# Large Segmental Bone Defects Treated with 3D Printed Scaffolds and Autologous Stem Cells Demonstrate Increased Stiffness Following Hardware Removal

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## Introduction

- Large critical size segmental long bone defects do not reliably heal despite numerous surgical treatment options.
- Biomimetic scaffolds, produced from micro-CT images of trabecular bone, result in 500% increased bone regeneration compared to scaffolds with simple geometric pores.
- Biomimetic scaffolds coated in beta-tricalcium phosphate, and seeded with autologous adipose derived stem cells, can bridge a 4.2cm mid-diaphyseal femoral defect in a sheep within 3 months.
- Strain gauges incorporated into the scaffolds can measure loads passing through the scaffold during healing.
- The purpose of the current study was to compare the mechanical properties of bone regenerated using biomimetic stem cell seeded sensate scaffolds when the supporting metal hardware in non-dynamized, dynamized, or following complete removal of the hardware.

## Methods

- Polybutylene terephthalate (PBT) scaffolds 4.2 cm in length were 3D printed with an internal structure based on micro-CT images of sheep femoral heads.
- Three, 1000 Ω strain gauges were waterproofed and attached to each scaffold. Scaffolds were compressed to 294 N at 294 N/s to generate linear stress-strain calibration curves.
- Scaffolds were coated in beta tricalcium phosphate, sterilized in ethylene oxide, and seeded with autologous adipose derived stem cells obtained from the tail fat pad of sheep two weeks prior to surgical placement.
- Scaffolds were surgically placed into a 4.2 cm mid-diaphyseal femoral defects in three sheep. Scaffolds were stabilized using an intramedullary nail with a locking screw placed proximal and distal to the defect.
- Following surgery sheep were walked on a treadmill for 15 minutes up to three times per week. Radiographs of the femur were taken monthly.
- Scaffolds were dynamized by removal of the proximal locking screw at six months, and the intramedullary nail was removed nine months following scaffold placement.
- Following sacrifice, the femora were explanted for mechanical testing, micro-CT imaging and histological analysis of regenerated bone.

## Results

- All sheep demonstrated bridging bone across the anterior, medial and lateral sides of the defect by three months.
- Monthly radiographs demonstrated evidence of remodeling in the regenerated bone three months after dynamization, with continued remodeling three months after hardware removal (Figure 1).

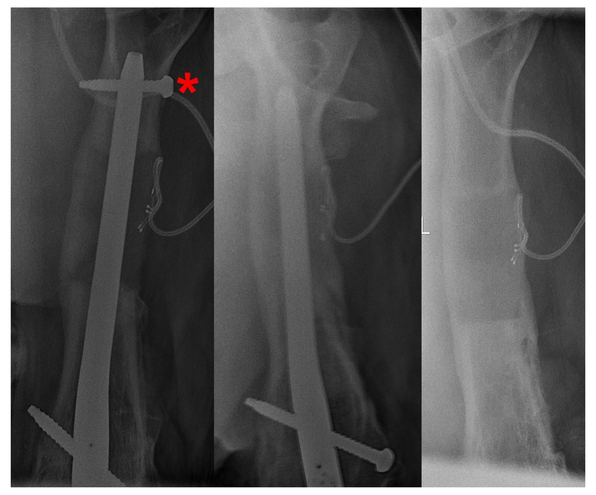

**Figure 1**: Representative monthly radiographs of sheep femora. A radiograph at 6months (left) demonstrates bridging bone prior to dynamization. The proximal screw removed during dynamization is marked with an asterisk. Three months following dynamization (middle) additional bone formation is visible on the lateral side of the scaffold. Three months following removal of all hardware (right) the scaffold outline is visible within remodeled bone.

- Scaffold loads did not demonstrate a significant change in loading prior to and immediately following dynamization, but demonstrated decreased peak loads 3 months following dynamization, just prior to hardware removal (Figure 2).

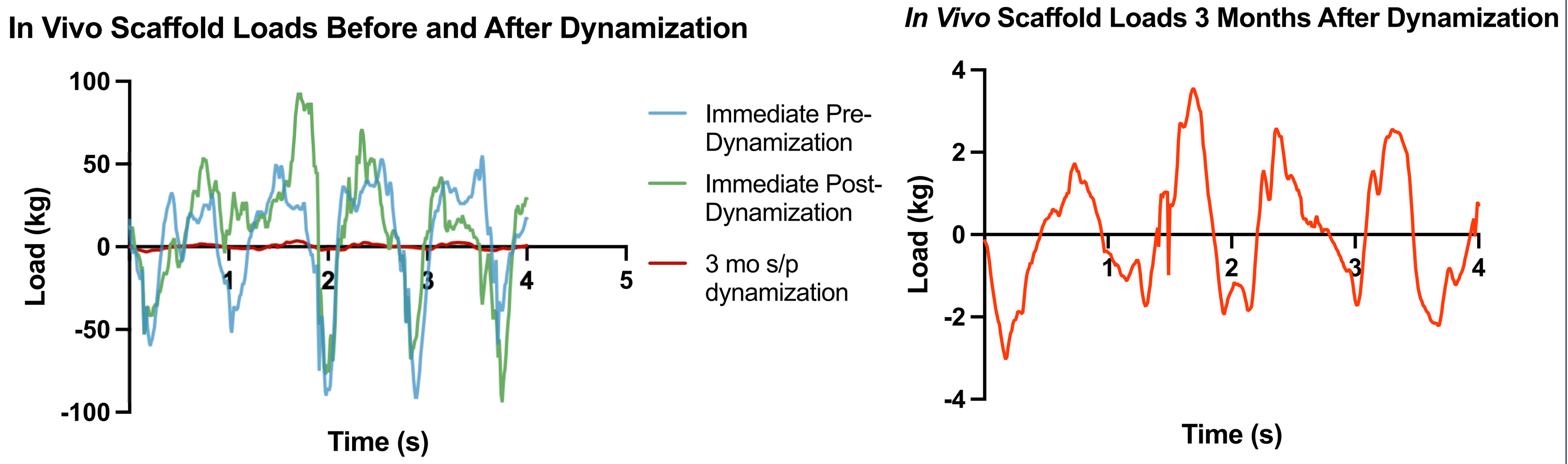


**Figure 2**: Loads measured from calibrated scaffolds during treadmill walking (left) demonstrate the same peak loads prior to (blue) and following (green) dynamization of the intramedullary nail. The peak loads measured 3 months following dynamization of the intramedullary nail decreased (red). The graph on the right demonstrates is the same as that on the left, but only shows the loads measured 3 months following dynamization. In this case the loading pattern during treadmill walking is the same as it was prior to and immediately following dynamization, but the peak loads were lower indicating that the regenerated bone was entirely supporting limb loading.

- Sheep femora demonstrated significantly increased axial stiffness compared to control and non-dynamized femora (Figure 3).

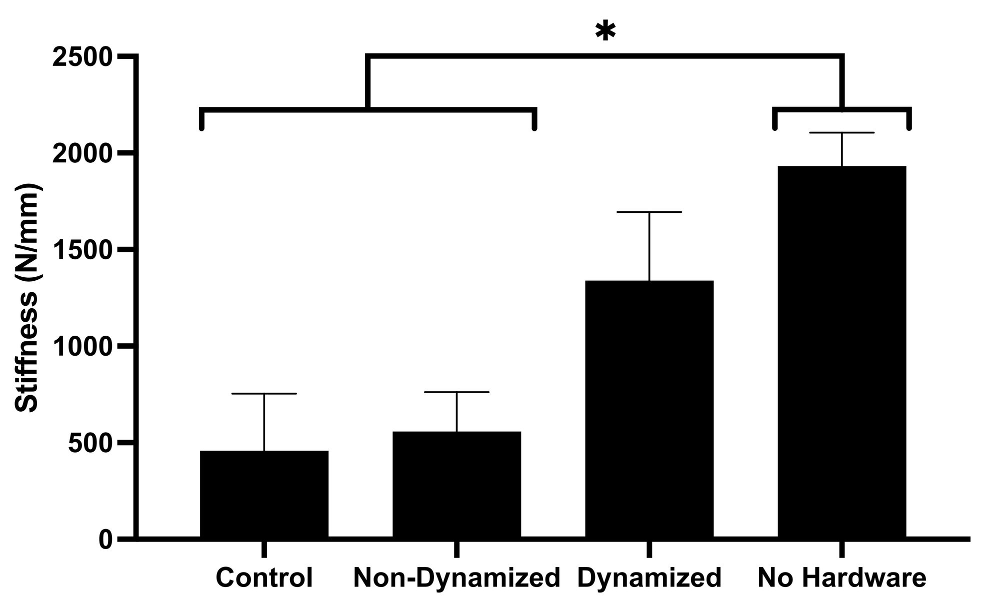


**Figure 3**: *Ex vivo* mechanical testing demonstrated axial stiffness of 0.44+/-0.52 kN/mm in control femora, 0.56+/-0.50 kN/mm in non-dynamized femora, 1.34+/-0.87 kN/mm in dynamized femora, and 2.10+/-0.13 kN/mm in femora following hardware removal. Femoral stiffness following hardware removal was significantly higher than control and non-dynamized femora ($p<0.05$). Femora showed a trend toward increasing stiffness following dynamization, but this change was not significantly different than the other groups ($p>0.05$).

- CT imaging and histology (Figure 4) demonstrate extensive bone formation.

## Results

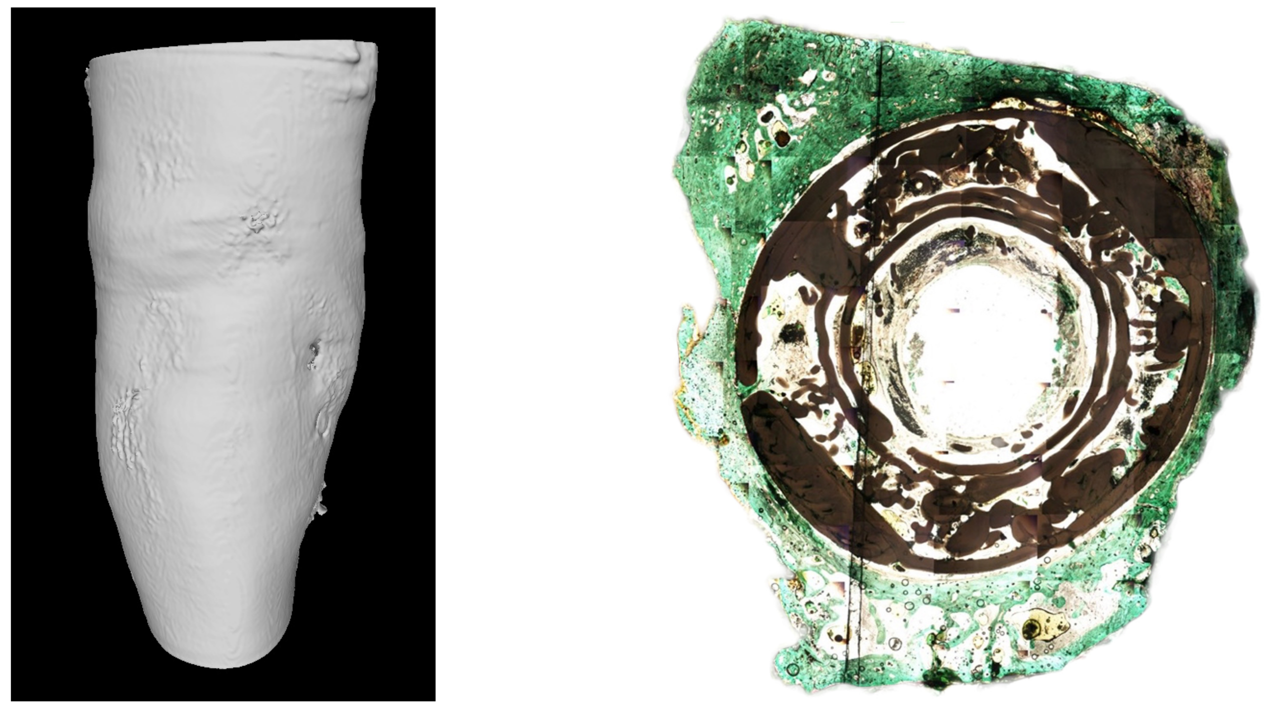

**Figure 4**: A 3D reconstructed CT image (left) and transverse histological section (right) showing circumferential bone regeneration around the scaffold following creation of a 4.2cm defect and placement of a biomimetic scaffold with stem cells.

## Discussion

- Sensate biomimetic PBT scaffolds coated in tricalcium phosphate particles and seeded with autologous adipose derived stem cells are able to regenerate a large 4.2 cm mid-diaphyseal femoral.
- Six months following scaffold placement the regenerated bone is supporting a significant proportion of limb loading.
- Dynamization of the construct facilitates additional bone formation and remodeling around the scaffold.
- Regenerated bone is supporting limb loading by 9 months, allowing safe removal of all hardware.
- Regenerated bone demonstrates an increased stiffness in axial loading following removal of all supporting hardware.

## Acknowledgements

- The Army Medical Research Acquisition Activity, Fort Detrick MD 21702-5014 - awarding and administering acquisition office. Work supported by Assistant Secretary of Defense for Health Affairs endorsed by the DoD, Broad Agency Announcement for Extramural Medical Research; Award W81XWH-18-1-0490. Opinions, interpretations, conclusions and recommendations are not necessarily endorsed by the DoD. Animal research adhered to the laws of the U.S. and regulations of the Department of Agriculture.

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