Stiffness and Topography Modulates TRPV4 Activation in AF Mechanotransduction

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INTRODUCTION: Low back pain (LBP) has been shown to directly correlate with intervertebral disc (IVD) degeneration. The process of IVD degeneration is characterized by the degradation of the extracellular matrix (ECM), ultimately leading to structural changes in the annulus fibrosus (AF), including substrate stiffening and disorganization of highly aligned lamellar collagen architecture [1,2]. These changes in stiffness and topography have been shown to drive downstream cell behavior in many cell types through mechanoreceptors, such as transient receptor potential (TRP) channels [3]. Amongst TRP channels, TRPV4 is of specific interest due to its high expression in the AF, substrate stiffness- and topography-dependent activation, and correlation with pain and inflammation [4-6]. However, despite its socioeconomic importance, AF cell-substrate interaction with TRPV4 has yet to be investigated, hence the objective of the study. We hypothesize that the expression and activation of TRPV4 by pharmacological activators (GSK101790A) or mechanical cues (cyclic strain) will be affected by substate stiffness and topography, resulting in different calcium (Ca2+) flux levels leading to the modulation of ECM, inflammatory, and degeneration-associated downstream targets.

METHODS: For the fabrication of cell stretching chambers with different substrate stiffnesses, the ratio of polydimethylsiloxane (PDMS) Sylgard 184 and 527 was varied at 0, 14, and 24 wt% and cured at 65°C for 24 hours in an aluminum mold. The PDMS stiffnesses (Young's Modulus) were obtained by uniaxial tensile testing (Univert Cell scale) on dog bone-shaped samples with a 10 mm/s strain rate until rupture (n=5). Substate biocompatibility was then assessed through cell viability with an alamarBlue assay using bovine AF cells (n=5). In addition to material characterization and viability, the effects of substrate stiffness on total and maximal Ca2+ influx were studied by exposing AF cells (cultured on different stiffness) to 0.5 µM GSK101790A. The ratio of the intracellular and extracellular Ca2+ ion content was measured with a Fura-2 QBT assay (n=5). To simulate the degeneration-associated loss of collagen alignment, PDMS with aligned and random topographies were fabricated using a coaxial spinning. A PDMS core and a polyvinylpyrrolidone (PVP) sheath were spun, and an additional 100°C in situ and 24-hour post-cure was implemented. PDMS fibers were obtained by dissolving the PVP sheath with 100% ethanol (EtOH) for 20 minutes. Removal of PVP was validated via nuclear magnetic resonance (NMR). Lastly, the PDMS fibers were sputter-coated with gold-plated palladium and imaged with a scanning electron microscope (SEM). The fiber diameter and alignment within 15 degrees were measured using FIJI. Statistical analysis was conducted using a Kruskal-Wallis and Shapiro-Wilk test to check for normality and one-way ANOVA using GraphPad Prism.

RESULTS SECTION: PDMS chambers were successfully fabricated with 9, 63, and 240 kPa stiffness (Fig. 1A) and AF cell viability was greater than 80% in all conditions (Fig. 1B). In addition to stiffness and viability, chambers of varying stiffness portrayed differing surface strains mimicking anatomical conditions. Upon TRPV4 activation, AF cells cultured on PDSM chambers with higher stiffness showed overall higher total and maximal Ca2+ flux levels (Fig. 2). This stiffness-dependent Ca2+ flux modulation shows the mechanosensitive characteristics of TRPV4 in AF cells and may modulate downstream targets. PDMS fibers were also successfully fabricated. After dissolving PVP with a 20 min EtOH wash, the PDMS fibers have similar diameters to collagen fibers (2.97 ± 0.49 µm), as shown in Figure 3. PVP removal was validated via the lack of PVP peaks after the 20-minute EtOH wash in the NMR (Fig. 3).

DISCUSSION: Our biocompatible cell-substrate interaction model successfully mimics the different stages of degeneration. Increasing in vitro stiffness, as observed in vivo during degeneration, led to an increase in TRPV4 activation, as evidenced by enhanced Ca2+ flux; however, additional trials must be conducted for validation. Our preliminary data suggests a possible correlation between TRPV4 activation and stiffness-associated degradation. As changes in intracellular Ca2+ are known to modulate cell behavior and can promote e.g. catabolism, the modulation of Ca2+ flux through TRPV4 represents an interesting pathway to study AF ECM synthesis and remodeling, thus creating a crucial feedback loop. To further improve the cell-substate interaction model to study IVD degeneration, incorporating topography to mimic healthy (aligned) and degenerated (random) AFs is also highly significant. Ongoing experiments are currently working to identify relevant ECM, inflammatory, and degeneration-associated downstream targets and improving the fabrication of aligned PDMS fibers. In the future, experiments will also investigate stiffness and topography in combination with mechanical activation of TRPV4 through cyclic stretching.

SIGNIFICANCE/CLINICAL RELEVANCE: Stiffening and disorganization of the AF are factors directly associated with disc herniation leading to LBP. A better understanding of AF cell-substrate interaction and the mechanotransduction process may contribute to developing new tissue engineering models and novel TRP-based therapeutics.

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IMAGES AND TABLES:

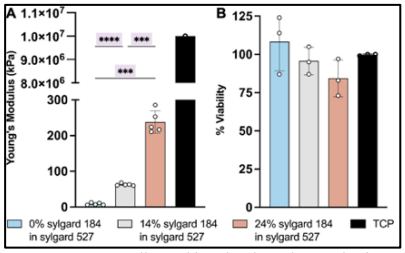

Figure 1. PDMS cell stretching chambers characterization in comparison to TCP. A) Stiffness. (*** $p \leq 0.001$, *** $P \leq 0.0001$). B) AF cell viability comparison.

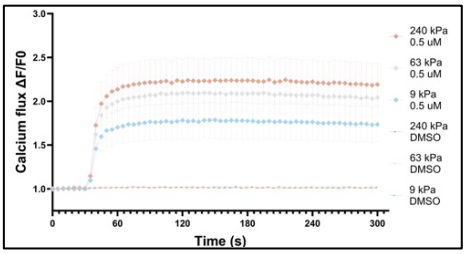

Figure 2. Stiffness dependent calcium flux response after a 0.5 µM GSK treatment and a DMSO control.

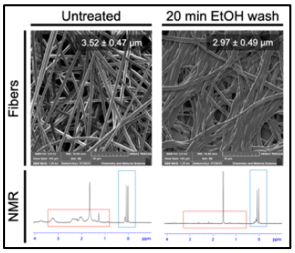

Figure 3. SEM images with fiber diameter analysis and NMR characterization of random electrospun PDMS/PVP core-sheath fibers before and after PVP removal with a 20 min EtOH wash.