# Porcine Cartilage Interface Shear Stress with Four Weeks of Physioxic Culture

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INTRODUCTION: Focal cartilage defects are complex but common clinical occurrences that may progress into cartilage disease [1]. Focal defect repair removes the damaged tissue and replaces it using enzymatic matrix digestion, allograft tissue, autograft tissue, or cellular products [2]. These repairs result in an interface between the graft repair tissue and the surrounding host tissue. Often, this interface fails to fully integrate due to loss of local matrix-secreting chondrocyte cells [2]. Matrix growth between graft and host is sometimes mechanically assessed by push-out tests which measure the strength of the interface. However, other forces such as friction and adhesion may contribute to the measured strength. Therefore, the aim of this study was to evaluate whether biological processes significantly affect early mechanical strength or repair in untreated in-vitro cartilage-cartilage interfaces. This was accomplished using tissue culture and mechanical push-out tests typically used for evaluating engineered cartilage repair treatments [3] but applied to native untreated porcine cartilage to simulate the normal physiological response at the tissue defect.

METHODS: Porcine patella explants (five 4-6 mo. pigs, sex unknown and assumed random) were dissected into cartilage discs of 6 mm diameter and trimmed to uniform thickness (1.79±0.21 mm) to remove the subchondral tissue. Using biopsy punches, a 3 mm-diameter disc-ring interface defect was introduced such that half the samples had a 0.25 mm gap between disc and ring (gap) and the other half had no gap (no-gap) with interface contact maintained throughout the experiment (Fig. 1a). Disc-ring constructs were seated in a 3D-printed Formlabs high temp resin holder previously shown to be biocompatible [4] and placed articular-surface down into 12-well plates. Constructs were each cultured for 28 days in 2 mL chemically defined chondrocyte media (DMEM + 1% ITS + 10 ug/L rTGFbeta1), at 5% $O_2$, which is physioxic for cartilage [5] (Oasis 6404-6, Caron; 37°C, 5% $CO_2$, 5% $O_2$). Every 2-3 days, media was changed and the articular surface was imaged (brightfield (BF), 4x magnification; IX-71, Olympus). Fluctuations in gap width were tracked by averaging three manual width measurements at each time point. After 28 days in culture, interface strength was measured using displacement-controlled push-out tests at 0.1 mm/s to 2 mm on a custom fixture (3230-AT series III test Electroforce, TA Instruments) (Fig. 1b). Load (using a 22 N load cell) and displacement were recorded over time. Cross-sectional images of the disc-ring interface were also taken for observation with a microscope-mounted actuator. Peak load ($P$) was calculated as the maximum magnitude load achieved after smoothing raw force-time data using Savitzky-Golay filtering (1.5 s window, 4th order polynomial) (Fig. 1C). Maximum shear stress ($\tau$) was calculated from sample thickness ($t$), sample diameter ($d = 3\ mm$), and maximum load: $\tau = \frac{P}{\pi dt}$. Cultured samples (n = 6 gap, n = 6 no-gap) were compared by rank-based 2-way ANOVA to control samples (n = 5 gap, n = 5 no-gap), which were mechanically tested by push-out immediately following disc-ring interface dissection in 1x phosphate buffered saline. Data analysis and plotting was conducted in MATLAB R2022b.

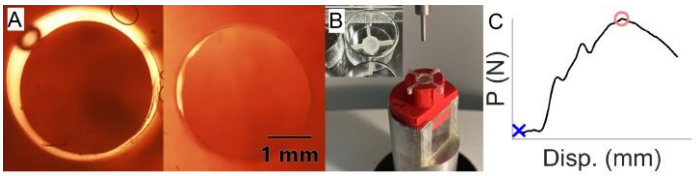


***Figure 1.*** *(A) BF image of articular surface with gap (left) and no-gap (right) day 2 samples. (B) custom devices used, with a resin crosshair holder and push-out mount. (C) example smoothed load-displacement curve for push-out tests, with start (blue X) and peak load (red circle).*

RESULTS: No-gap samples had significantly larger shear stress than gap samples ($p < 0.01$), but culturing did not significantly affect shear stress ($p = 0.68$) (Fig. 2). Interaction between the two factors, gap and culture, was not significant ($p = 0.12$). The variance in shear stress increased in the cultured condition compared to control, particularly for the control no-gap (3.8±1.2 kPa) versus culture no-gap (8.2±6.2 kPa) conditions. Cross-sectional imaging revealed that the interfacial surface was uneven along the height of the cultured gap samples. No overall change in gap width was measurable from the samples imaged over the 28 day culture.

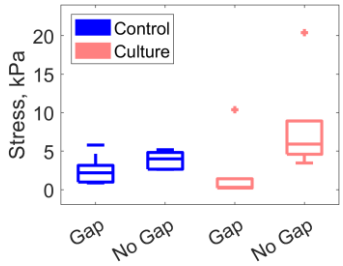


***Figure 2.*** *Box plot of maximum shear stresses in each sample group from push-out tests.*

DISCUSSION: The stresses attained indicate that non-biological mechanisms, potentially adhesion and friction, are a significantly greater determinant of cartilage-to-cartilage interface strength than biological mechanisms within the time-frame and culture conditions studied. A similar experiment with treated bovine cartilage at 4 weeks achieved push-out shear stresses of 39.7±5.6 kPa, similar to the magnitudes observed in this study [3]. The present study utilized physioxic incubation conditions to better approximate the articular environment [5], a practice which has not been consistent among other similar experiments [3,6]. In addition, the use of chemically defined ITS media supplement, rather than the popular underdefined fetal bovine serum supplement [3,6,7], will make this data compatible with future studies. A pilot test confirmed chondrocytes remained viable in ITS-supplemented culture (81.74% viability at 4 days). While studies utilizing engineered tissues and digestive enzymes have shown improved integration of cartilage interfaces [2,3,6,7], untreated tissue defects rarely exhibit any self-repair whether *in vivo* [1] or *in vitro* [7]. Therefore, the findings of this experiment are consistent with current understanding of articular cartilage defects. Additionally, novel low-cost culture devices were developed and 3D-printed for securing the disc-ring cartilage constructs. Future iterations of this experiment could implement various improvements. Mechanical stimulation has been shown to improve explant integration in culture [3]. Treatments such as isolated chondrocyte seeding [3] could form a positive control group to clarify whether culture conditions suppressed integration. Lastly, tuning the local osmolarity during push-out testing would reveal the differences in friction more clearly. Further research will determine the mechanical interactions at the interface in greater detail.

SIGNIFICANCE/CLINICAL RELEVANCE: A more thorough understanding of mechanics at soft tissue interfaces can inform future interventions, such as tissue-engineered repair of articular cartilage defects. It can also reveal the mechanical interactions that accompany long-term tissue conditions such as disease progression at articular cartilage interfaces.

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