FIBROBLAST-MEDIATED COLLAGEN FIBRIL REORIENTATION IN THREE DIMENSIONS USING CONFOCAL REFLECTION MICROSCOPY

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Structural anisotropy is an important feature of many native connective soft tissues that allow them to best perform their mechanical function. However, the chemical and mechanical signals that guide cells to remodel the extracellular matrix (ECM) to the appropriate morphology during tissue development and repair are poorly-understood. While, for example, it is well-established that contractile cells can produce anisotropy in mechanically-constrained collagen gels via cell traction-induced fibril reorientation [1], a greater understanding of the thermodynamics of the relevant chemo-mechanical processes is required in order to better engineer tissue replacements and repair strategies. It is hypothesized that fundamental thermodynamic principles like maximum dissipation are satisfied during the evolution of such processes [2]. The conditions that must be met for satisfaction of these principles have been worked out for this representative example of collagen gel remodeling. These conditions possess the attractive feature of retaining their validity independent of specific constitutive models for the mechanical response of the cytoplasm and ECM, the details of which are elaborated upon. The experimental verification of these conditions requires detailed, three-dimensional (3D) information on the evolution of collagen fibril orientations to fully characterize the thermodynamic system.

To this end, reflection-mode confocal laser scanning microscopy (CLSM; e.g., [3]) and image analysis methods [4] are adopted and extended. Specifically, a gradient-of-Gaussian filtering technique is applied to image-stack volumes of collagen gel microstructure obtained by CLSM. This field data is then transformed into angle spectra that describe the 3D orientation distribution of gel fibrils. The algorithm is validated in two dimensions with representative images containing known feature orientation distributions. The methodology is demonstrated via the time course of cell-mediated uniaxial compaction of a fibroblast-populated collagen gel. While focusing experimentally on this particular case of soft tissue remodeling, the approach forms the basis for future work quantifying engineered tissue mechanics by providing the 3D orientation data essential to describing the dissipative character of the system.

References

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