Bone Ingrown Dynamized Long Bone Segment Regeneration Scaffolds Successfully Support Full Body Weight Loading within 9 Months

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Introduction and Statement of Purpose:

Extensive skeletal injury due to trauma, or resection of cancerous bone often leaves a large, long bone defect which will not heal without surgery. The most common currently used procedure involves the placement of cadaver bone into the defect site. The cadaver part can develop cracks and often fails before it can be replaced with living bone.

We developed a technique utilizing biomimetic 3D printed scaffolds infiltrated with 5 µm calcium particles and endogenous pluripotent cells (Figure 1). The scaffolds have induced extensive, rapid bone growth when placed in sheep critical sized femoral defects. On-board sensors were used to monitor load changes during bone formation.

The purpose of this part of the study was to collect *in vivo* loads as a predictive tool to assess when dynamization and rod removal could safely be done and to show that bone infiltrated scaffolds support each sheep's full body weight.

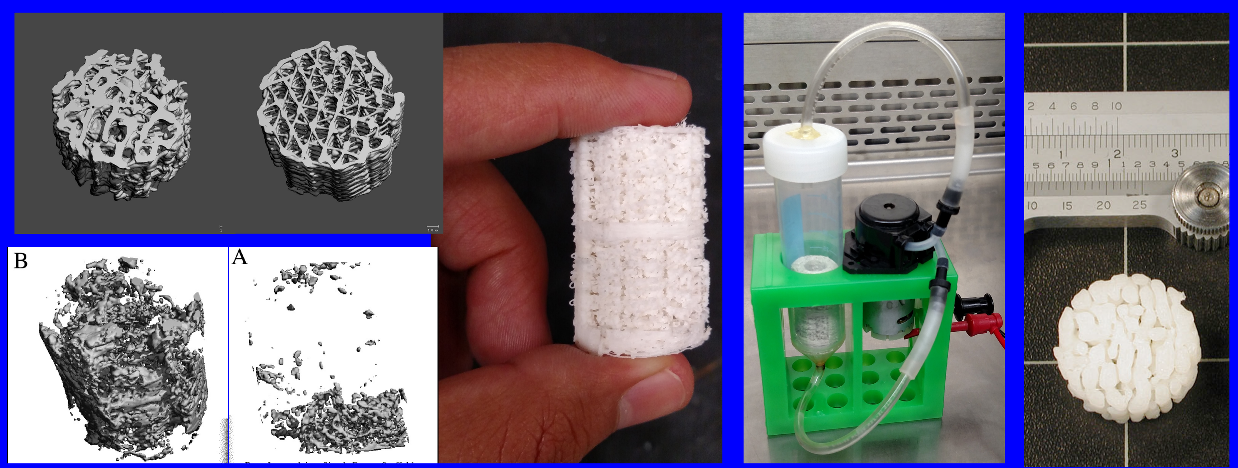

Figure 1: (Left most) B. A biomimetic porous scaffold design and below it a µCT scan of bone ingrowth into the biomimetic pattern at 6 months in a dog. A. 3D printed pattern of a simple geometric porous scaffold design and below it a µCT scan of bone ingrowth into the geometric patterned scaffold at 6 months in a dog. (Second from Left) This picture shows a critical sized defect length 3D printed scaffold. (Second from Right) Cell and tricalcium phosphate (TCP) infiltration bioreactor. (Right most) Inverse biomimetic polybutylene terephthalate scaffolds printed using free form fabrication utilizing µCT data sets collected from trabecular bone and modified to the size of the femoral mid-diaphysis of a sheep. Scaffold is identical to the inverse trabecular pattern of the sheep bone, to induce ingrowth that has the form of a trabecular pattern.

Methods, materials and analytical procedures:

Polybutylene terephthalate (PBT) scaffolds were printed with reverse trabecular patterns collected from 12-micron resolution µCT images of sheep femoral heads. Scaffolds were printed 4.2 cm in length (a critical sized defect in this model).

1000-ohm custom made rosette gauges (EK-06-125BZ-10C/W; Vishay Measurements Group, Raleigh NC) were assembled, waterproofed, and attached to scaffolds using a published procedure (1). Gauges were used to assess scaffold stiffness when compressed to 294 N at 294 N/s in an MTS and stress-strain curves were used as calibration curves for *in vivo* measurements.

Groups of four sheep received scaffolds infiltrated with tricalcium phosphate ceramic (TPC) particles and fat derived endogenous pluripotent cells. During an IACUC approved procedure, scaffolds were surgically placed in sheep femora and stabilized using a modified intramedullary humeral rod locked with 2 locking screws. Multiconductor cables attached to rosettes were led to the back and exteriorized. Our control sheep received a locked rod but no scaffold. Radiographs and blood draws were collected monthly. The experimental group was exercised on a treadmill up to 3 times a week. At 6 months rods were dynamized (Figure 2 and 3)and load measurements collected immediately pre- and post-op. At 9 months, rods were extracted in sheep with sufficient healing. Activity of sheep was monitored with video and implanted sensors. Post-sacrifice µCT and histomorphometry were used to examine bone formation.

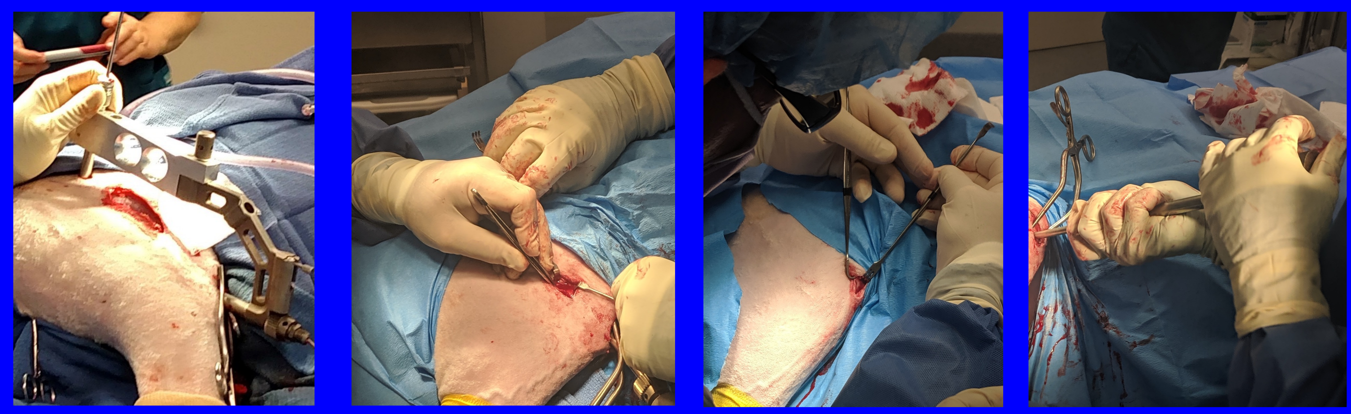

Figure 2: The dynamization and rod removal procedure involved making an incision at the stifle to attach a screw targeting device, marking the location of the screw on the skin and making an incision over the location of the screw. The screw was located and removed with a hand-held screwdriver.

Results:

A scaffold stiffness of 2.8 GPa was measured preimplantation. Surgeries were uneventful and sheep were load bearing in 24 hours.

Three months after surgery, peak loads of ~ 35kg (~ 40% body weight) were observed. Pre-dynamization x-rays at 6 months showed extensive bone formation in all sheep with scaffolds and cells. Prior to proximal screw removal (dynamization), peak loads of 45kg (~ 50% body weight) were measured. Post-dynamization loads did not significantly change (Figure 3). Gait load measurements showed no significant changes pre- and post- rod removal after 9 months in sheep. Mechanical testing of implanted bones and contra-lateral intact bones indicated relative bone stiffness of 72% to 83% of intact. Early radiographs showed complete anterior bone bridging within a month. Post sacrifice µCTs showed long segment scaffolds infiltrated with cells facilitated rapid bone growth through-out the length of the defect within 3 months. After 6 months, remodeled lamellar bone was apparent suggesting sufficient strength to support physiological loads. *In vivo* measurements pre and post dynamization did not change significantly. At 9 months bone in and around scaffolds provided sufficient support for sheep to fully load the experimental limbs after rod removal and during gait (Figure 4). A sheep held for over a year (i.e. more than 3 months past rod removal) has been fully weigh bearing and active with an ingrown scaffold (Figure 5).

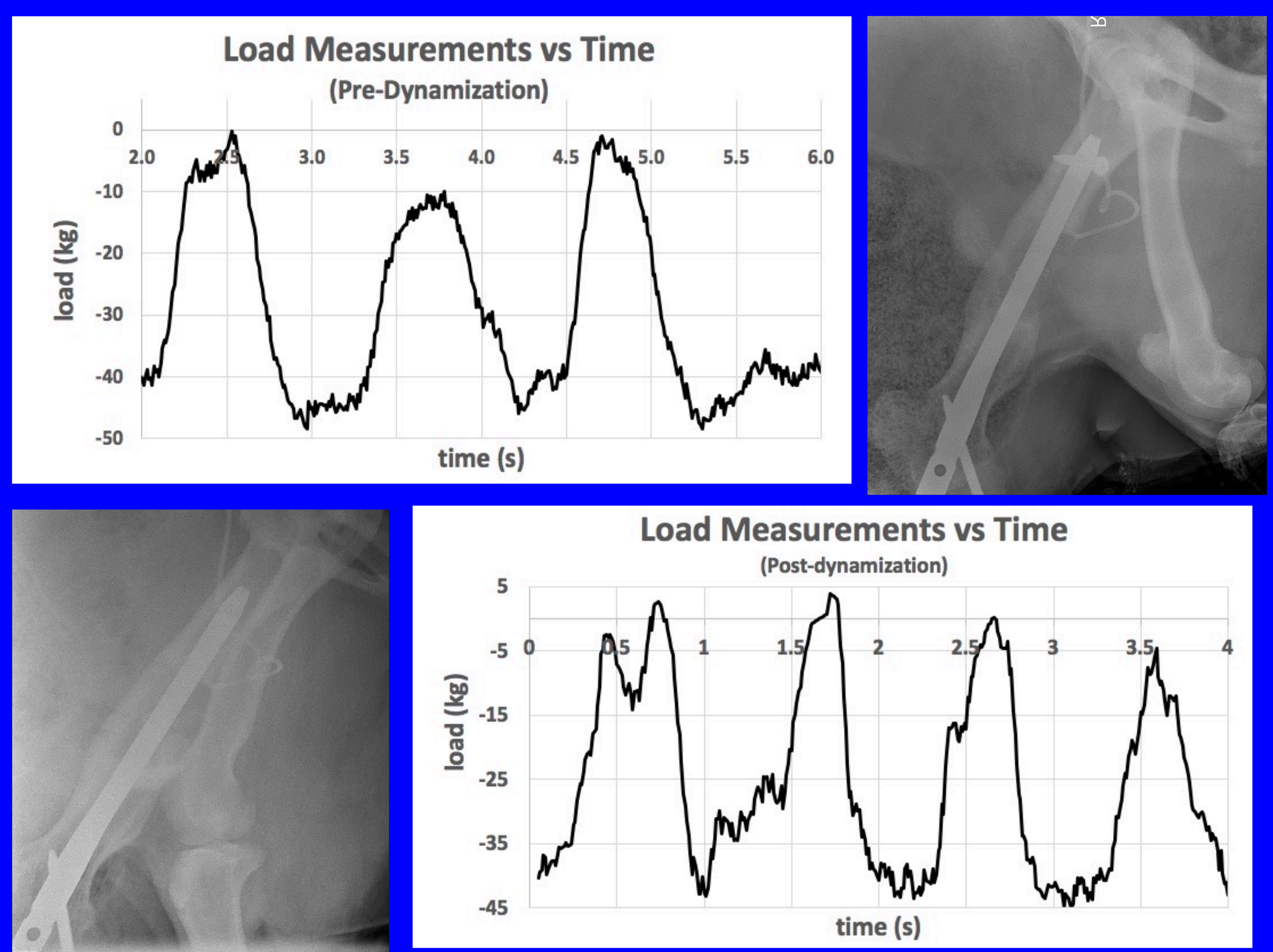

Figure 3: Radiographs showing femur of one sheep prior to and following dynamization at 6 months. Note that rods were placed retrograde and the orientation of these radiographs is with the proximal femur at the top of each image. In order to dynamize the rod the proximal screw was removed . Load changes measured before and after dynamization. (Top graph) Pre-dynamization loads. (Bottom) Post dynamization loads, from same sheep showed slight increases following dynamization.

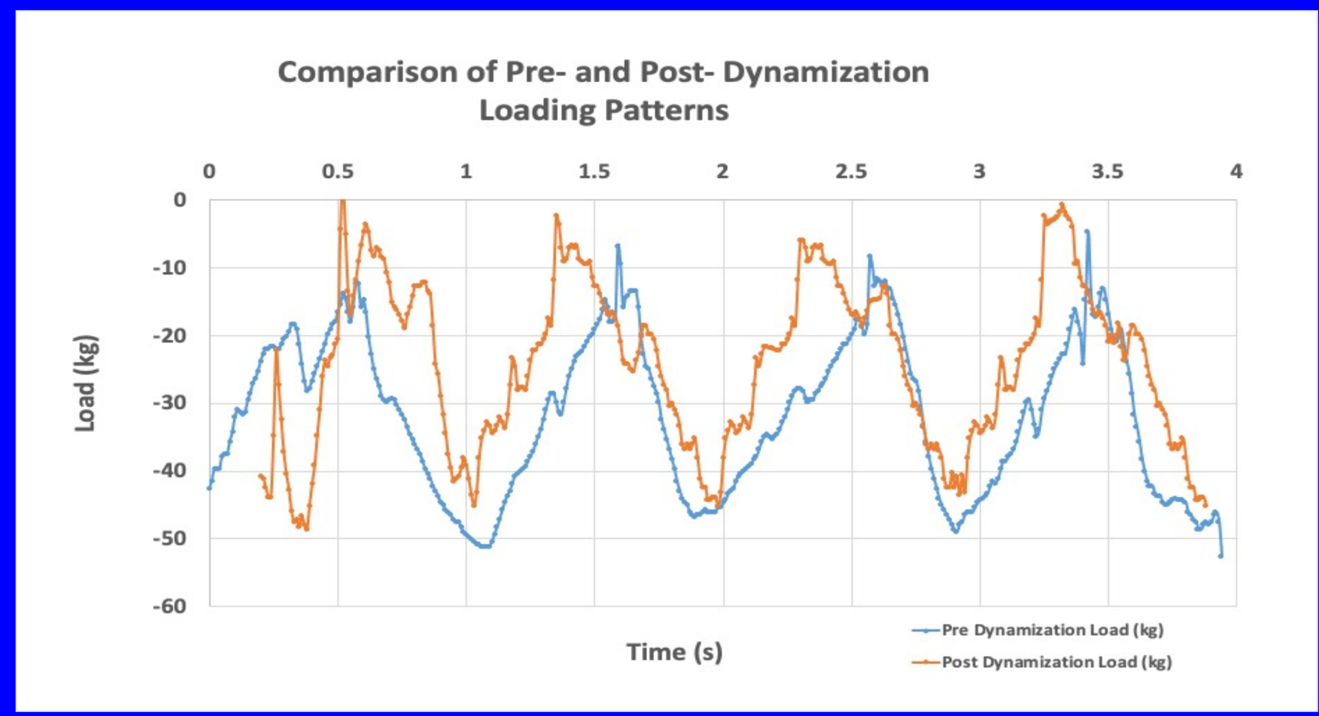

Figure 4: An example of gait pattern loads pre - and post -rod removal after 9 months post- scaffold implant. Patterns have been time shifted to facilitate load comparisons.

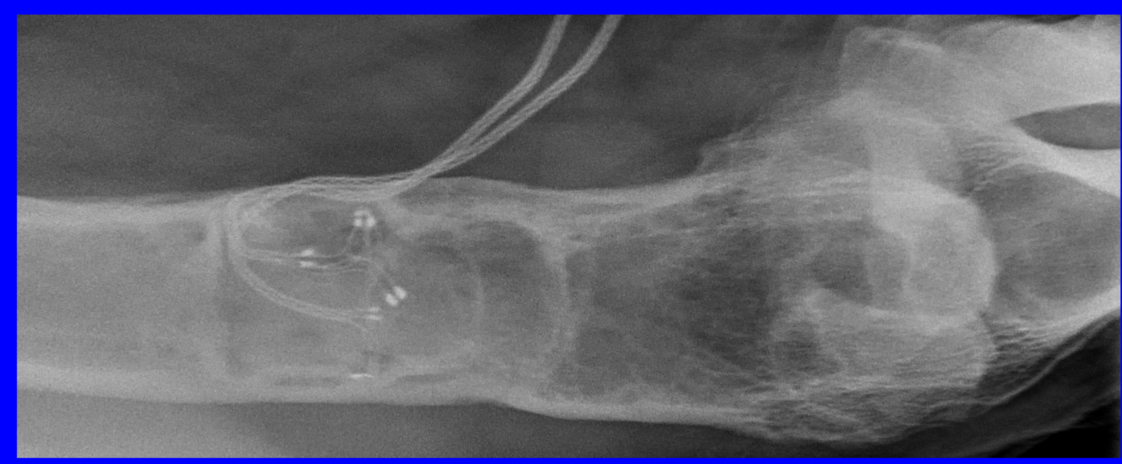

Figure 5: Radiographs (AP) of a sheep femur in a sheep that is 14 months post scaffold placement showing substantial bone ingrowth and overgrowth. The wiring to the rosette strain gauge on the scaffold is clearly visible.

Discussion and Conclusions:

Calibrated strain gauges provided load measurements following scaffold placement, prior to and following dynamization and following rod removal providing information to assess when dynamization and rod removal could safely be carried out. Evidence of our ability to use this tool was noted since no significant change was noted in measurements immediately following dynamization and none following rod removal. This suggests there was no need for load sharing of bones with rods which provided initial stability. Mechanical testing of explanted bones confirmed that bone formation by 9 months provided sufficient stiffness to support full body weight. Evidence at 14 months indicates the bone formed by this point provides ongoing structural support.

Significance: Developing a scaffold and placement approach that ensures rapid and robust bone formation in a critical sized defect provides a first step toward a clinical solution for patients that will insure rapid bone regeneration in large bone defects. Utilizing a sensor to monitor loading changes and the effect of exercise on bone regeneration, will ensure an understanding of the best approach to induce bone healing in patients and a tool to monitor rehabilitation.

References: 1. J B Mater Res–B, 66B, 514-19, 2003.

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