly higher following hardware removal. Although not statistically significant, the increased bone volume observed in the dynamized group would demonstrate increased bone formation that subsequently undergoes remodeling to stiffer bone after hardware removal.

SIGNIFICANCE/CLINICAL RELEVANCE: There is currently no viable treatment that results in consistent healing of large critical size bone defects. This study demonstrates that biomimetic 3D printed PBT scaffolds can rapidly regenerate bone in a large critical size defect and can provide functional mechanical properties by 9 months as the regenerated bone can withstand physiological loading after removing all stabilizing hardware.

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IMAGES AND TABLES:

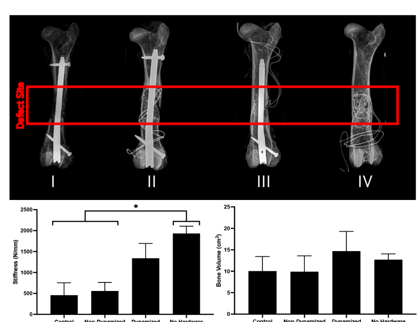

Figure 1. (**Top**) Representative images of ex vivo radiographs of I (Control), II (Non-Dynamized), III (Dynamized), and IV (No Hardware). Red square represents the site where the large critical defect was created. (**Bottom Left**) Mechanical testing results for axial compression. Results show a significant increase in compressive stiffness in the hardware removal group compared to the control and non-dynamized groups ($p<0.05$). (**Bottom Right**) MicroCT results for total bone volume within the defect show a trend in increase in total bone volume after dynamization that decreased after hardware removal ($p>0.05$).