Viscoelastic hydrogels regulate adipose-derived stem cells for nucleus pulposus regeneration

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INTRODUCTION: Low back pain is a leading cause of disability worldwide, often attributed to intervertebral disc (IVD) degeneration with loss of the functional nucleus pulposus (NP). In the United States, it is estimated that over 100 billion dollars is spent on LBP treatments annually and spine pain treatments were ranked as the highest healthcare expenditure in 2016 [1]. Combining biomaterials and stem cell therapies have shown promising results for IVD regeneration. However, many challenges exist such as the limited cell differentiation capacity and the poor cell survival and retention rate in the harsh tissue environment. Thus, novel technologies are demanded to develop more supportive biomaterial systems that can protect and instruct transplanted cells development for IVD regeneration. Human NP tissue is highly viscoelastic, relaxing stress rapidly under deformation. However, the impact of tissue-specific viscoelasticity on the activities of adipose-derived stem cells (ASC) remains largely unexplored, and so far, no study has characterized its influence on stem cell therapies for IVD repair. In this study, we aim to investigate the role of matrix viscoelasticity in regulating ASC behavior and differentiation towards an NP-like cell phenotype and determine whether it can be leveraged to potentiate ASC for NP regeneration.

METHODS: Alginate was chosen as a model hydrogel in our study considering its minimal material cues with no mammalian cell receptors and low protein absorption property, as well as its highly tunable viscoelasticity by modulation of polymer molecular weight (MW) and ionic crosslinking density [2] (Fig. 1a). Three viscoelastic alginate hydrogels with the same initial shear modulus ~ 1 kPa (mimicking the stiffness of non-degenerative human NP tissue [3]) but different stress relaxation time scales ranging from 100s (low-MW, fast-relaxing) to 1000s (high-MW, slow-relaxing), covering the range reported for most biological tissues [2], were developed and used to culture human ASCs in 3D for 21 days (Fig. 1b). The mechanical properties of hydrogels were determined using rheological shear tests and compared to those tested with native human NP tissues isolated from donors (approved by the Research Ethics Office of McGill University: IRB #2019-4896). Human ASCs cell viability and metabolic activities were tested by LIVE/DEAD® viability assay and alamarBlue® assay. Immunostaining and quantitative polymerase chain reaction (qPCR) assays of NP markers were used to analyze cell morphology changes (single cell circularity), NP-like extracellular matrix (ECM) secretion (aggrecan and type-II collagen), and the NP-like cell phenotype development. To evaluate the proteoglycans secreted by ASCs, dimethylmethylene Blue (DMMB) assay and Safranin-O staining were conducted to quantify the sulfated glycosaminoglycans (sGAG) released during 21-day culture and visualize their deposition in various viscoelastic hydrogel matrices. All data were presented as mean ± SD, and analyzed using non-parametric, one-way ANOVA with the Kruskal-Wallis test. The statistically significant difference was set as $p < 0.05$.

RESULTS SECTION: Our results showed that the viscoelastic hydrogels recapitulated the stiffness of non-degenerative NP tissue (Fig. 1b, shear modulus ~ 1 - 1.5 kPa) and the fast-relaxing hydrogel ($\tau_{1/2}$ ~ 100s) has the stress relaxation process the closest to the non-degenerative NP tissue behavior. Moreover, the fast-relaxing hydrogel significantly enhanced ASCs long-term cell survival (Fig. 1c, 98.59 ± 0.66% through 21-day culture vs 95.31 ± 1.96% in the slow-relaxing group, n = 4), metabolic activities and promoted NP-like ECM secretion of aggrecan and type-II collagen. Gene expression analysis by qPCR revealed a substantial upregulation of the mechanosensitive ion channel marker *TRPV4* and NP-specific markers such as *SOX9*, *HIF-1α*, *KRT18*, *CDH2* and *CD24* (an indicator of NP-committed cell fate) in ASCs cultured within the fast-relaxing hydrogel, compared to slower-relaxing hydrogels (Fig. 1c, n = 4 - 6). Furthermore, DMMB and Safranin-O staining assays revealed a consistent trend where the sGAG and proteoglycans secretion was the highest in the fast-relaxing hydrogel group (Fig. 1c, 575 ± 36 ng/ng DNA, n = 4, comparable to that in human NP tissue reported as 500 µg/µg DNA in the donor age group of 41-50 years [4]).

DISCUSSION: In this study, we systematically investigated the role of tissue-mimicking viscoelasticity on ASC development for NP regeneration. Our study demonstrated that the fast-relaxing hydrogel matrix mimicking both the native human NP tissue stiffness and time-dependent stress relaxation process promoted the long-term ASC viability and most importantly, exhibited a pronounced enhancement in ASC differentiation towards an NP-like cell phenotype, coupled with a more homogeneous secretion of ECM proteins of type-II collagen and proteoglycans. These findings indicate that a fast-relaxing matrix closer to healthy human NP tissue behavior is more conducive to ASC differentiation and NP-like ECM formation. Admitting that mechanistic investigation in this study is limited, our results suggest a role of TRPV4 as a molecular sensor for matrix viscoelasticity, in accordance with previous studies [5], with its expression significantly enhanced in the fast-relaxing hydrogel indicating ASC sensing matrix viscoelasticity during cell development.

SIGNIFICANCE/CLINICAL RELEVANCE: Our work underscores the important role of matrix viscoelasticity in governing ASC development for NP regeneration and highlights the potential synergy between indispensable biomechanical cues and regenerative strategies. We anticipate this research will contribute to the understanding of fundamental ASC mechanobiology and advance the design of novel hydrogel technologies to improve the therapeutic outcomes of stem cell therapies for IVD repair and LBP treatment.

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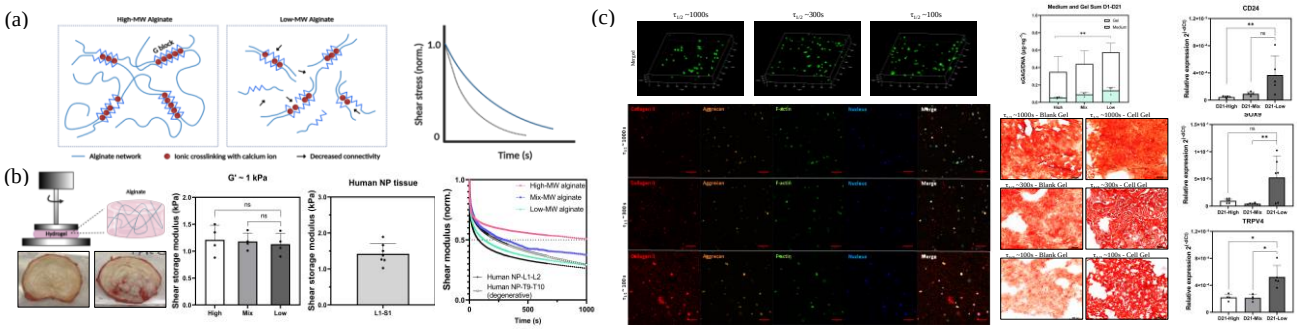

Figure 1. (a) Schematic showing the mechanism of tuning alginate viscoelasticity. (b) Mechanical tests of viscoelastic hydrogels (n=4) and human lumbar NP tissue isolated from donors (n=7, age: 31-56 years). (c) Cell viability, immunostaining (scale bars: 100 µm), DMMB assays, Safranin-O staining (scale bars:200 µm) and qPCR analysis of ASCs development towards NP-like cells.