Active Microrheology of Magnetically-aligned Fibrin Gel to Measure Stiffness Anisotropy

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Statement of Purpose

Cells migrate and align in response to aligned fibrils in the ECM.[1] This preferential orientation of cells is called contact guidance. Contact guidance of cells in response to aligned fibrils is crucial to the development of aligned tissue-equivalents resulting from cell-contracted fibrin gels.[2] However, the mechanism underlying cell contact guidance in an aligned fibrin gel is unknown. Mechanical anisotropy, chemical anisotropy, and steric anisotropy of the fibril network are long-standing hypotheses for the mechanisms.[3] This work is the basis for assessing whether stiffness anisotropy is the major contributing mechanism to contact guidance. Understanding the mechanisms of contact guidance will allow them to be used and manipulated to better engineer tissue-equivalents.

Methods, Materials, and Analytical Methods

Active microrheology (AMR) was used to characterize viscoelastic anisotropy of a fibrin gel's fibril network after magnetic alignment and subsequent cross-linking. Previous work has shown that AMR can be used to characterize the storage modulus (G') and the loss modulus (G") of the fibrin network [4], magnetic alignment creates a uniformly aligned fibrin gel [5] and that fibrin gels can be photo-crosslinked for stiffening using a Ruthenium-catalyzed dityrosine formation without loss of cell viability.[6] AMR was performed on replicate fibrin gels in two groups: gels aligned at 7T, and gels aligned at 7T and then photo-crosslinked.

Results

AMR results are shown in Fig. 1. The values of G'x/G'y ,where x is the direction of fibril alignment, are plotted for the non-crosslinked aligned gel, and the crosslinked aligned gel. The non-crosslinked aligned gels exhibited anisotropic stiffness and the crosslinked aligned gels exhibited increased anisotropic stiffness (as shown). Both crosslinked samples were different from the non-crosslinked samples based on 2-sided t-test.

Discussion

These results show that gels that are otherwise identical except for stiffness anisotropy might be created using magnetic alignment with and without subsequent Ruthenium-catalyzed photo-crosslinking. If cells entrapped during gel formation exhibited stronger alignment with the aligned fibril direction in the case of subsequent photo-crosslinking (leading to a greater stiffness anisotropy), then sensing stiffness anisotropy of an aligned fibril network could be concluded to be a dominant mechanism of the contact guidance response. This would require suitable control experiments showing that the photo-crosslinking does not affect cell adhesion or network microstructure (i.e. the chemical anisotropy and steric anisotropy are unchanged). These experiments are ongoing, with no change of steric anisotropy evident from analysis of confocal reflectance images (not shown).

Conclusions

AMR successfully measures local stiffness anisotropy in magnetically-aligned fibrin gel. Ruthenium-catalyzed photo-crosslinking can be used to create fibrin gels with varying anisotropic stiffness without changing the fibrin composition. This combination of methods will allow the stiffness anisotropy hypothesis of contact guidance to be thoroughly tested.

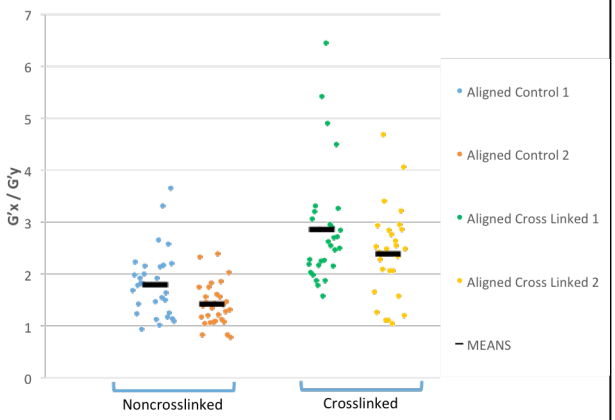

Fig. 1 AMR results of local elastic modulus (G'x, G'y) measurements for control, magnetically-aligned, and magnetically-aligned + Ru-crosslinked fibrin gels, plotted as the stiffness anisotropy, G'x/G'y. The anisotropy is greater in the aligned gels after crosslinking, which establishes feasibility of testing the stiffness anisotropy hypothesis of contact guidance.

References

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