Dynamic Axial Loading Disrupts Lateral Integration of Arthritic Human Articular Cartilage and Synthetic Tissue Engineered Scaffolds in an Ex Vivo Ring Model

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Introduction: Common surgical procedures including mosaicplasty, microfracture, and autologous chondrocyte implantation to promote articular cartilage regeneration account for ~40% of knee arthroscopy.[1] These procedures are reliant on proper integration between newly placed tissue and surrounding native cartilage to provide a repair capable of reducing shear during articulation and resisting axial loads. There are varied post-operative regimens including immobilization and restrictions on weight bearing. To better understand the tissue response to loading following introduction of a tissue engineered implant, we have developed a novel ex vivo experimental system consisting of a lateral integration ring construct and axial loading multi-chamber bioreactor.
Methods: Arthritic human cartilage was obtained from the loadbearing region of the femoral condyles of patients undergoing total knee replacement surgery. Samples were handled in accordance with protocols approved by Albany Medical Center and Rensselaer Polytechnic Institute IRB committees. Tissue was maintained in saline at 4^{o}C before use.
Static (non-loaded) control samples were cultured in a multi-well plate with chondrocyte growth media at 37^{o}C. Samples receiving dynamic stimulation are loaded into the bioreactor. Rather than pursuing the promotion of an optimal chondrocyte phenotype through applied loading, a physiologically relevant regime was chosen of 10% strain at 1 Hz. These values correspond to peak physiological strain estimates used in previous studies that incorporate physiological loading levels as well as the mean frequency of a walking stride with a normal gait cycle [2]. These samples were exposed to sinusoidal axial loading for 6x cycles of one hour of movement and one hour of rest for 12 combined hours per day. The bioreactor (MACH-1, Biomomentum, Laval, QC) was entirely enclosed in an incubator maintained at 37^{o}C.
A push-out test was utilized to help quantify the strength of bonds in lateral integration in the samples. During the push-out test, a plunger was lowered at 10 μm/sec until the central plug was completely dislodged from the outer ring. The resulting peak force was normalized across interfacial area to calculate interfacial strength. Day zero control samples were used to establish a baseline for evaluation of the influence of new tissue growth only on interfacial strength by enabling isolation and subtraction of the frictional forces incurred between the plug and ring during the push-out procedure. (Figure 1B) Raw interfacial strength was calculated for samples after 1, 4, and 6 weeks of culture (Figure 1A), and subtracted from day 0 controls (Figure 1C).

Histology samples were evaluated for visual evidence of biological activity at the interface of lateral integration. After processing, 12 µm cross-sectional samples showing the integration zone between the central plug and outer ring were obtained with the HM505E cryostat (Microm. Walldorf, Germany) at -20 ºC. Histological sections were taken in cross-section to highlight the interfacial space between the central plug and outer ring. Samples from weeks 1, 4, and 6, were stained with H&E and Alcian blue

Results: Push-out test results revealed distinctions in interfacial strength between scaffold and tissue samples. Scaffold samples possessed significantly ($p<.01$) higher push-out strength than tissue samples for all time points (Figure 1 B, C). Static samples possessed significantly (p.05) (Figure 1).

Discussion: Significant differences in interfacial strength between scaffold and tissue samples utilized in this project is likely due to the press-fit nature of the scaffold as well as the biologic activity observed on histology. PCL samples must be press-fit into surrounding tissue to ensure a minimal gap at the interfacial zone. This results in a reaction force between the scaffold and tissue ring that increases frictional forces thereby increasing maximal push-out strength to dislodge the scaffold. Due to this discrepancy, a better visual representation of interfacial strength due to novel tissue formation can be found in the control-subtracted bar graph. (Figure 1C)

The reduction in push-out strength from static to dynamic samples is in agreement with earlier data obtained in mechanical testing of ex vivo cultured bovine tissue integration, and in vivo studies that indicate loading may be detrimental to the lateral integration of articular cartilage.[3] One potential mechanism, pertinent for future study, is the potential for micro-motion between opposing faces of tissue/tissue engineered scaffold.

Chondrocytes embedded in the arthritic tissue appear large (~30 µm diameter) and fairly hypertrophic in morphology, consistent with the appearance mid-to-late stage OA.[4] Chondrocytes were observed lining walls of macropores of the PCL/HA scaffold across all three time points. Although indicative of positive attachment to the scaffold, this morphology is consistent with previous study of monolayer dedifferentiated chondrocytes on PCL scaffolds, which suggests a lack of re-differentiation amongst seeded monolayer-cultured cells. [5]

Significance: This work incorporates cyclic axial lading with culture of human tissue and tissue engineered scaffolding materials to determine the effects of loading on lateral integration. Major findings of this study may help guide future in vivo work to investigate post operative surgical regimens including the implementation and duration of weight bearing restrictions after articular regenerative repairs of the knee.

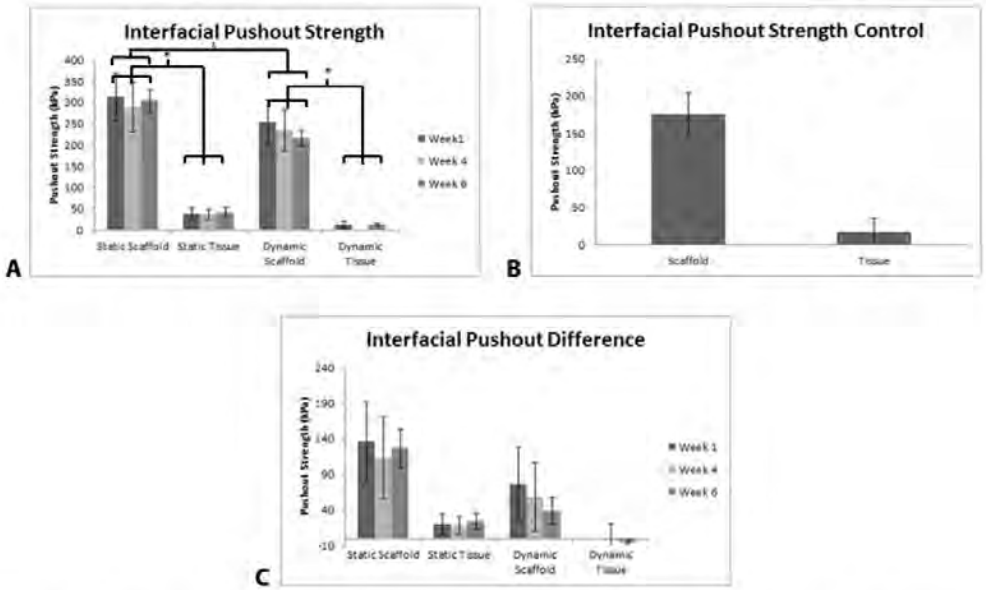


Figure 1: (A) Interfacial pushout strength between the central plug and outer ring of static and dynamic, scaffold and tissue samples after 1, 4, and 6 weeks of culture. (B) Day 0 pushout strength control experiment performed on newly punched non-cultured tissue and scaffold samples. (C) Difference between interfacial strength obtained in (A) and control obtained in (B). ANOVA, Tukey HSD significance values * p<.01, § p<.05

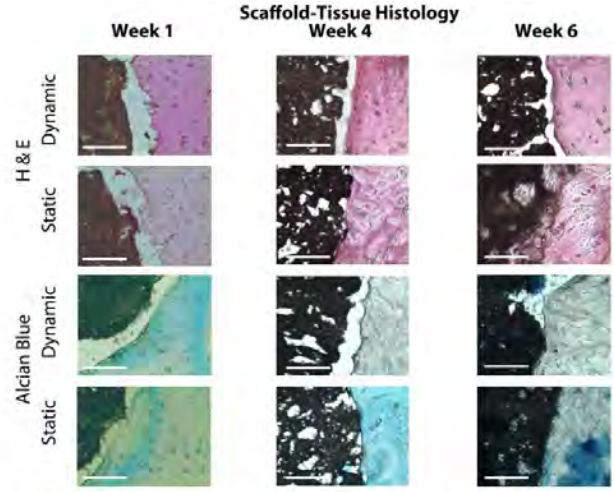


Figure 2: Scaffold sample histology images were obtained with a 20x objective lens. Cross-sectional micrographs are displayed with the integration area aligned vertically, the central scaffold on the left, and the outer tissue ring on the right. H&E staining shows chondrocytes in blue and tissue in red/pink. Alcian blue stains GAGs and proteoglycans blue. Scale bars indicate 200 μm.