In Situ and *In Vivo* Mechanoactivation of Anti-Inflammatory Tension-Activated Repair Patches

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Introduction

Two percent of the world is affected by disc herniations, which are associated with tears the annulus fibrosus (AF) due to injury or advanced intervertebral disc degeneration. The management of disc herniations through microdiscectomy surgery can alleviate symptoms but leaves the annulus unrepaired. Due to the poor capacity of the AF to heal following injury, 10-30% of patients experience recurrent disc herniation.[1] The lack of repair and the acute inflammation that arise after injury further compromises the disc and can result in disc-wide degeneration in the long term. To address this clinical need, we developed tension-activated repair patches (TARPs) for annular repair. TARPs transmit physiologic strains to mechanically-activated microcapsules (MAMCs) embedded within, which activate and release encapsulated biomolecules in response to physiologic loading.[2,3] In this study, we assessed in vitro and in situ activation thresholds for the MAMCs within the TARPs. Furthermore, we evaluated in vivo expression of physiologically relevant proinflammatory cytokines and neurofilament proteins in the anterior and posterior AF after TARP repair to determine if TARP- mediated delivery of an anti-inflammatory drug (IL-1Ra, Anakinra) improved repair.

Methods

In Vitro and In Situ TARP Mechanoactivation: TARPs were fabricated by melt-stamping MAMCs between two hydrated PCL-PEO scaffold strips, 10 mm in length and 3.5 mm in width. Mechano-activation strain thresholds for MAMCs were established in vitro via 1,800 cycles of tensile loading at varying strain levels (0%, 2%, 4%, 6%, 8%, n = 5 samples/strain level, Figure 1A). For in situ testing, a 5 mm × 2.5 mm cruciate laceration was created in the anterior annulus of goat cervical vertebra-disc-vertebra motion segments, with full thickness needle puncture (2.1 mm diameter) to the nucleus. TARPs were sutured to the AF overlying the defect using 6-0 Gortex suture. Seven motion segments were then subjected to 1,800 cycles of cyclic compression from 0 to 300N at 1Hz (Figure 1E-F). Four additional motion segments were utilized as unloaded controls. Following in situ and in vitro mechanical loading, each TARP was gently peeled apart and fluorescent microscopy was utilized to image the outer shell (580nm) and the inner contents (AlexaFluor 488nm) to quantify the number of full versus empty MAMCs. In Vivo TARP Annular

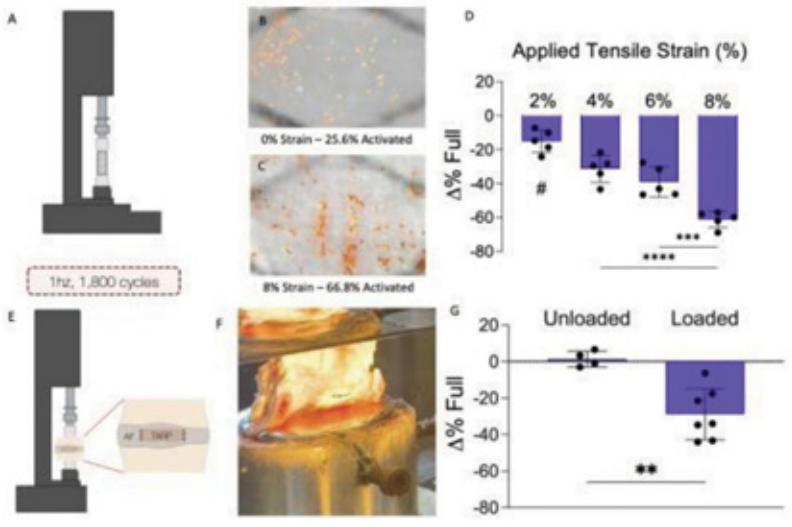

Figure 1. (A) Schematic of uniaxial tension loading of the TARPs. **(B-D).** Image and quantification of MAMC activation across a range of applied tensile strains. **(E-F).** Schematic and photograph of in situ testing of the TARP and **(G).** Quantification of MAMC activation. * $p<0.05$, # $p<0.05$ compared to all other groups.

Repair: To study the physiologic consequences of TARP mechanoactivation and local release of Anakinra (IL-1Ra), BSA-loaded TARPs (E-TARPs) and Anakinra loaded TARPs (A-TARPs) were implanted in a large animal cervical disc annular injury model.[2] Following IACUC approval, eight female goats underwent annular injury of the cervical intervertebral discs, as described above, followed by repair with either the E-TARP (n = 4) or A-TARP (n = 4) over the injury site at either C2-3. C3-C4 served as an injury-only control. Four weeks post-repair, animals were euthanized and isolated motion segments were processed for histology, sectioned in the sagittal plane at 10μm, and stained with picrosirius red and imaged with polarized light microscopy. Immunofluorescence was performed on additional sections to assess protein expression levels of inflammatory cytokines (TNF-α and IL-6) along with expression of Neurofilament Heavy Chain (NFH) and Protein Gene Product (PGP 9.5). Mean fluorescent intensity (MFI) and % fluorescent area were quantified in the anterior and posterior AF for each level using Image J. Statistical analysis was performed via one-way ANOVA with a Tukey's post-hoc test.

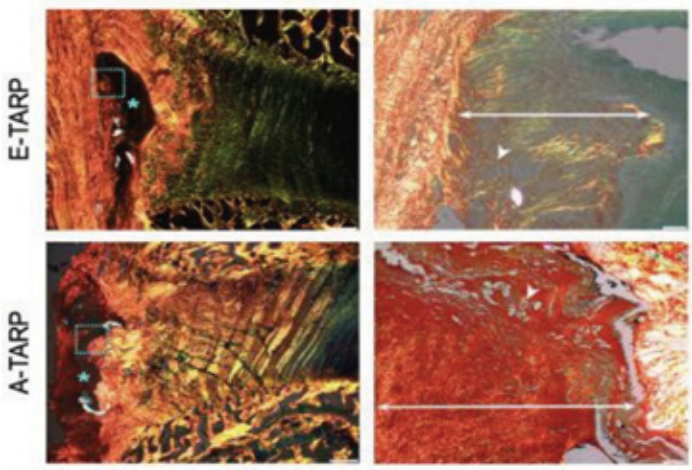

Figure 2. Polarized light microscopy of the anterior annulus of TARP repaired discs. The * denotes location of TARP, the right panel is a higher magnification (scale=100μm) of the area denoted in the dashed box on the left panel (scale=1mm).

Results

In vitro and in situ TARP mechanoactivation: Tensile loading of the TARP in vitro resulted in increasing MAMC activation with increasing levels of applied strain (Figure 1B-C). Compressive loading of spinal motion segments resulted in circumferential strain transfer through the disc to the TARP, significantly increasing MAMC rupture compared to TARPs sutured to the AF but not loaded (Figure 1G). In Vivo TARP Repair: Polarized light microscopy revealed increased collagenous matrix accumulation in the anterior annulus of the A-TARP group, compared to the E-TARP group, at 4 weeks post-repair (Figure 2). Post hoc analysis demonstrated a substantial reduction in the % area and MFI of inflammatory and nerve markers between the injury and E-TARP repaired levels, averaging 96% and 76%, respectively ($p < 0.05$). When comparing the A-TARP repair to the injury model, there was an 82% reduction in inflammation ($p = 0.053$) and a 76% decrease in nerve markers ($p = 0.24$), as assessed via MFI (Figure 3).

Discussion

Our studies demonstrated that MAMC rupture within the TARPS occurs in response to directly applied tensile strain and under tensile strains translated to the TARP in situ during compressive loading of the disc. In vivo, we observed an increase in collagenous matrix deposition in the anterior annulus of the A-TARP group, suggesting that the Anakinra released from the TARPS may have contributed towards enhanced AF repair. Furthermore, TARP repair demonstrated a significant attenuation of innervation and inflammation in the annulus compared to the unrepaired injury in both TARP groups. Interestingly, we observed a trend towards increased innervation and inflammation in the A-TARP group compared to the E-TARP group. Our prior studies in other joints suggest the most MAMC cargo is released over 2 weeks,[4] so it may be that the time course of inflammation and repair is shifted in the A-TARP group. Amid limited clinical alternatives, this work advances a novel annular repair strategy, bringing it closer

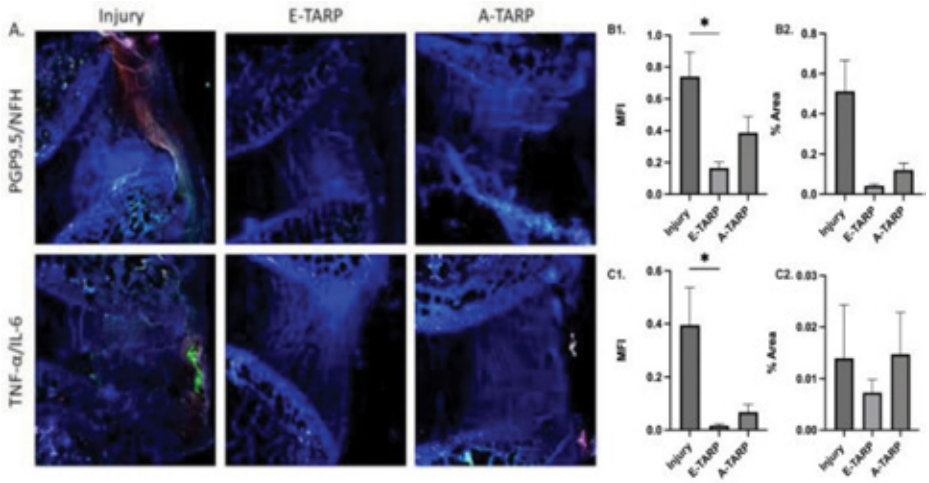

Figure 3. (A) Immunofluorescence microscopy of the annulus across Injury, E-TARP, and A-TARP groups (red = NFH & TNF-α, green = PGP 9.5 & IL-6). Quantification of MFI and % area in the anterior and posterior annulus for **(B)** PGP 9.5 & NFH and **(C)** TNF-α and IL-6. *p<0.05.

to clinical implementation for patients grappling with back pain resulting from disc herniation.

Acknowledgements

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