A Dextran Based Hydrogel System for Studying Fibrotic Extracellular Matrix Cues

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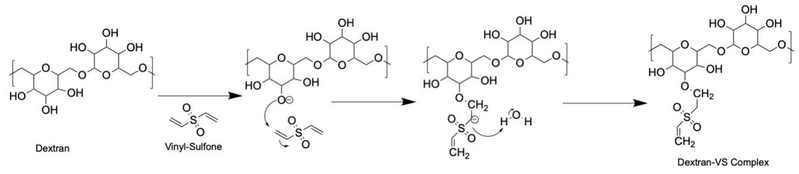
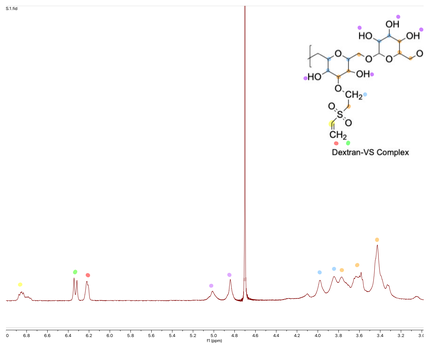
Introduction

Fibrosis can impact almost every tissue in the body, resulting in excessive deposition of collagen and dysfunctional tissue stiffening. Excessive fibrotic tissue is challenging to treat due to its resistant properties to therapies and the long half-life of crosslinked collagen networks. To better understand the development and treatment of fibrotic tissue remodeling, it is crucial to first understand the triggers of fibrotic signaling. This project implemented a tunable hydrogel system to study fibrotic signaling in fibroblasts. First, the synthesis and characterization of a Dextran-Vinyl Sulfone polymer provided the basis for a tunable hydrogel system with controllable peptide presentation. After the synthesis of Dextran-Vinyl Sulfone, it was crosslinked to create hydrogels and modified with different extracellular matrix derived adhesive peptides. 3T3 fibroblasts were seeded on these gels to assess the activation of a mechanosensitive transcription factor that plays an important role in fibrosis (YAP). Quantification of YAP nuclear localization showed increased activation on fibronectin derived peptides compared to laminin derived peptides. Additionally, a fluorescence resonance energy transfer tension sensor (FRET-TS) will be used to analyze force on the central adapter protein in focal adhesions (Talin), an essential linkage for cell adhesion formation that provides force dependent adhesion reinforcement and acts as a mechanosensitive signaling hub.

1. Synthesis of DexVS Complex

The synthesis of DexVS from the reaction presented in *One-Step "Click" Method for Generating Vinyl Sulfone Groups on Hydroxyl-Containing Water-Soluble Polymers. [1]"*





H-NMR Analysis:

- Approximately 6.3 ppm (red)
- Approximately 6.5 ppm (green)
- Approximately 7 ppm (yellow)
- Modification:6.2 ppm and 4.9 ppm
- Final product found to be approximately 47% modified with vinyl-sulfone groups.

Figure 1: H-NMR of Dextran-Vinyl Sulfone

2. YAP Activation on Peptide Modified Gels

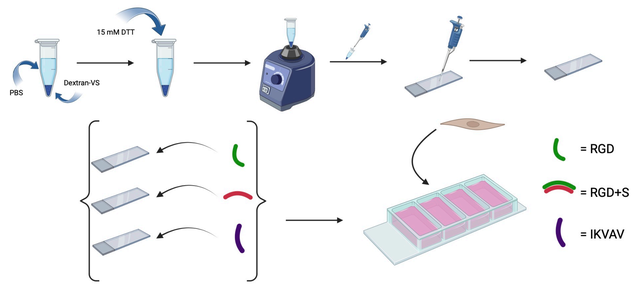


Figure 2: Peptide Modification of DexVS.

3T3 fibroblasts were seeded onto modified Dextran-VS hydrogels to assess activation of a transcription factor that plays a critical role in fibrotic signaling (YAP). The data was analyzed using an ANOVA test to determine the differences among the sample means between the three peptides being tested. With this analysis, the study was trying to determine the nuclear to cytoplasmic ratio of YAP for each group. Analysis indicated that (Nuclear:Cytoplasmic) is significantly higher in the RGD+Synergy peptide modified gels, indicating the highest level of fibrotic signaling activation on these gels.

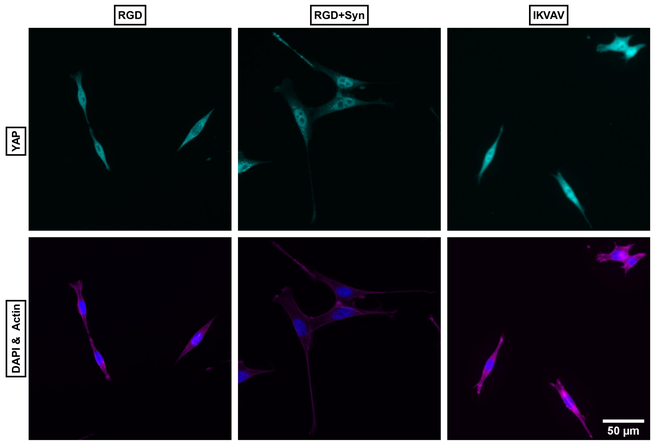


Figure 3: YAP, DAPI, & Actin staining of RGD, RGD+Syn, & IKVAV trial groups.

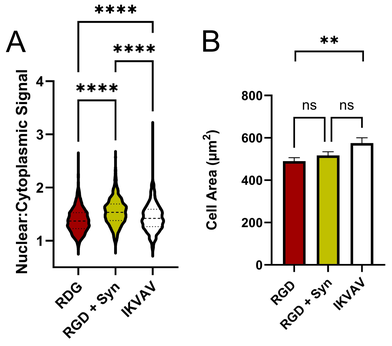


Figure 4: YAP Nuclear/ Cytoplasmic Ratio quantification (A) & Cellular Area (B) of 3T3 cells seeded on peptide modified dextran gels. Bars indicate mean +/- SEM, n>100 cells/grp. One way ANOVA with Tukey's post hoc, **** p<0.0001.

3. Talin FRET Tension Sensor

The final goal of this project will include a measurement of the force on the focal adhesion protein talin, measured using genetically encoded fluorescence resonance energy transfer tension sensor (FRET-TS). This testing relies on the distance-dependent transfer of energy from a donor molecule to an acceptor molecule. TalinTS FRET is dependent on distance between the fluorophores and decreases with force [2,3].

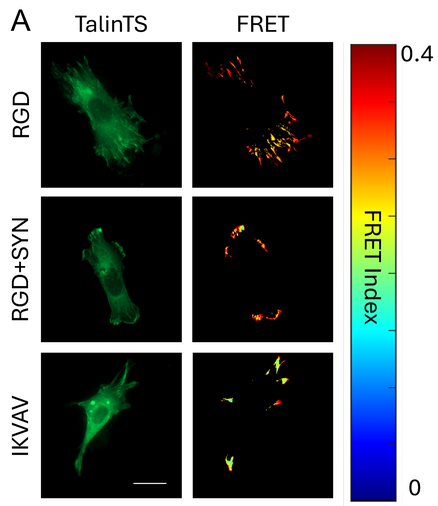


Figure 5: (A) Representative images of Talin (green) and FRET heatmaps for cells on 8% DexVS gels modified with peptides (Scale = 25µm). (B) Average FRET Index for TalinTS adhesions for 15 cells/grp.

Conclusions and Future Works

The first objective of this project was to modify dextran with vinyl sulfone groups to allow for crosslinking and addition of cell adhesive peptides with the goal of ~50% modification, which was achieved (Figure 1). The second objective was to investigate if the synergy domain of fibronectin regulates the activation of YAP in fibroblasts. Specifically, the strong binding interactions between this fibronectin domain and $\alpha_5\beta_1$ integrin has potential to regulate adhesion based mechanosensing. We observe that RGD+Syn increased YAP activation compared to IKVAV, a laminin mimetic peptide that has previously been shown to have antifibrotic effects. Interestingly, force on talin was relatively similar between groups. Overall, the results of this experiment contribute valuable insight into the integrin regulation of YAP, which enhance the understanding of how fibronectin derived peptides (RGD, RGD+Syn) and the laminin peptide (IKVAV) regulate fibrotic signaling.

Acknowledgements

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References

[1] Y. Yu and Y. Chau, *Biomacromolecules* (2010), [2] LaCroix+, *eLife* (2018) [3] Driscoll+, PNAS (2020)