FINITE ELEMENT MODELING OF COLLAGEN FIBERS IN THE MECHANICAL INTERACTION BETWEEN CELLS AND THE EXTRACELLULAR MATRIX

DISSERTATION

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By

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Abstract

Cells can sense, signal and organize via mechanical forces. The ability of cells to mechanically sense and respond to the presence of other cells over relatively long distances across extracellular matrix (ECM) has been attributed to the strain-hardening behavior of the ECM. In this study, we explore an alternative hypothesis that the fibrous nature of the ECM makes long-range stress transmission possible, which could provide a mechanism for long-range cell-cell mechanical signaling. To test this hypothesis, we built 2-D and 3-D finite element models of stress transmission within cell-seeded collagen gels. To examine the role of collagen fibers in lateral stress transmission, confocal reflectance microscopy was used to develop 2-D image-based finite-element models. Models that account for the gel’s fibrous nature were compared with homogenous linear-elastic and strain-hardening models to investigate the mechanisms of stress propagation. To examine the role of collagen fibers in cell thickness sensing, 3-D finite element models with idealized fiber networks were built, and the stress transmissions in fibrous and homogeneous ECM were compared. Finite-element analysis revealed that stresses generated by cell contraction are concentrated in the relatively stiff ECM fibers and are propagated farther in a fibrous matrix as compared to linear elastic or strain-hardening homogenous materials. These results support the hypothesis that ECM fibers, especially aligned ones, play an important role in long-range stress transmission. Further, fluid-
structure interaction models were built to investigate the interplay between collagen fibers and interstitial fluid. The results suggest that in cell culture, cell movement is the key factor in defining fluid-flow development at the microscopic ‘cellular’ level, and the cross-links are the key factor that determines the micro-mechanical environment.
Dedication

This dissertation is dedicated to my family.
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Chapter 1 : Introduction

All living organisms, unicellular or multicellular, have an organized structure: they are composed of cells that are the fundamental structural and functional units. Multicellular organisms have additional hierarchy above the cells: the organized cells form a tissue, the tissues are organized into organs, the organs function together within systems that comprise the different systems of the body.

Living organisms have the ability to adapt to changing environmental conditions through natural means. In recent years, there has been a growing appreciation of the importance of the biochemical and biomechanical interactions between cells, the fundamental structural and functional units, and their local environment, the extracellular matrix (ECM), on a myriad of cellular functions relevant to development, homeostasis, and disease. Many experiments have shown that the biomechanical interaction between cells and the ECM involves in a broad range of cellular functions, from differentiation to proliferation and viability. The study in this dissertation focused on computational modeling of the mechanical interaction to understand some underlying mechanisms.

1.1 The Structure of the Extracellular Matrix

The extracellular matrix (ECM) is a complex structural entity surrounding and supporting cells that are found within all tissues and organs. This matrix is composed of a
variety of proteins and polysaccharides that are secreted locally by neighboring cells. The proteins and polysaccharides are assembled into an organized meshwork in close association with the surfaces of the cells that produced them (Alberts et al., 2002).

Figure 1.1 The connective tissue underlying an epithelium. This tissue contains cells and extracellular matrix components. Redrawn from Alberts et al., 2002.

The ECM includes the basement membrane and the interstitial matrix. “The basement membrane is a condensed matrix layer that is formed adjacent to epithelial cells, and other covering cell sheets (e.g. mesothelium), muscle and Schwann cells, adipocytes” (Bosman & Stamenkovic, 2003). It is generally agreed that the basement membrane is mainly synthesized by the cells resting on them. Although their precise composition varies from tissue to tissue, even within the same tissue at different developmental stages, and during tissue remodeling, most mature basement membranes
contain type IV collagen together with proteoglycans (primarily heparin sulfates) and the glycoproteins laminin and nidogen/entactin (Alberts et al., 2002). Collagen IV and laminin individually form a superstructure by self-assembly, and nidogen/entactin and proteoglycans bridge the laminin and type IV collagen networks. Minor components include agrin, fibulin, type XV collagen and type XVIII collagen, SPARC/BM-40-osteopontin. Specific composition of these minor components contributes to basement membrane tissue specificity and heterogeneity. The major differences in basement membrane from one tissue to the other, though, are likely dependent on tissue-specific expression of type IV collagen protomers and laminin subtypes (LeBleu et al., 2007).

Two main classes of extracellular macromolecules make up the interstitial matrix: proteoglycans (PGs) and fibrous proteins. PGs are formed by Glycosaminoglycans (GAGs) and proteins associated through covalent and non-covalent interactions. They look like bottle-brushes, all attached to a central chain, made of hyaluronic acid. To a long strand of hyaluronic acid, several proteins, called core proteins, attach non-covalently. From these core proteins, which come out of the hyaluronic acid strand, several other GAGs are linked covalently forming glycosidic bonds. GAGs are long unbranched polysaccharide chains made of repeating disaccharide units, and one of the two sugars in the repeating disaccharide is an amino sugar that in most case is sulfated. Because there are sulfate or carboxyl groups on most of their sugars, GAGs are highly negatively charged, and they attract a cloud of cations causing large amounts of water to be sucked into the matrix. On the other hand, negative charges cause GAGs to be repelled by each other and make them too stiff to fold up into the compact globular structures that
polypeptide chains typically form. As a result, PGs occupy large amounts of the extracellular interstitial space within the tissue in the form of a hydrated gel.

The main fibrous ECM proteins are collagens, elastins, fibronectins, and laminins. Collagen is the most abundant fibrous protein within the interstitial ECM and constitutes up to 30% of the total protein mass of the multicellular animal (Frantz et al., 2010). The characteristic feature of a typical collagen molecule is its long, stiff, triple-stranded helical structure in which three collagen polypeptide chains (α chains), are wound around each other to form a ropelike superhelix. About 25 distinct α chains have been identified so far, each is encoded by a different gene and is molecularly and immunologically unique. Combinations of these genes are expressed in different tissues and result in the production of about 20 different types of collagen molecules, derived from the mixtures of various collagen α chains forming a distinct helix. The type, distribution and orientation of collagen molecules reflect the function of the tissue in which it is found. The main types of collagen found in connective tissues are types I, II, III, V, and XI, type I being the principal collagen of skin and bone, and type II collagen forming the cartilage found in joints. “After being secreted from the cell, these collagen molecules assemble into ordered polymers called collagen fibrils, which are thin structures (10-300 nm in diameter) many hundreds of micrometers long in mature tissues. Collagen fibrils often aggregate into larger, cable-like bundles, several micrometers in diameter, which can be seen in the light microscope as collagen fibers” (Alberts et al., 2002).

An extensive network of elastic fibers is found in tissues that require greater compliance, such as skin, lung, and blood vessels. In this elastic fiber network, individual
elastin molecules are highly cross-linked with each other, and they remain unfolded such that random coils are formed. It is this cross-linked, random-coil structure of the elastic fiber network that allows it to stretch and recoil like a rubber band. Long, inelastic collagen fibers interwoven with the elastic fibers limit the extent of stretching and prevent tissue tearing.

The extracellular matrix contains numbers of non-collagen proteins that typically have specific binding sites for other matrix macromolecules and for receptors on the surface of cells (Alberts et al., 2002). The first of them to be well characterized was fibronectin. Fibronectin is a large, fiber-forming glycoprotein composed of two disulfide-bonded subunits. Each subunit has a multi-modular structure and comprises repeats of three distinct types of motifs, called fibronectin I, II and III. The protein exists in the ECM in fibrillar superstructures with a characteristic compliance – it can be stretched several times over its resting length by cellular traction forces (Smith et al., 2007). Fibronectin binds to integrins through a cell adhesive site comprising modules III8-III10. The most critical site is the Arg-Gly-Asp (RGD) sequence in III10. The fibronectin molecule also has many other adhesive sites for different substances, including fibrin, heparin, and collagen (Alberts et al., 2002; Magnusson & Mosher, 1998).

Laminins are major proteins in the basement membrane. They are large, flexible proteins composed of three very long polypeptide chains (α, β, and γ) arranged in the shape of an asymmetric cross and held together by coiled-coil and disulfide bonds. Like many other proteins in the extracellular matrix, the laminin in basal lamina consists of
several functional domains that bind to laminin receptor proteins on the surface of cells (Alberts et al., 2002).

1.2 The Function of the Extracellular Matrix

In addition to providing essential physical scaffolding for the cellular constituents, the ECM serves many other functions. As a major component of the microenvironment of a cell, the ECM takes part in most basic cell behaviors, from cell proliferation, adhesion and migration, to cell differentiation and death (Lu et al., 2011) “since cellular responses are tissue and context dependent in terms of both biochemical and biomechanical cues” (Cox & Erler, 2011). The multifunction feature of the ECM depends on its highly dynamic structure and remodeling. Many disease phenotypes caused by dysregulated ECM illustrate the importance of correct biochemical and biophysical ECM properties on the regulation of cell and tissue homeostasis (Cox & Erler, 2011).

1.2.1 The Biochemical Regulation of the Extracellular Matrix

The ECM can initiate biochemical cues that regulate many facets of cell behavior through a number of mechanisms. Firstly, by binding cytokines, proteases, and protease inhibitors through proteins, the ECM acts as a reservoir for multiple growth factors and cytokines (Cox & Erler, 2011; Kyriakides & Bornstein, 2003). For example, tissue perturbations lead to a rapid release of transforming growth factor-β (TGFβ) and allow for rapid and localized changes in TGFβ activity without de novo synthesis (ten Dijke &
Arthur, 2007). Secondly, the basement membranes act as molecular filters, semipermeable selective barriers to the movement of cells, and guide the formation and regeneration of tissues, as basement membrane proteins possess a number of binding sites for cell adhesion molecules (Alberts et al., 2002). Mutations in different basement components have been related to multiple diseases. For example, human hereditary kidney disorder (Alport syndrome) has been connected with mutations in type IV collagen α–chain genes. Investigations of Drosophila mutants have shown that the loss of apicobasal polarity caused basement membrane disruption is a driver of tumorigenesis (Cox & Erler, 2011).

PGs can also serve as selective filters to regulate the traffic of molecules and cells according to their size, charge, or both, as their GAG chains can form gels of varying pore size and charge density. In addition, PGs are thought to have an important role in chemical signaling between cells. They can be found on the surface of cells as coreceptors to help cells respond to secrete signal proteins. They also bind a number of secreted signal molecules and can enhance or inhibit their signaling activity. For example, SLRPs (small leucine-rich proteoglycans) are found to be involved in many signaling pathways including binding to and activation of epidermal growth factor receptor (EGFR), insulin-like growth factor 1 receptor (IGFIR) and low-density lipoprotein-receptor-related protein 1 (LRP1), and regulation of inflammatory response reaction (Frantz et al., 2010).

Matrix glycoproteins regulate cell adhesion, support chemotaxis and migration, and direct tissue development (Hurskainen et al., 2010; Rozario & Desimone, 2011) by
initiating critical biochemical cues. For example, it was found that enhanced binding of integrin to collagen inhibited mammary gland branching (Alford et al., 1998). Researchers also found out that fibronectin plays a role in organizing the polarity of myocardial precursor cells (Rozario & Desimone, 2011).

1.2.2 The Biomechanical Regulation of the Extracellular Matrix

“Cells in multicellular tissues are subjected to various types of forces, including tensile, compressive, hydrostatic pressure, fluid shear stress, and hydrostatic pressure, each of which plays an critical part in shaping, development, and maintenance of the tissue” (DuFort et al., 2011). The process, by which physical forces are converted into biochemical signals at the cell surface and then cellular responses, is defined as mechanotransduction. “A critical component of the mechanotransduction process is the ECM and its interface with resident cells” (Pizzo et al., 2005). Comprised of integrins and a multiplex of adaptor and signaling proteins, including talin and vinculin, the focal adhesion complex is viewed as a mechanosensor link between the actomyosin cytoskeleton and the ECM. A sophisticated mechanosensing machinery, constructed by focal adhesions, cytoskeleton, nuclear matrices, nuclear envelope, and chromatin, defines how cells sense and react to force from the ECM (DuFort et al., 2011).

The biomechanical regulation function of the extracellular matrix does not only stay on the cellular level -- given that cell behaviors depend tightly upon the context of their surroundings. In addition, tissue function also emerges from the coordinated behavior of cells in both time and space. As a result, small local changes in cell-cell or
cell-ECM connectivity, resulting from mechanical cues, can have large influences on global tissue structure and function.

In summary, mechanical cues originated or transmitted by the ECM are integrated in cells and tissues, and thus play critical roles in regulating tissue morphology, differentiation, and locomotion. The significance of the biomechanical interaction between cells and the ECM will be explained in detail in the following section.

1.3 The significance of cell/ECM biomechanical interactions

1.3.1 Cell/ECM biomechanical interactions in morphogenesis

“The ECM provides both elastic and rigid elements that likely participate in the propagation and/or resistance of forces needed to sculpt tissues into functioning organ structures” (Rozario & Desimone, 2011). Morphogenesis is a mechanical process given that it is defined by the movement and deformation of a material structure (Koehl, 1990). In any material, the applied forces, and the resistance to those forces, determines the rate and magnitude of deformation. This is where the ECM is important during tissue morphogenesis.

At the tissue (macro) level, the mechanical interaction between cells and the ECM likely contributes mechanical stiffness for morphogenesis to proceed normally. Human mammary epithelial cells (MECs) form polarized acini with cleared lumens when grown within compliant collagen gel. As the matrix is progressively stiffened, MECs assemble colonies in which cell-cell junction and tissue polarity are compromised, and luminal
clearance fails (Lopez et al., 2008). Another study has shown that when grown in mechanically loaded collagen gels, mammary epithelial cells fail to support acini morphogenesis and functional differentiation (β-casein expression). Furthermore, using glutaraldehyde cross-linked basement membrane gels calibrated for specific elasticity, results showed that highly cross-linked ECM promotes cell spreading rather than cell rounding, and thereby compromises tissue morphogenesis (Paszek & Weaver, 2004).

At the cell-ECM (micro) level, local anisotropies in the distribution of tension could determine morphogenetic movements. For instance, it was suggested that the anisotropy of the micromechanical environment of cells plays a significant role in controlling tissue structure formation in Drosophila embryos (Rauzi et al., 2008). Asymmetric induction of sulfated GAGs (SGAGs) at the mammary gland terminal end bud was suggested as being responsible for altering branch direction and/or end bud bifurcation. Deposition of SGAGs, followed by accumulation of collagen I, creates a thickened and relatively inelastic ECM that acts as a girdle around the end bud at a time when constriction to ductal dimensions occurs (Hinck & Silberstein, 2005). Studies have also demonstrated that local thinning of the basement membrane precedes the branch initiation in the lung, mammary gland, and salivary gland. Further, a stress-induced local increase in the production of ECM degrading matrix metalloproteinases (MMPs) was found at branching sites. This local increase in MMP activity necessitates the pre-existence of spatial non-uniformities in mechanical stress (Gjorevski & Nelson, 2010).
1.3.2 Cell/ECM biomechanical interactions in differentiation

In addition to the role in morphogenesis through regulating cell adhesion, motility and polarity, ECM also functions in the commitment of cell lineage. Recent discoveries have indicated that stem cells can differentiate into various types of mature and functional tissue cells when exposed to intrinsic properties of the ECM, for example, matrix elasticity (Reilly & Engler, 2010). Engler et al. (Engler et al., 2006) and McBeath et al. (McBeath et al., 2004) showed that the stiffness of the ECM substrate can determine mesenchymal stem cell (MSC) fates. MSCs grown on polymer gels with similar elasticity to the brain express neuronal markers and morphology, while those grown on gels that are semi compliant like smooth and skeletal muscle tissues or rigid like bone express muscle or bone proteins respectively.

In addition, by modulating cell shape the ECM can indirectly affect cell differentiation. Beqaj et al. (Beqaj et al., 2002) showed that MSCs differentiated into smooth muscle cells while grown in laminin culture conditions that enrich elongated cells. When cultured to favor rounded cells, expression of smooth muscle markers was inhibited. Other research has shown that by stiffening the substrate, undifferentiated rounded human mesenchymal stem cells (hMSCs) spread and led to a mixed population of adipogenic and undifferentiated cells at day 8 (Guvendiren & Burdick, 2012).

Also, physical forces acting on the ECM itself can affect how cells receive cues from the environment by influencing the shape, size, and composition of focal adhesions (DuFort et al., 2011; Rozario & Desimone, 2011). Stretching of fibronectin reveals cryptic integrin binding sites that reinforce integrin adhesion and promote focal adhesion
complex assembly. These changes have been shown to influence differentiation. For example, Martino et al. (Martino et al., 2009) found out that a fibronectin fragment containing a Leu 1048 to Pro mutation in the cell-binding region stabilizes the conformation of this fragment to favor high affinity binding specifically to α5β1. This fragment was further shown to promote osteogenic differentiation of MSCs.

1.3.3 Cell/ECM biomechanical interactions in homeostasis

The biomechanical interactions between cells and the ECM control many cell activities that are critical for maintaining tissue homeostasis. Mechanical cues initiated from or transmitted through the ECM can trigger the mechanotransduction pathways, and then regulate cell adhesion, migration, proliferation, gene expression, differentiation, and survival. Cells also dynamically remodel the ECM through protein secretion and cellular contractile force, which in turn changes the set of cues cells receive from their environment (mechanical feedback) (Kim et al., 2009).

Cellular response to the mechanical microenvironment has been discussed in previous sections. Cells also play an active role in sculpting the surrounding ECM. “Cells use MMPs to remove steric barriers during migration, to produce cleavage fragments with unique biological activities, and to remodel intercellular junctions during morphogenesis” (Gjorevski & Nelson, 2010; Page-McCaw et al., 2007). The ECM is also mechanically remodeled by cells’ contractile forces. Experiments have shown that cells pull on their substrate when plated on silicone rubber substrate (Harris et al., 1980), and compact their matrix when embedded in 3-D collagen gels (Stevenson et al., 2010).
Mechanical remodeling of the ECM is thought to be the result of cell-matrix interaction. Cell contraction leads to activation of the Rho pathway and the subsequent assembly of stress fibers, which result in integrin clustering, focal adhesion maturation, and elevated traction forces at the cell-matrix interface (Engler et al., 2006). These processes eventually lead to matrix traction and compaction, as well as local ECM stiffness elevation. This mechanical stiffening of the ECM is balanced by negative regulation, in the form of mechanically induced biochemical matrix remodeling. In particular, studies have shown that upon mechanical loading of matrices, the levels of MMP2 and MMP9 dramatically increase (Gjorevski & Nelson, 2009; Prajapati et al., 2000). This tightly regulated cell-matrix crosstalk could be an essential mechanism by which tissues adapt to changes during development, disease progression, or aging.

1.4 Finite element analysis of cell/ECM biomedical interaction

1.4.1 A brief introduction to finite element analysis

Finite element analysis (FEA) is a computer-aided technique for constructing approximate numerical solutions to the partial differential equations that predict the response of physical systems subjected to external influence. It has been widely used as a powerful tool in the last few decades to understand and predict biomechanical phenomena (Erdemir et al., 2012). This computational analysis is considered one of the most comprehensive methods currently available to calculate the complex conditions of stress distributions as are found in biological systems.
The concept of FEA is to obtain the solution to a complex physical problem by dividing the problem domain into a collection of much smaller and simpler domains (elements) in which the field variables can be interpolated with the use of shape functions. The material properties and the governing equations are assumed over the elements and expressed mathematically in terms of unknown values at specific points in the elements called nodes. An assembly process is used to link the individual elements to the given system, and when the effects of loads and boundary conditions are imposed a set of algebraic equations is obtained. Solution of these equations gives the approximate behavior of the entire problem domain.

In FEA, choosing the appropriate mathematical model, element type and degree of discretization are important to obtain accurate as well as time and cost effective solutions (Bathe et al., 1990). Given the right model definition, FEA is capable of computationally simulating the stress distribution and predicting the sites of stress concentrations. Other advantages of this method compared with other research methodologies are the low operating costs, reduced time to carry out the investigation and the means to find information that cannot be obtained by experimental studies (Soares et al., 2011).

1.4.2 FEA of cell/ECM biomechanical interaction

Given the complexity of cell/ECM biomechanical interaction, FEA has been used to help further our understanding of experimental findings. Finite element modeling has been introduced to quantify forces exerted by cells through their movement and
pseudopodial activities (Roy et al., 1999), describe the local stress and strain fields of cells in the ECM (Guilak & Mow, 2000; Kim et al., 2009; Sen et al., 2009; Zeng et al., 2006), investigate the effects of focal adhesion mechanical properties and substrate stiffness on cell migration (Wong & Tang, 2011), as well as to examine the matrix gel contraction by embedded cells (Ohsumi et al., 2008; Sander et al., 2009).

Some of the models mentioned above introduced a biphasic theory (Guilak & Mow, 2000; Kim et al., 2009), which depicts hydrated tissues as a homogeneous mixture of porous, permeable linear elastic solid (proteoglycan, collagen, and chondrocytes) and a movable inviscid interstitial fluid (water and dissolved ions). Others employed a multiscale modeling technique (Sander et al., 2009) in which the Galerkin finite element method is used for the macroscopic (tissue-level) problem, and a microscopic scale is introduced to relate the macroscopic deformation of the tissue to the macroscopic stress. The microscopic network problem is solved at the points where the stress is needed to get the finite element solution. In this set up, the microstructure of the matrix was idealized as a single network of uniform diameter collagen fibers, and other ECM components were excluded.

As described in previous sections, while tissues may appear homogeneous at a macroscopic scale, they are composed of discrete structural entities at the cellular/subcellular scale. So assuming the ECM uniform and homogeneous is an oversimplification. The ECM is also not a simple collagen fiber network; compliant non-fibrous material (contains PGs and hyaluronic acid) play an important role in the mechanical interaction between cells and the ECM and cannot be ignored. In addition, the ECM is known to
exhibit nonlinear, viscoelastic behavior. The viscoelasticity of the ECM is directly related to its complex structure and to its specific macromolecular components. At the macroscale, it is well known that both collagen fibers and the interstitial fluid contribute to the ECM’s viscoelastic response (Chandran & Barocas, 2004; Sander et al., 2009), with the relaxation time in the range of 10 – 200 s (Chandran & Barocas, 2004; Huang et al., 2001). At the molecular level, it has been speculated that the stretching of cross-links between collagen molecules and other macromolecules (such as proteoglycans) leads to the elastic behavior of the ECM, whereas the sliding of molecules and fibrils by each other results in the viscous behavior (Silver et al., 2000), with the relaxation time in the range of 0.2 – 0.6 ns (Gautieri et al., 2012). We proposed here an image based finite element method that incorporates the heterogeneity features of the ECM, and investigate the importance of mechanical property mismatch between the fibrous and non-fibrous materials in the mechanical interaction between cells and the ECM. We also investigated the role of interstitial fluid using time dependent solid-fluid interaction study with simulation time 0 – 200 s. However, the viscoelasticity of collagen fibers was neglected, as has been done in previous studies (Bischofs & Schwarz, 2003; Ohayon & Chadwick, 1988; Wagenseil & Okamoto, 2007).

1.5 Specific Aims

Previous studies suggest that cells can signal and organize via mechanical forces. For example, during embryonic development, corneal fibroblasts exert physical forces to organize extracellular matrix into a unique pattern that provides structural support (Hay,
Similarly, after injury, corneal fibroblasts induce wound contraction and matrix remodeling and they play a critical role in determining corneal clarity, mechanical integrity, and refractive properties (Alaminos et al., 2006). In a reciprocal manner, mechanical cues serve as important signals that influence and regulate cell phenotype in both health and disease. For example, by influencing the production of ECM molecules and the expression of specific genes, mechanical stretch stimulates fetal lung cell proliferation and affects fetal lung maturation (Liu & Post, 2000). Also, the pathophysiological mechanical overload of the heart has been related to excess deposition of cardiac ECM (fibrosis) by cardiac fibroblasts (MacKenna et al., 2000). As a result, accurate and quantitative descriptions of reciprocal mechanical interaction between cells and surrounding collagen matrix are essential for exploring the underlying mechanisms, and can provide information on biophysical parameters that cannot be measured experimentally in situ at the cellular level, e.g., the stress and strain distribution and variations, and fluid flow in the immediate vicinity of the cell. Multi-phasic and multi-scale models have been used to simulate cell-matrix interactions while trying to model the mechanical environment of cells within a matrix (Guilak & Mow, 2000; Kim et al., 2009; Maini & Olsen, 2002; Sander et al., 2009). However, the relationship between the stress, strain and fluid-flow fields at the microscopic ‘cellular’ level is still not fully understood.

The purpose of this study was to develop a multi-phasic finite element method to investigate the stress-strain and fluid-flow environment in cell culturing. We hypothesize that the presence of collagen fibers significantly change the micromechanical
environment of the matrix in which the cells are present. Different properties of the collagen matrix, including fiber number, cross-links and entanglements, and fiber length, all play important roles in determining mechanical properties of fiber networks, and we further hypothesize that cross-links are the key factors.

The overall goal is understand, simulate, and predict how cells and the surrounding collagen matrix interact mechanically. Towards this end, the specific Aims are:

**Aim 1. Develop a multi-phasic FE model to simulate the cell-matrix interaction in cell culture.**

Confocal reflectance microscopy images will be processed to get the topological structures of fibers and cells distributing in cell culturing matrices. Then a 2-D finite element model will be built based on the topological structures and locations. Loading and boundary conditions will be set to match the experimental conditions. The model will be validated by comparing the simulation results with analytical solutions. Non-observable features, e.g., stress, force propagation, and stress gradient, of the cell-matrix interaction will be investigated and quantified.

**Aim 2. Determine the effect of different properties of collagen matrix on cell’s mechanical microenvironment.**

A series of idealized 2-D and 3-D models will be built in which a randomly generated fiber network floating in water representing the matrix. While the waviness of each fiber in the networks will be assigned randomly, fiber number, length, orientation,
and cross-links will be varied to determine the effect of each parameter on cell’s mechanical microenvironment.

Aim 3. Examine the propagation of cell contraction induced force through collagen fiber networks.

Idealized 3-D models will be built with randomly generated fiber networks are embedded in compliant non-fibrous material. Cell contraction induced force propagation will be investigated to understand the mechanism by which cells may sense the stiffness of cell substrates through gel thickness.

1.6 Outline

The contents of the following dissertation chapters are as follows:

Chapter 2 describes the study that investigated the role of collagen fibers in long-range lateral stress transmission between cells. Confocal reflectance microscopy was used to develop image-based finite-element models of stress transmission within fibroblast-seeded collagen gels. Models that account for the gel’s fibrous nature were compared with homogenous linear-elastic and strain-hardening models to investigate the mechanisms of stress propagation. Experimentally, cells were observed to compact the collagen gel and align collagen fibers between neighboring cells within 24 hours. Finite-element analysis revealed that stresses generated by a centripetally contracting cell boundary are concentrated in the relatively stiff ECM fibers and are propagated farther in a fibrous matrix as compared to linear elastic or strain-hardening homogenous materials. These results support the hypothesis that ECM fibers, especially aligned ones, play an
important role in long-range stress transmission.

Chapter 3 describes the study that developed 2-D and 3-D multi-phasic finite element models to examine the stress, strain, and fluid-flow environment in cell cultures. MATLAB (MathWorks, Inc., Natick, MA) programs were used to randomly generate fiber networks in which fiber number, length, and orientation were varied while the waviness of each fiber in the networks was assigned randomly. The fiber networks were then imported into the COMSOL 3.5a (COMSOL Inc., Boston, MA) finite element analysis program as geometry objects. 2-D and 3-D fluid-solid coupling models were built based on the fiber and surrounding fluid geometry. The results quantified the responses due to different properties of the collagen matrix, including fiber length, number, density, cross-links and entanglements that all play important roles in determining mechanical properties of fiber networks with cross-linking and entanglement details being most significant.

Chapter 4 includes the study that explored the importance of collagen fibers in cells thickness sensing. 3-D finite element models of cell-ECM mechanical interactions were developed and used to investigate differences in force transmission within homogeneous and inhomogeneous matrix models. The algorithm developed in chapter 3 was used to randomly generate idealized fiber network geometries, and 3-D finite element models were built based on the fiber network geometry using COMSOL 3.5a (Boston, MA) to explore force propagation and cell-ECM interactions. When forces were applied on a cell on the top of a fibrous matrix, stresses were mostly concentrated in fibers, and up to ninety percent of applied forces propagated along fibers several tens of
microns away from cell/ECM interface. In contrast, for a homogenous ECM without stiffer fibers, stresses induced by cell distributed locally around cells and decayed to insignificant magnitudes within several microns of the site of cell-ECM interaction. These results suggest that the presence of fibers within the ECM may allow cells to mechanically sense their environment far beyond the length scale of cell-matrix adhesion contacts and cell dimensions, and make long-range thickness sensing possible.

Chapter 5 summarizes the major findings of this dissertation and provide the conclusions of the studies carried out. Future work was also proposed to gain a better understanding of the role of collagen fibers in the biomechanical interactions between cells and the ECM.
Chapter 2: Fibers in the Extracellular Matrix Enable Long-Range Stress Transmission between Cells

The content in this chapter is in a manuscript submitted to the *Biophysical Journal:* Xiaoyue Ma, Maureen Weber, Mark D. Stevenson, Alisha L. Sarang-Sieminski, Keith J. Gooch, Samir N. Ghadiali, Richard T. Hart, “Fibers in the extracellular matrix enable long-range stress transmission between cells.”

2.1 Introduction

A rapidly growing body of literature suggests that the passive mechanical environment (e.g., the local viscosity (Edwards et al., 1996) and elastic modulus (Peyton et al., 2007)) impacts cellular function. For example, substrate stiffness affects the rate and direction of migration (Lo et al., 2000; Wang & Pelham, 1997), focal adhesion (Wang & Pelham, 1997) and stress fiber formation (Halliday & Tomasek, 1995; Yeung et al., 2005), as well as responsiveness to exogenous growth factors (Arora & McCulloch, 1999) for cultured fibroblasts. The spreading of smooth muscle cells is dependent on both the density of surface ligands and the material’s compliance with spreading favored on stiffer surfaces (Engler et al., 2004). Additionally, neurons show increased neurite branching densities when cultured on malleable substrates while glial cells, which are normally co-cultured with these neurons, do not survive on deformable substrates
Biomaterial stiffness also influences the differentiation of mesenchymal stem cells (Engler et al., 2006), a cell’s force generation (Paszek et al., 2005) and its own stiffness (Byfield et al., 2009; Engler et al., 2006).

Polyacrylamide (PA) gels are frequently used for studies of the role of substrate stiffness because of the ability to control its mechanical properties (Arora & McCulloch, 1999; Engler et al., 2004; Engler et al., 2006; Flanagan et al., 2002; Halliday & Tomasek, 1995; Lo et al., 2000; Paszek et al., 2005; Peyton et al., 2007; Wang & Pelham, 1997; Yeung et al., 2005). Computational modeling (Sen et al., 2009) and experimental studies (Maloney et al., 2008; Merkel et al., 2007) suggest that cells grown on top of PA gels are responsive to their local mechanical environment but cannot sense substrate stiffness beyond ~20 microns away from the cell surface (Buxboim et al., 2010). For example, when human mesenchymal stem cells (hMSCs) are grown on a 70-micron thick layer of PA adhered to a glass slide, they respond to the stiffness of the PA, not the glass (Engler et al., 2006). Similarly, endothelial cells cultured on PA gels appear to mechanically sense the presence of other cells within 25 microns and respond by migrating towards nearby cells, but they do not respond to those farther away (Reinhart-King et al., 2008).

In contrast to cells on PA gels, cells grown in or on extracellular matrix (ECM) gels may be able to sense and propagate mechanical signals over longer distances. For example, hMSCs grown on type-I collagen gels appear to respond to the stiffness of a glass slide through more than 1000 microns of collagen gel (Leong et al., 2010). Fibroblasts grown on fibrin gels sense the presence of other cells up to 250 microns away and respond by aligning themselves relative to one another. Similarly, hMSCs cultured on fibrin gels
have been shown to sense other cells up to 450 microns away (Winer et al., 2009), while the same cells on PA can only detect cells several tens of microns away (Sen et al., 2009).

Identifying the mechanisms underlying why cell-substrate and cell-cell interactions occur over different length-scales when cells are seeded on/in different biomaterials could help improve our understanding of several experimental observations and help guide the selection of biomaterials for tissue engineering applications. The ability of cells to mechanically sense rigid substrates (e.g. a glass slide) and other cells over longer distances in fibrin and collagen gels relative to short distances in PA gels has been attributed to the strain-hardening behavior of the ECM as opposed to the purely elastic behavior of PA (Leong et al., 2010; Winer et al., 2009). Here we explore an alternative hypothesis - that cells transmit more mechanical stresses through fibrous materials, which might allow them to sense stresses and organize ECM or cells over significantly larger distances than would be possible in a homogenous material with or without strain-hardening behavior.

Previous experimental studies have suggested that ECM fibers can effectively transmit mechanical stresses over long distances. It has been proposed that during vascular morphogenesis, the elastic interactions between fibrils allow the transmission of stress over a significant range so that the ECM serves as a 3-D scaffold for individual endothelial cells (ECs) and clusters of ECs transducing mechanical forces to other ECs at a considerable distance (Vernon & Sage, 1995). Thus, ECs may establish stress-based guidance pathways that provide a mechanism for them to organize into large multicellular structures and communicate over a long distances without the initial requirement of cell-
cell contact (Davis & Camarillo, 1996; Davis & Senger, 2005). Consistent with this view, capillary sprouts in vivo preferentially follow ECM fibers (Anderson et al., 2004). Additionally, isolated corneal fibroblasts align and compact collagen fibers parallel to the axis of greatest effective stiffness. This effect was observed several millimeters away from the location of the physical constraints suggesting that ECM fibers are effective conduits for long-range mechanical force transmission (Karamichos et al., 2007). Experimentally, it is difficult to decouple the relative contributions of strain-hardening and the presence of fibers to an observed cellular response since biologically relevant fibrous materials (e.g. ECM or cytoskeletal elements) are also strain-hardening (Storm et al., 2005; Winer et al., 2009). In this study, we have used confocal reflectance microscopy images of cells and their surrounding network of collagen fibers to generate finite-element models of stress transmission in fibrous networks and have used these models to investigate the relative contributions of fibrous materials or strain-hardening behavior to long-range stress propagation.

2.2 Materials and Methods

2.2.1 Cell Culture

NIH 3T3 fibroblasts (ATCC, Manassas, VA) were cultured in a 5% CO\textsubscript{2}/balance air incubator with Dulbecco’s modified Eagles medium (ATCC, Manassas, VA) containing 10% fetal bovine serum (FBS) (Invitrogen, Carlsbad, CA) supplemented with 1% penicillin/streptomycin (Invitrogen).
2.2.2 Collagen Gel Formation

Acidic bovine collagen (Advanced BioMatrix, San Diego, CA) was mixed with 10X phosphate buffered saline (PBS) (Invitrogen) containing 30 mM phenol red, sterile water, and enough NaOH to turn the mixture neutral or slightly basic. Collagen gels (~40 µL) were cast into the coverslip portion of 35 mm glass-bottom dishes (MatTek, Ashland, MA), sealed with parafilm, and placed in a 37°C incubator overnight to allow a gel to form. This setup allowed the full thickness of the gels (~100-150 µm) to be imaged in our confocal microscopy system. The next day, approximately 5000 fibroblasts in 2 mL of medium were added to each dish.

2.2.3 Confocal Microscopy and Image Analysis

Imaging was performed between four and eight hours and after one day of cell seeding. Reflectance images of the collagen fibers were collected every 1 µm through the depth of the cell to create image stacks of the collagen fibers adjacent to the cell. In addition to the reflectance imaging, a differential interference contrast (DIC) image was taken of each cell in the imaging area. All imaging was performed using a Zeiss LSM 510 Confocal Laser-Scanning Microscope and a 63x objective. Reflectance imaging used a 488nm argon laser as the source and reflected light greater than 480 nm was collected by the detector. The pinhole was set to one airy disk, based on the wavelength used for reflectance imaging. DIC imaging used a 633 nm HeNe laser with the microscope set for
DIC imaging. All images contained 1024x1024 pixels, corresponding to a 202x202 \( \mu m^2 \) region.

ImageJ 1.45b (Rasband, NIH, Bethesda, MD) was used to remove confocal imaging artifacts from the collagen reflection images by subtracting an image of a blank field of view. The optical sections with the highest pixel intensity were chosen from each stack of images by a custom program written in MATLAB (The Mathworks, Inc., Natick, MA). Images were then thresholded at a chosen intensity value that most closely coincides with the outline of the fiber networks in ImageJ.

2.2.4 2-D Geometry Modeling and Meshing

Thresholded microscopy images were converted from TIFF to DXF formatted graphics files using Print2CAD (BackToCAD Technologies, Atlanta, GA). After smoothing the geometries using SolidWorks (SolidWorks Corps, Concord, MA) DXF files were imported into the COMSOL Multiphysics finite-element program (COMSOL, Inc., Burlington, MA). Non-fibrous material, collagen fibers, and cells were separately registered as two-dimensional (2-D) solid geometry objects. Thus, 2-D solid plane stress models with three sub-domains were built, based on their 2-D morphologies representing fibroblasts seeded on top of type-I collagen gels with dimensions of 202 \( \mu m \) by 202 \( \mu m \); the thickness was set to be 10 \( \mu m \). The combined 2-D solid objects were meshed using quadratic triangular elements in COMSOL Multiphysics (Fig. 2.2c). The complex 2-D geometries required a large number of triangular elements to generate good quality meshes due to the curvature and roughness of the contour of collagen fibers and cells.
The meshes generally consisted of 422,000 – 762,000 triangular elements and approximately 1,670,000 – 3,050,000 degrees of freedom. The degree of required mesh refinement was objectively determined using a convergence test, and results demonstrated that the default mesh needed to capture the geometric features was sufficient for computing accurate displacements.

2.2.5 Material Properties

To examine the effect of stiff collagen fibers vs. a strain-hardening matrix on long-range stress propagation, the ECM was modeled using 3 different sets of assumptions: 1) fibrous material with two subdomains: collagen fibers and non-fibrous material, 2) linearly-elastic homogeneous non-fibrous material, or 3) strain-hardening homogeneous non-fibrous material.

For the first set of assumptions, the 2-D models of the collagen fiber networks had several characteristics:

(i) Each intersection of collagen fiber segments was assumed to be a cross-link (Chandran & Barocas, 2005), and modeled as a welded joint.

(ii) The collagen fibers, non-fibrous material and cell sub-domains were assumed to be linearly elastic neglecting the viscoelasticity of collagen fibers and cells, as has been done in previous studies (Bischofs & Schwarz, 2003; Ohayon & Chadwick, 1988; Wagenseil & Okamoto, 2007).

(iii) The material constants were chosen from the literature for the collagen fibers (Bischofs & Schwarz, 2003; van der Rijt et al., 2006), non-fibrous material
(Arevalo et al., 2006; Yang et al., 2009) and cells (Solon et al., 2007; Storm et al., 2005) (Table 2.1).

<table>
<thead>
<tr>
<th></th>
<th>Young’s Modulus $E$ (Pa)</th>
<th>Poisson’s Ratio $\nu$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen Fiber</td>
<td>$300 \times 10^6$</td>
<td>0.45</td>
</tr>
<tr>
<td>Non-fibrous Material</td>
<td>42.6</td>
<td>0.49</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>1000</td>
<td>0.49</td>
</tr>
</tbody>
</table>

Table 2.1 Material properties chosen for collagen fibers, non-fibrous material, and fibroblasts when modeling fibrous gel.

For the second set of assumptions, that of a homogeneous ECM, simulations were based on the same images but the moduli of the fiber- and non-fiber extracellular regions were assumed to be equal ($E=42.6$ Pa) and linearly elastic (Table 2.2).

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<th>Young’s Modulus $E$ (Pa)</th>
<th>Poisson’s Ratio $\nu$</th>
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<tbody>
<tr>
<td>Collagen Fiber</td>
<td>42.6</td>
<td>0.49</td>
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<tr>
<td>Non-fibrous Material</td>
<td>42.6</td>
<td>0.49</td>
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<tr>
<td>Fibroblasts</td>
<td>1000</td>
<td>0.49</td>
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Table 2.2 Material properties chosen for collagen fibers, non-fibrous material, and fibroblasts when modeling homogeneous linear elastic gel.
For the third set of assumptions, the Young’s modulus was chosen to simulate the strain-hardening material seen in collagen gels. A plot of published cone-and-plate rheometry data of shear modulus versus strain for collagen (Storm et al., 2005) was digitized using a customized MATLAB routine (Fig. 2.1). The shear modulus was converted to Young’s Modulus using the following equation (2.1):

\[ E = 2G(1 + \nu) \]  

(2.1)

where \( \nu \) is Poisson’s Ratio and \( G \) is the shear modulus. A nonlinear fitting routine was used to fit the data with a four-parameter Hill curve (Eq. 2.2). The four-parameter Hill curve was chosen since the shape matched the data well and each parameter has a physical meaning. The parameter \( d \) is the minimal modulus, \( a \) is the difference between the minimal and maximal modulus, \( c \) is the strain at which \( E \) has changed by 50% of \( a \), and \( b \) is related to how abruptly the modulus changes from its low to high values with increasing strain.

\[ E = d + \frac{a \times \gamma^b}{c^b + \gamma^b} \]  

(2.2)

The results of the nonlinear fitting routine found values for \( a = 549.2 \) Pa, \( b = 3.357 \), \( c = 0.2035 \), and \( d = 42.61 \) Pa. Figure 2.1 shows the change in Young’s Modulus with shear strain for the experimental data and the curve used to fit the data. Table 2.3 shows the detailed material properties.
Figure 2.1  Plot of experimental data from Storm et al. (Storm et al., 2005) and the nonlinear model of collagen.

<table>
<thead>
<tr>
<th>Material</th>
<th>Young’s Modulus $E$ (Pa)</th>
<th>Poisson’s Ratio $\nu$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen Fiber</td>
<td>Strain hardening</td>
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<tr>
<td>Non-fibrous Material</td>
<td>Strain hardening</td>
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<tr>
<td>Fibroblasts</td>
<td>1000</td>
<td>0.49</td>
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</tbody>
</table>

Table 2.3  Material properties chosen for collagen fibers, non-fibrous material, and fibroblasts when modeling strain-hardening gel.
2.2.6 Loading and Boundary Conditions

To investigate mechanical stress transmission we focused on and created models for images in which two cells were located ~100 um apart. We chose to simulate cell contraction of one cell by imposing a centripetal stress on its membrane while monitoring the resultant displacement, strain, and stress fields on a second cell and in the surrounding materials. This scheme was intended to provide a simplified representation of the loading experienced by the cell and collagen gel under the experimental culture conditions (Stevenson et al., 2010).

Figure 2.2  2-D model set up. (a) Fixed boundary conditions with the ROI surrounded by soft non-fibrous material. (b) A uniform contractile stress was imposed on the void simulating a contracting cell. (c) A dense mesh was used to compute stress distributions in the fibers.

Cell contraction was simulated by applying an inward normal load on the boundary of the first cell with a constant stress $\sigma_n = 1 \ Pa$. Note that the first cell was
modeled as a void to allow precise loading of the surrounding material (see Fig. 2.2b), where \( n \) was the normal direction of the boundary. The 202 \( \mu \)m by 202 \( \mu \)m images of cells and ECM were defined as the region of interest (ROI) for the simulations. To minimize the potential contribution of edge effects, the ROI was surrounded by a linear elastic material with the same properties as the non-fibrous material and with a width equal to that of the ROI. Increasing the width of surrounding region did not influence the results in the ROI. With the chosen width for the linearly elastic boundary, the computational results for cells with a homogeneous linear elastic matrix matched the analytical solution (Fig. 2.9). The outer boundaries of this surrounding material were held fixed (Fig. 2.2a) and prevented rigid body motion of the system.

### 2.2.7 Solution Procedures and Post-processing

Three sets of images were modeled for two different experimental conditions: a pair of nearby cells (~100 \( \mu \)m apart) at 4 hours and a pair of nearby cells at 24 hours. The models were solved as static problems after load application using COMSOL3.5a. Post-analysis results for stress distribution were obtained using COMSOL post-processing features. All calculated values were compared across models using the same conditions to explore differences in the distance of stress propagation, maximum stress and maximum stress gradient on the second cell.
2.2.8 Characterization of Force Distribution between the two cells

We characterized the forces between the two cells. The cell-cell (c-c) axis was determined by connecting the centroids of the two cells and served as a local coordinate system. The force distribution along the c-c direction was calculated by numerically integrating traction stresses over a 20-micron wide cross-section perpendicular to the local c-c-axis at 5 µm increments in the c-c-direction (Fig. 2.3). The mesh on the cross-sections was refined for accurate numerical integration results.
Figure 2.3  The mesh was refined for accurate integration of stresses.
2.2.9 Stress on the boundary of the unloaded cell

We also characterized the normal and tangential stresses on the boundary of the unloaded 2\textsuperscript{nd} cell. To examine stress transmission, stress components on the boundary of the second cell were obtained from COMSOL post-processing features and normal and tangential stresses on the boundary of the second cell were calculated using the following equation:

\[
\begin{align*}
\sigma_n &= \sigma_{nx} n_x^2 + 2\tau_{xy} n_x n_y + \sigma_{ny} n_y^2 \\
\sigma_t &= \sigma_{tx} t_x^2 + 2\tau_{xy} t_x t_y + \sigma_{ty} t_y^2
\end{align*}
\]

(2.3)

where \(n_x\) and \(n_y\) were \(x, y\) components of the normal vector, and \(t_x\) and \(t_y\) were \(x, y\) components of the tangential vector.

2.2.10 Model Validation

The analytical solution for the stress field surrounding a pressurized circle in an infinite, linear-elastic medium is (Hill, 1998):

\[
\sigma_r = - p \left( \frac{a_0}{r} \right)^2
\]

(2.4)

where \(\sigma_r\) is the radial stress, \(p\) is the applied stress, \(a_0\) is the radius of the pressurized circle, and \(r\) is the distance from the center of the circle. An idealized finite element model was used to validate our computational method (Fig. 2.4). The model contained a block of homogeneous material with a 20 \(\mu\)m void representing the first (contracting) cell and a 20 \(\mu\)m diameter cell located 150 \(\mu\)m away in the c-c direction. Contraction of the
first cell was simulated by applying a 1 Pa inward normal stress, fixed boundary conditions were applied on the block edges and the second cell was linear elastic with the same material properties as surrounding homogeneous material.

Figure 2.4  Idealized finite element model - geometry and boundary conditions.

2.2.11 Statistical Analysis

One-way ANOVA was used for comparison of results between more than two groups with p<0.05 considered as being statistically significant.
2.3 Results

2.3.1 Experimental results

Images taken 4 hours after seeding fibroblasts on top of collagen gels revealed relatively circular cells (Fig. 2.5a, c, e) and collagen fibers distributed evenly with little evidence of a preferred direction of fiber alignment (Fig. 2.5b, d, f). In contrast, by ~24 hours after seeding, fibroblasts had elongated (Fig. 2.6a, c, e) and reorganized the collagen fibers leading to areas of higher fiber density near the cells, with highly aligned collagen fibers in the area between the cells (Fig. 2.6b, d, f). The reorganization observed 1 day after seeding was similar to observations reported by others (Stevenson et al., 2010; Vader, Kabla, Weitz, & Mahadevan, 2009).
Figure 2.5 Confocal reflectance (b,d,f) and DIC (a,c,e) microscopy images of cell-seeded type-I collagen gels showing pairs of nearby cells at 4 hours.
Figure 2.6 Confocal reflectance (b,d,f) and DIC (a,c,e) microscopy images of cell-seeded type=I collagen gels showing pairs of nearby cells at 24 hours.
2.3.2 Image process results and mesh

Figure 2.7 shows the results from transferring the original DIC and confocal reflectance microscopy images to thresholded TIFF files. Figure 2.8 shows the mesh for overall geometry, Figure 2.9 shows a close-up view of the mesh for the ROI, and Figure 2.10 shows a close-up view of the mesh for the region close to the contracting cell. The mesh in this region is more refined to capture the complex geometry of the cell.

Figure 2.7  DIC (a) and confocal reflectance (b) microscopy images of cell-seeded type-I collagen gels (24 hours case 2) were thresholded and saved as TIFF files in ImageJ (c,d).
Figure 2.8  A sample of the mesh for overall geometry.
Figure 2.9 A sample of the mesh for the ROI.
Figure 2.10  A sample of the mesh for the region close to the contracting cell. The mesh in this region was further refined.
2.3.3 Model validation results

Using the test model described in section 2.2.10, computational models were validated against the analytical solution. The computational result matched the analytical solution (Fig. 2.11).

Figure 2.11  Plot of computational result and analytical solution for the stress fields around a pressurized cell in an infinite, linear elastic medium.

2.3.4 Computational results

Stresses developed during centripetal contraction by a single cell (the ones on the tops of the images), were assessed by performing simulations based on images of two nearby cells on top of the collagen gel. For simulations based on images of the cells and collagen fibers taken 4 hours after seeding, stresses were concentrated in fibers near the
contracting cell (Fig. 2.12a); after 24 hours stresses were transmitted through aligned fibers between the two cells that were ~120 microns apart (Fig. 2.12d). In contrast, in simulations based on the same images in which the moduli of the fiber and surrounding matrix regions were set to be equal ($E=42.6$ Pa) and linearly elastic (i.e., homogenous conditions), peak stresses around the cell were much lower and decayed quickly with distance from the cell (Fig. 2.12b, e). Changing the matrix properties to a strain-hardening material with a stress strain relationship based on analysis of experimental measurements for collagen did not visibly change the calculated stresses (Fig. 2.12c, f) from that of the linearly elastic material. Figures 2.13-2.15 show the stress plots for cases 1-3 for a cell pair at 4 hours, stresses were concentrated in fibers near the contracting cell. Figures 2.16-2.18 show the stress plots for cases 1-3 for a cell pair at 24 hours. Stress patterns varied as the cell shapes, cell orientations, fiber alignments were different from one case to another, but stresses were consistently transmitted through aligned fibers between the two cells.

Figure 2.19 shows the calculated tensile (Fig. 2.19a) and compressive (Fig. 2.19b) forces between the two cells for case 2 at 24 hours. There were significant compressive forces carried by fibers that connecting the two cells as a consequence of the frame-like behavior of the fiber network with rigid cross-links (Fig. 2.19b and Fig. 2.20).
Figure 2.12  Stress plots show the mechanical inhomogeneity feature of gels, that contained a cell pair, were remodeled to different extents with fibrous material (a,d), homogeneous materials (b,e), and homogeneous materials with non-linear strain-hardening properties (e,f) at 4 hours (a,b,c) for case 1 and 24 hours (d,e,f) for case 2. The contracting cells are on the left-hand side in the images. The unit of stress is Pa. The color bar is from 0 to 1 Pa. Any stress value that exceeds the largest value in the color bar is white. The contracting cells are manually colored green, and the second cells are manually colored cyan.
Figure 2.13  Stress plots for case 1 at 4 hours with fibrous material (a), homogeneous materials (b), and homogeneous materials with non-linear strain-hardening properties (c). The contracting cells are the ones on the upper side in the images. The unit of stress is Pa. The color bar is from 0 to 1 Pa. Any stress value that exceeds the largest value in the color bar is white.
Figure 2.14  Stress plots for case 2 at 4 hours with fibrous material (a), homogeneous materials (b), and homogeneous materials with non-linear strain-hardening properties (c). The contracting cells are the ones on the lower side in the images. The unit of stress is Pa. The color bar is from 0 to 1 Pa. Any stress value that exceeds the largest value in the color bar is white.
Figure 2.15 Stress plots for case 3 at 4 hours with fibrous material (a), homogeneous materials (b), and homogeneous materials with non-linear strain-hardening properties (c). The contracting cells are the ones on the upper side in the images. The unit of stress is Pa. The color bar is from 0 to 1 Pa. Any stress value that exceeds the largest value in the color bar is white.
Figure 2.16  Stress plots for case 1 at 24 hours with fibrous material (a), homogeneous materials (b), and homogeneous materials with non-linear strain-hardening properties (c). The contracting cells are the ones on the upper side in the images. The unit of stress is Pa. The color bar is from 0 to 1 Pa. Any stress value that exceeds the largest value in the color bar is white.
Figure 2.17  Stress plots for case 2 at 24 hours with fibrous material (a), homogenous materials (b), and homogenous materials with non-linear strain-hardening properties (c). The contracting cells are the ones on the upper side in the images. The unit of stress is Pa. The color bar is from 0 to 1 Pa. Any stress value that exceeds the largest value in the color bar is white.
Figure 2.18  Stress plots for case 3 at 24 hours with fibrous material (a), homogenous materials (b), and homogenous materials with non-linear strain-hardening properties (c). The contracting cells are the ones on the upper side in the images. The unit of stress is Pa. The color bar is from 0 to 15 Pa. Any stress value that exceeds the largest value in the color bar is white.
Figure 2.19 Calculated forces between the two cells in case 2 at 24 hours with fibrous material shows that there were tensile and compressive stresses carried by fibers between the two cells.

Figure 2.19 (a) Integral of compressive stress [Pa.mm] vs. Distance from the cell boundary [mu]. (b) Integral of tensile stress [Pa.mm] vs. Distance from the cell boundary [mu].
Figure 2.20  Stress plots for case 2 at 24 hours with fibrous material shows that there were tensile and compressive stresses carried by fibers between the two cells.
A close-up view of a simulated contracting cell with fibrous material for case 1 at 24hrs (Fig. 2.21) shows that stresses are focused in the stiff fibers rather than in the surrounding matrix. The magnitude of stress in fibers attached to the contracting cell were thus even greater than the stress applied to the boundary of the contracting cell (1 Pa). Note that this level of applied stress gave a local cell boundary displacement up to 1.3 µm, consistent with measurements by others (Z. Yang, Lin, Chen, & Wang, 2006).

Figure 2.21 Stresses were concentrated in stiffer fibers (close-up view of the contracting cell with fibrous material (a)), but decayed quickly with distance from the contracting cell with strain-hardening (b) materials at 24 hrs. The color scale for both plots is 0-10 Pa. Any stress value exceeds the largest value in the color bar is white. The contracting cells are manually colored green.

The close-up view of the second, non-contracting, cell with fibrous material for case 1 at 24 hours shows that stresses are concentrated in the fibers, whereas models without fibers – including material with strain-hardening properties – do not have stress concentrations around the second cell (Fig 2.22).
Figure 2.22  More stresses were transmitted to the second cell through fibrous material (close-up view of the second cell with fibrous material (a)) than through strain-hardening (b) materials at 24hrs. The color scale for both plots is 0-1 Pa. Any stress value exceeds the largest value in the color bar is white. The second cells are manually colored cyan.

Figure 2.23 – Figure 2.28 shows the normal and tangential stress distributions on the boundary of the second cell at 4 hours and 24 hours. With fibrous material in the ECM, the normal and tangential stress distribution presented a complex pattern with several spikes or bursts of spikes in normal stress corresponding to the connections between cell boundary and fibers both at 4 hours and 24 hours. When the ECM was modeled as homogeneous and without fibers, the stress patterns were significantly smoother.
Figure 2.23  Plot of normal (a) and tangential (b) stresses on the boundary of the second cell for case 1 at 4 hours.
Figure 2.24  Plot of normal (a) and tangential (b) stresses on the boundary of the second cell for case 2 at 4 hours.
Figure 2.25  Plot of normal (a) and tangential (b) stresses on the boundary of the second cell for case 3 at 4 hours.
Figure 2.26  Plot of normal (a) and tangential (b) stresses on the boundary of the second cell for case 1 at 24 hours.
Figure 2.27  Plot of normal (a) and tangential (b) stresses on the boundary of the second cell for case 2 at 24 hours.
Figure 2.28  Plot of normal (a) and tangential (b) stresses on the boundary of the second cell for case 3 at 24 hours.
Analysis of data similar to that shown in Figure 2.23 – 2.28 for all three models indicated that the normal and tangential stresses gradient on the boundary of the second cell were up to 4 orders greater with fibrous ECM than non-fibrous ECM at both 4 and 24 hours (Fig. 2.29). There were significant differences in maximum normal and tangential stress gradients between fibrous and strain-hardening ECM both at 4 hours ($p=0.004$ and 0.002 respectively) and 24 hours ($p=0.011$ and 0.017 respectively), and between fibrous and homogeneous ECM both at 4 hours ($p=0.003$ and 0.002 respectively) and 24 hours ($p=0.008$ and 0.012 respectively).
Figure 2.29  Plot of peak normal (a) and tangential (b) stresses gradient on the boundary of the second cell for all 3 cases (Mean±STD, n=3). $p<0.05$ is considered statistically significant.
Peak normal and tangential stresses on the boundary of second cells with fibrous material were greater than with non-fibrous material using either linear elastic or strain-hardening properties. With fibrous ECM, the peak normal and tangential stresses on the boundary of the second cell were up to 10 times the applied stress at 24 hours; while with non-fibrous material, the peak normal and tangential stresses on the boundary of the second cell were only 25% of applied stress. At 4 hours, the peak normal stress on the boundary of the second cell was up to 140% of stress applied to the first cell, and the peak tangential stress on the boundary of the second cell was up to 50% of applied stress. With non-fibrous ECM, the peak normal and tangential stress on the boundary of the second cell was only 2% of applied stress (Fig. 2.30). At 24 hours, mean normal and tangential stresses on the boundary of the second cell with fibrous ECM were up to 3 times greater than that with non-fibrous materials. At 4 hours, mean normal and tangential stresses on the boundary of the second cell with fibrous ECM were comparable with non-fibrous materials (Fig. 2.31). There were significant differences in maximum tangential stress between fibrous and strain-hardening ECM both at 4 hours ($p=0.006$) and 24 hours ($p=0.029$), and between fibrous and homogeneous ECM both at 4 hours ($p=0.004$) and 24 hours ($p=0.02$). And there were significant differences in maximum normal stress between fibrous and strain-hardening ECM at 4 hours ($p=0.029$), and between fibrous and homogeneous ECM at 4 hours ($p=0.02$).
Figure 2.30  Plot of peak normal (a) and tangential (b) stresses on the boundary of the second cell for all 3 cases (Mean±STD, n=3). $p<0.05$ is considered statistically significant.
Figure 2.31  Plot of mean normal (a) and tangential (b) stresses on the boundary of the second cell for all 3 cases (Mean±STD, n=3). $p<0.05$ is considered statistically significant.
Our image-based models did not show a significant difference between the homogeneous material property and the strain-hardening material property. To test whether a different strain-hardening material could lead to an increase in stress propagation, we varied parameters $a$, $b$, and $c$ from equation 2.2 and tested them using an idealized geometry shown in Fig. 2.4. Details about the parameter ranges tested can be found in appendix A. As a measure of the amount of strain hardening present in a specific run, we calculated the ratio of Young’s modulus at the first cell boundary versus the Young’s modulus at the second cell boundary on the c-c-axis. Figure 2.32 shows the mean normal stress on the second cell versus the modulus ratio for all 125 parameter combinations. Stress transmission of all strain-hardening materials decreased relative to the amount of strain hardening that occurred in the simulation. Stress transmission for strain-hardening materials was never greater that the stress transmission for the homogeneous case. These studies indicate that the inability of the collagen-strain hardening material property to increase stress transmission in our image-based computational models is not due to the specific numerical values we chose for the strain hardening material or boundary conditions. Our image-based models did show significant differences in peak stress between fibrous and homogeneous material properties. To test how different in stiffness the fibrous and non-fibrous material have to be for more stress transmission, we used an idealized geometry described in the Appendix B (Fig. B.1), and completed a parameter study. The result showed that one order difference is needed for more stress transmission in fibrous material than that in homogeneous material (Fig. B.2).
2.4 Discussion

The results presented in this study suggest several possible mechanisms by which fibers could enhance the transmission of mechanical signals between cells. Our results show that centripetal contraction of a cell with fibers that connect it to a second cell located ~ 100 microns away can focus stress into the stiff fibers and lead to a local increase in fiber stress near the first cell boundary that can be higher than the applied stress. Stress concentration in fibers could explain the higher peak stresses and stress gradients observed on the surface of the second cells. The increase in stress gradients is
noteworthy because previous researchers have shown that stress gradients play an important role in cellular mechanotransduction (Huang et al., 2010; Tschumperlin et al., 2002; Zhou et al., 2012). Specifically, at 24 hours when the collagen fibers are aligned, the cell contraction derived stress is preferentially directed towards the second cell – this does not occur at 4 hours before the fibers are aligned or if a mechanically homogenous matrix is used in the simulations. The stress concentration and higher peak stresses and stress gradient in fibrous materials were found consistent across simulation models and geometry independent, while the values of peak stresses and stress gradient were geometry dependent.

Previous investigators have suggested that the strain-hardening properties of the fibrous materials such as collagen and fibrin gels might be responsible for long-range mechanical communication in these systems (Leong et al., 2010; Winer et al., 2009). A major advantage of the finite element calculation for cell-matrix mechanical interactions presented here is that it allows for decoupling contributions of the fibers and strain-hardening. As shown in Figure 2.1, in this study we investigated this possibility by first specifying homogenous strain-hardening material properties mimicking collagen (Fig. 2.1). Relative to a linear elastic homogeneous material, this strain-hardening material did not alter the amount of stress or stress gradients present at the second cell. It is possible that the strains in our experiments and or simulations may not have been great enough to elicit increased stress transmission via strain hardening or perhaps, if another strain hardening substrate was used stress transmission would increase. To address these possibilities, we conducted an extensive parameter variation study in which the
parameters of the strain-hardening material model were altered to capture a wide range of material behaviors over a wide range of strain values (Fig. A.1). For all combinations of parameters and therefore material behaviors, we did not observe any significant increase in stress propagation to the second cell. Based on these results, we suggest that isotropic strain-hardening is at most a secondary factor. Fibers appear to be the essential element for the long-range transmission of stress in the simulated ECM models.

Our finite element study of cell-matrix interactions used geometries of the collagen fibers and cells based on their in vitro morphologies. Several previous finite element studies of mechanical interactions between cells and substrates used homogeneous substrates and idealized cell geometries (Appelman et al., 2011; Krishnan et al., 2008). While there is at least one report where images of collagen fibers were used as the basis of computational simulations of the deformation of cell-free collagen gels to externally applied loads (Stein et al., 2010), we believe that we are the first to use image-based models to understand cell-matrix interactions. In our image-based models of cell-matrix interactions, we made several simplifying assumptions. We assumed uniformly distributed contractile stress on cell membrane and continuous adhesions between the cell and the ECM despite evidence of variation stresses and adhesion on the subcellular length scale (McGarry et al., 2009). While this assumption might significantly influence the predicted values close to the contracting cell, it should have less influence farther away from the contracting cell (e.g., at the second cell which is ~100 microns away) or when values are averaged or summed over a region (e.g., when calculating force transmitted to the second cell). In addition, in our current models, collagen fibers were
assumed to be in their unstressed state when imaged 4 or 24 hours after seeding the cells. This appears to be a reasonable first approximation given the viscoelastic nature of type I collagen gels and the modest (but clearly non-zero) recoil observed in such gels that have begun to compact if cellular contraction is inhibited (Grinnell, 2000). To more accurately account for the zero-stress state of the collagen fibers, time-lapse microscopy could be used to follow the displacement of fibers from the time cells are initially seeded. Such time-lapse microscopy could also give insight into the best way to model fiber-fiber interactions. In the current model, these are assumed to be welded (physically cross-linked); others have modeled them as torsional springs (Stein et al., 2010), but sliding of fibers that appear to be in contact might also occur. In our models, connected fibers acted more like a frame structure than a net as proposed by others (Wolinsky & Glagov, 1964). As a result there were substantial compressive stresses in some regions with multiple-connected fiber segments. In real collagen networks, substantial compressive loads are unlikely to occur as the individual fibers are on the order of 100 to 100 nm (Sander et al., 2009). In our image based modeling, we considered contiguous regions with a fluorescent intensity greater than the threshold as a single collagen fiber, even if their diameter suggested that the image actually consisted of multiple thinner fibers. Higher resolution of microscopic images would make possible modeling individual collagen fibers. In our current work, we considered a 2-D plane stress finite element model based on a single confocal microscopy plane, which may be an appropriate first approximation for cells grown on the 2-D surface of a 3-D collagen gel. Future work could account for the 3-D nature of the fibrous gel. Finally, in our simulations we considered a wide range of the
isotropic strain hardening cases but never observed a significant affect of force and stress propagation to the second cell. It is possible that relative to isotropic cases, anisotropic strain hardening might yield increased stress propagation.

Recognizing the role of ECM fibers on stress transmission gives insight about the selection of biomaterials for tissue-engineering applications. Especially relevant are cases with cells seeded on or in a biomaterial that are expected to organize and form multicellular structures, e.g., microvascular networks (Liu & Chen, 2007; Sieminski et al., 2000) and islet like cell clusters (Boretti & Gooch, 2006; Hardikar et al., 2003). For example, initially isolated endothelial cells form microvascular networks in collagen (Sieminski et al., 2005; Vernon et al., 1995; Vernon & Sage, 1996; Vernon & Sage, 1995) but they do not do so within Matrigel, which lacks a stromal ECM fiber structure (Sieminski et al., 2002). Endothelial cells within PEG hydrogels functionalized with RGD, VEGF, and a MMP-degradable motif form interconnected, elongated structures, though the cells do not appear as elongated or interconnected as observed for endothelial cells in collagen gels or in vivo (Leslie-Barbick et al., 2009). Since PEG chains exhibit a random coil structure, as opposed to the fibrous structure of collagen, one might expect that PEG would not support the long-range fiber mediated directional force propagation thought to be crucial for MVN formation in collagen gels (Vernon et al., 1995; Vernon & Sage, 1996). Consistent with this notion, endothelial cell density used in these studies with PEG gels was 30-fold greater than typically used for collagen gels, suggesting that higher cell densities and hence decreased intercellular distances might have been necessary to
compensate for the impaired ability to transmit forces over long dimensions in non-fibrous materials.

Taken together, these results suggest that fibrous materials such as stromal ECM concentrate stresses in their fibers but that this concentration of stresses per se is not adequate to account for the enhanced force transmission to nearby cells, which appears to require the alignment of fibers. Treating the ECM as a homogenous isotropic strain-hardening material could not account for the increased force transmission -- suggesting an important role for the fibers themselves. The ability of fibers to facilitate the transmission of forces and stresses over distances greater than several cell diameters likely impacts cell-cell (Guo et al., 2012) and cell-interface (Klebe et al., 1989) interactions and should be considered in the design of biomaterials.
Chapter 3: Finite Element Modeling of the Effect of Fiber Properties on Collagen Gel Mechanics

The content in this chapter is in a manuscript prepared for submission to the Journal of Biomechanical Engineering: Xiaoyue Ma, J. Edward F. Green, Keith J. Gooch, Richard T. Hart, “Finite element modeling of the effect of fiber properties on collagen gel mechanics.”

3.1 Introduction

Previous studies suggest that cells can signal and organize via mechanical forces. Corneal fibroblasts generate physical forces that uniquely pattern the extracellular matrix (ECM) to provide structural support during embryonic development (Hay, 2005). Similarly, during wound healing and the creation of engineered tissues, cell exert mechanical forces that remodel their local environment by reorganizing the ECM and thus play a critical role in determining tissue properties and mechanical integrity (Alaminos et al., 2006; Barocas et al., 1998; Barocas & Tranquillo, 1997; Chaponnier & Gabbiani, 2005). In a reciprocal manner, mechanical cues serve as important signals that influence and regulate cell phenotype in both health and disease. For example, mechanical stretch of extracellular matrix stimulates fetal lung cell proliferation (Liu & Post, 2000). In addition, substrate stiffness influences corneal fibroblast morphology
(Karamichos et al., 2007), the differentiation of mesenchymal stem cells (Engler et al., 2006), as well as the rate and direction of migration for cultured fibroblasts (Lo et al., 2000; Wang & Pelham, 1997).

Various approaches have been proposed to identify, define, and evaluate specific signaling pathways and mechanisms involved in the mechanical interactions between cells and the ECM. Experimental systems (Wang & Pelham, 1997; Balaban et al., 2001; Pizzo et al., 2005; Eastwood et al., 1998; Paszek et al., 2005; Varedi et al., 2000) and mathematical modeling (Maini & Olsen, 2002; Ramtani, 2004; Zaman et al., 2005) have been used to explore how compositional and physical aspects of the extracellular microenvironment affect cell behavior. Finite element models of cells and tissue are especially helpful in that they can be used to provide insights for evaluating biophysical parameters that cannot be measured experimentally in situ at cellular level. They are also potentially useful for making predictions on the outcome of manipulating key biological parameters. Studies have shown that the local mechanical environments of cells have complex profiles, and demonstrate dependence on macroscopic loading features and material properties of the ECM and cells themselves (Guilak & Mow, 2000; Kim et al., 2009; Ohsumi et al., 2008; Sander et al., 2009; Stein et al., 2010; Sen et al., 2009). For examples, the finite element model developed by Guilak and Mow (Guilak & Mow, 2000) suggested that the local mechanical environment of chondrocytes, that were under simple compressive loading conditions, had a very complex profile – the local mechanical environment consisted of tension, compression, shear, and fluid pressure. It was also suggested that the time-varying responses for microscopic cellular mechanical
variables such as axial strain were governed by the macroscopic loading period. Also, alterations in the ECM material properties, due to osteoarthritis, resulted in significant changes in cellular strain amplification profiles, especially in the regime of peak amplification (Kim et al., 2009).

The experimental systems described above all lack quantitative measurements of the interaction between cells and ECM. Most current analytical or finite element models, mostly in two dimensions, treat the ECM as a homogeneous material in which no detailed information regarding fiber distribution and fiber/fluid interplay is provided. For example, there were several studies that utilized homogeneous elastic materials to model the ECM (Sen et al., 2009). Some of the models mentioned above introduced a biphasic theory (Guilak & Mow, 2000; Kim et al., 2009), which depicts the ECM as a homogeneous mixture of porous, permeable linear elastic solid and a movable inviscid interstitial fluid. Others described the microstructure of the matrix as an idealized network with uniform diameter collagen fibers, and the fluid phase was neglected (Sander et al., 2009; Stein et al., 2010). As a result, the mechanism by which particular structural and mechanical properties of the ECM influence its interaction with cells is not well understood. This lack of knowledge precludes establishment of biomimetic microenvironments for efficacious tissue engineering that aims to improve and expedite tissue repair and replacement with use of engineered extracellular microenvironments.

In this work we present a biphasic finite element method to simulate the micro-mechanical environment of idealized ECM. The aim of this study is to investigate the stress-strain and fluid-flow environment of the ECM, and to evaluate the role of different
properties of collagen matrix, including fiber number, orientation, cross-links and entanglements, and fiber length in determining the mechanical microenvironment of fiber networks. The ability of this approach to simulate complex solid-fluid interactions in the ECM and its potential for use with advanced tissue-engineering methods is discussed based on the results obtained with our models.

3.2 Methods

A finite element method consisting of linear elastic fibers and a Newtonian fluid undergoing Stokes flow was developed. This method included the contribution of fluid-solid interactions. The presence of cells was not explicitly considered but their indirect effects on fluid motion, either resulting from cell motion or compaction of the collagen gel and the resulting exudation of the incompressible fluid phase was approximated as an extensional flow.

3.2.1 Modeling of Collagen Fiber Gels

In this study, collagen gels were treated as composite materials with solid collagen fibers immersed in viscous fluid. This assumption was made based on the structural composition of collagen fiber gels and reported diffusion properties of gel samples (Kudo et al., 2008). Custom MATLAB (MathWorks, Inc., Natick, MA) routines were used to generate fiber networks in which the number of fibers $c$, length $l$, and orientation $\theta$ were variably controlled while the waviness of each fiber in the networks
was assigned randomly. In brief, the routines generated anchor points on partial sine curves with starting points, phases, and amplitudes selected at random while the characteristic lengths were specified. An arc-length parameterization method was then used to create splines based on the anchor points. These splines served as the axes of cylindrical fibers that were oriented randomly or at specified angles. This method of generating fiber networks satisfies the intended purpose of creating a wide range of fiber networks of known structure, similar in appearance to those found in reconstituted gels.

The fiber networks were then imported into the COMSOL (COMSOL Inc., Boston, MA) finite element program as geometry objects (Fig. 3.1). 2D and 3D fluid-solid coupling models were built based on the geometry objects using COMSOL 3.5a. In 2D models, because any overlapping fibers are cross-linked, it was difficult to decouple the effect of the cross-links from other fiber properties. In 3D models, fibers were placed in six different thin layers that were stacked along the z-axis so that the number of cross-links could be varied from 0 (no fiber overlapping in any of the 6 layers) to 20 (total number of cross-links in the 6 layers) while maintaining the same overall geometry in the x-y plane. This method allowed for investigation of the effect of cross-linking (Fig. 3.2).
The model of the collagen fiber networks had the following characteristics:

(i) The fibers were assumed to be linear elastic as some numerical and computational studies suggested (VanBavel et al., 2003; Thomopoulos et al., 2006).

(ii) The elastic modulus of collagen fibers was set to be $E=1$ kPa which was more consistent with that of gel and soft tissue (Bischofs & Schwarz, 2003), while the
Young’s modulus for individual fibers was reported on the scale of MPa to GPa (Stein et al., 2010); and the Poisson’s ratio was set to be $v = 0.33$ (Genin et al., 2009).

(iii) Each intersection of collagen fiber segments was assumed to be a cross-link (Chandran & Barocas, 2005), and modeled as a welded joint.

(iv) In 3-D models, the collagen fibers were assumed not to interact between different layers, meaning cross-linking between separate layers was not considered (Boerboom et al., 2003).

3.2.2 Fluid-Solid Interaction Modeling with Moving Mesh

The multiphysics coupling combined structural mechanics with fluid flow by using a Moving Mesh application mode to capture the fluid-structure interactions. The fluid flow was formulated using a Eulerian description and a spatial frame, while solid mechanics was formulated using a Lagrangian description and a reference frame. The fluid structure interaction couplings appeared on the boundaries between the fluid and the solid. The arbitrary Lagrangian-Eulerian (ALE) feature of COMSOL was used to regulate the dynamics of the deforming geometry and the moving boundaries with a moving grid, by computing new mesh coordinates at each time step in the fluid phase based on the movement of the structure’s boundaries and the Winslow mesh smoothing method (COMSOL, 2009).
3.2.3 Boundary conditions

On the fiber–fluid interface, fibers experience a load (defined as total force per area) from the fluid, given by

\[ F_T = -n \cdot \sigma = -n \cdot (-pI + \eta(\nabla u + (\nabla u)^T)) \]  \hspace{1cm} (3.1)

where \( n \) is the unit outward normal to the boundary, \( \sigma \) is the stress tensor for the fluid, \( p \) is the pressure, \( I \) denoted the unit diagonal matrix, \( \eta \) is the dynamic viscosity, and \( u \) is the velocity field of the fluid. This load represents a sum of pressure and viscous forces. The velocities of the deforming interface equaled the deformation rate:

\[ u = u', \]  \hspace{1cm} (3.2)

where \( u' \) on the right-hand sides refers to the displacement.

The boundary settings in the fluid mode of the 2D models was similar to Green and Friedman’s work (Green & Friedman, 2008), and is shown in Fig. 1; the upper and lower boundaries were set to be stress free so

\[ \tau = \sigma \cdot n = 0 \]  \hspace{1cm} (3.3)

The left boundary was set to have a zero velocity, while the right boundary was set to have an x-velocity to mimic the movement of a cell in the ECM (Wojciak-Stothard et al., 2007):

\[ u_x = 50 \mu m/hr \]  \hspace{1cm} (3.4)

The boundary conditions that were employed on the fluid application mode in 3D models were slightly different from that of 2D models, and are shown in Figure 3.3. The boundary conditions were chosen to closely approximate the 2D boundary conditions.
while reach a convergence in 3D simulations. The upper boundary was set to be the symmetry boundary, which prescribed no penetration and vanishing shear stresses:

\[
\begin{align*}
    u \cdot n &= 0, \\
    t \cdot (\sigma \cdot n) &= 0
\end{align*}
\]  

(3.5)

where \( u \) was the velocity field, \( n \) was the boundary normal, \( \sigma \) is the stress tensor for the fluid, and \( t \) was a tangential vector to the boundary. The bottom boundary was set to be stress free so that the total stress on the boundary was zero:

\[
\sigma \cdot n = 0
\]  

(3.6)

The front and back boundaries were set to have no slip so:

\[
u = 0
\]  

(3.7)

The left boundary was set to be an open boundary with no viscous stress as:

\[
\eta(\nabla u + (\nabla u)^T)n = 0
\]  

(3.8)

The right boundary was set having an x-velocity to mimic the movement of a cell in the ECM (Wojciak-Stothard et al., 2007):

\[
u_x = 50 \mu m/ hr
\]  

(3.9)

Figure 3.3  Boundary conditions applied in all 3-D models.
3.2.4 Evaluating the Stress-strain and Fluid-flow Environment

Navier-Stokes equations and the global equilibrium equations based on stress components for the 2D and 3D transient cases were solved for a simulation time of 0-200 seconds using COMSOL 3.5a. The models were solved using a direct solver, UMFPack, for the nonsymmetrical system, and a backward Euler difference approximation for time-dependent analysis. The convergence was examined by refining the mesh. The final meshes generally consisted of ~80,000 triangular elements and ~900,000 degrees of freedom in 2D simulations, and ~120,000 tetrahedral elements and ~1,700,000 degrees of freedom in 3D simulations. The average minimum element quality index across all models was ~0.35, satisfying the requirement for element quality in COMSOL (>0.1). In this study, emphasis was given to the characterization of the fluid flow profile and stress distribution on the fiber network when varying the following parameters: 1) fiber number, 2) fiber length, 3) fiber orientation, and 4) number of cross-links. The velocity field of fluid flow, x-displacement, total-displacement of collagen fibers, and von Mises stress on the fiber network were calculated to quantify the fluid profile and the stress distribution. The plots presented herein indicated the variation of calculated values with changed parameters.

3.2.5 Statistical Analysis

Where applicable, all data were expressed as mean ± standard deviation, and a simple sensitivity analysis was performed to see the effects of parameter changes on the calculated von Mises stress in the fibers.
3.3 Results

3.3.1 Results from 2-D models

In 2D simulations, fluid flow with fibrous approached a steady state in 1 to 2 seconds and was never fully developed to a Poiseuille flow (in contrast to cases with no fibers) because the entangled fibers acted as a solid block as shown on the left portion of the simulation field in figure 3.4.

Figure 3.5 shows the von Mises stress distribution and fluid flow profile in a sample model where fibers were randomly oriented ($0 \leq \theta < \pi$) at 200 seconds. Due to small length scale, the Reynolds number of the flow is small ($R = 6.4 \times 10^{-3} << 1$), and the flow stays laminar. Fibers close to the right boundary, which had a non-zero velocity boundary setting, displaced the most where the flow reached the peak velocity. Stress concentrations were found at the sites of crosslinks (Fig. 3.6). For the model shown in Figures 3.4, 3.5 and 3.6, the mean stress at non-crosslink sites was $3.32 \times 10^{-5}$ Pa as calculated by evaluating the stress on different points. The mean stress at sites of crosslinks was $6.76 \times 10^{-4}$, a 20-fold increase compared to the non-crosslink sites.
Figure 3.4  The velocity field in a sample 2-D model with random fiber orientation.
Figure 3.5 The von Mises stress distribution and velocity field profile in a sample 2-D model with random fiber orientation.
Figure 3.6  The close up view of the region indicated by the dashed box in Fig. 3.5 shows the von Mises stress distribution and velocity field in the region.
For fiber orientation, a range from 0 to 90 degrees and random orientations were explored. Changing from horizontally aligned fibers to a random distribution, showed a sharp increase in the mean von Mises stresses when fibers alignment was near 90 degrees, with an increase by a factor of ~1.5. However, there was no significant linear relationship between fiber orientations and mean von Mises stresses (Fig. 3.7).

Fiber number was varied from 20 to 100, and quintupling the number of fibers gave a trend that increased the mean von Mises stress by a factor of ~1.6. However, the trend was not a significant linear relationship (Fig. 3.8).

In contrast, doubling the fiber length from 100 µm to 200 µm resulted in an increase of mean von Mises stress by a factor of 13. This change was characterized by a strong linear relationship with $R^2$ being 0.91 (p-value=0.00). Maximum von Mises stress was also examined, and it followed the same trend that fiber length had the greatest influence on maximum stress developed in fibers than fiber orientation and fiber number (Fig. 3.9).
Figure 3.7  Plot of mean von Mises stress from sample models with varied fiber orientation (n=3) in 2D. There is no linear relationship between fiber orientation and stresses ($R^2=0.07$, p-value=0.57) although there is increase by a factor of $\sim1.5$ when the orientation is near 90 degrees.
Figure 3.8  Plot of mean von Mises stress from sample models with varied fiber number (n=3) in 2D. There is no linear relationship between fiber number and stresses ($R^2=0.37$, $p$-value=0.28), but the trend is to increase mean stress with increasing fiber numbers.
3.3.2 Results from 3-D models

Fluid-flow development had similar profiles in all 3D sample models. Fluid ingress was at the left boundary, and flowed rightward (x-direction) reaching the maximum velocity at the right boundary (Fig. 3.10). The flow approached a steady state in 1 to 2 seconds, similar to the 2D simulations. In contrast to the 2D simulations, the flow demonstrated a Poiseuille profile even in the presence of stiff fibers because fluid was free to flow over and under the fibers in 3D.
Figure 3.10  The velocity field profile in a sample 3-D model with random fiber orientation.
The x-component dominated the velocity field (Fig. 3.11a, b). As a result, the displacement in fibers was mainly parallel to the x-direction (Fig. 3.12a, b). There is a negative linear relationship between the number of crosslinks and the velocity field ($R^2=0.70$, p-value=0.08), as well as the number of crosslinks and the total displacement ($R^2=0.70$, p-value=0.08).

![Graph](image1)

**Figure 3.11** Plot of mean velocities in fluid domain at time 200s (n=3) for the 3D models. There is a negative linear relationship between number of cross-links and mean velocity field in fluid domain ($R^2=0.70$, p-value=0.08). The y-velocity is much smaller than the velocity field and x-velocity (a), and the x-component of the fluid velocity dominates the velocity field (b).
Figure 3.12  Plot of mean displacement in fibers at time 200s (n=3) in 3D models. There is a negative linear relationship between number of cross-links and mean total displacement in fibers ($R^2=0.70$, $p$-value=0.08). The y-displacement is much smaller than the total displacement and x-displacement (a), and x-component of the displacement dominates the total displacement (b).
As found in 2D models, stress concentrations were found at sites of cross-links (Fig. 3.13). In the model shown Fig 3.2, 3.10 and 3.13, by evaluating the stress on different points, the mean stress at non-crosslink sites was $5.27 \times 10^{-6}$ Pa; the mean stress at sites of cross-links was $1.60 \times 10^{-4}$, which was 30-fold increase. As the number of cross-links increased from zero to 20, the mean von Mises stresses in fibers increased approximately two fold (Fig. 3.14). There was a strong linear relationship between number of cross-links and the stresses ($R^2=0.91$, p-value=0.01).

**Figure 3.13** The close up x-y view of a region in the same sample model of Fig. 3.10 shows the von Mises stress distribution in fibers. Stresses are concentrated on the sites of cross-links.
Figure 3.14 Plot of mean von Mises stresses resulted from sample models with varied number of cross-links (n=3) in 3D. There is a strong linear relationship between number of cross-links and stresses ($R^2=0.91$, p-value=0.01); increasing from 0 to 20 cross-links almost doubled the mean von Mises stress in the fibers.

3.4 Discussion

The micro-mechanical environment of idealized ECM was simulated using 2D and 3D finite-element models with randomly generated fiber networks. The main findings of this study are as follows: 1) With fluid-solid interaction, collagen fibers in all models moved and deformed along with the development of the fluid flow. 2) von Mises stresses developed at the sites of cross-links in 2D and 3D models. In 2D models the results show that fiber length had the greatest influence on stress: doubling fiber length increased the mean von Mises stress by a factor of 13. However, in 2D, because fibers
were cross-linked when they overlapped, the fiber length and the cross-link effect were coupled. 3) In 3D models, there was a strong linear relationship between the number of cross-links and the mean von Mises stress developed in fibers. 4) The fluid-flow profile was determined by the boundary conditions employed on the fluid domain while fibers were floating within, and at sites where fibers were entangled and cross-linked, the fluid flow was not well developed.

There were substantial differences between the 2D and 3D models in the fluid flow profile although the boundary conditions were similar. In 3D, the fluid could flow in z-direction, so that the fiber network had less effect on the development of fluid flow compared to the 2D simulations where the fiber network acted as an almost “solid” block that hindered the development of fluid flow.

Collagen fiber orientation and density have been reported as playing a critical role in determining the mechanical behavior of tissue. As examples, it was suggested that the initial collagen orientation played a dominant role in bioprosthetic heart valve biomaterial fatigue response (Sellaro et al., 2007). Also, reduced bone strength was related to decreased collagen alignment and content (Silva et al., 2006). It is obvious that fiber orientation, length, number all play a role in defining the anisotropic mechanical properties (Silva et al., 2006; Xu et al., 2011), our work presented here provides a method to decouple the effect of crosslinks from other factors.

Simulation results showed stress concentrated at the sites of cross-links in 2D and 3D models, and stresses developed in fibers increased with increased number of crosslinks, which was consistent with the results of experiments testing the mechanical
properties of biological scaffolds (Barnes et al., 2007; Sell et al., 2008). Identifying the role of crosslinks affecting the mechanical environment in the ECM gives insight about the understanding of many experimental findings, and the development of therapeutic tools for diseases. For example, ECM crosslinking was considered as a critical regulator of tissue desmoplasia (Butcher et al., 2009; Levental et al., 2009). Stress focus at the sites of crosslinks might be a factor by which crosslinks increased mechanosignalling, promoted focal adhesions, and forced tumor progression. Susic (Susic, 2007) also claimed that collagen fibers non-enzymatically crosslinked by formation of AGEs (advanced glycation end-products) led to increased oxidative stress, release of growth factors and cytokines, and inflammatory reactions that led to cardiovascular damage. Other than the biochemical factors, stress concentration with increased crosslinks might also contribute to the impairment of ventricular cardiovascular structure and function. Therefore breaking AGEs induced crosslinks between collagen fibers appeared to be a promising therapeutic approach to cardiovascular disease.

The biphasic finite element models took into account the fluid-structure interaction, as it is known that collagen gel is a biphasic system consisting of a fibrillar loose lattice structure filled with a large excess of interstitial fluid (Abou Neel et al., 2006). Many studies have shown the important contribution of interstitial fluid. It actively plays a role in gel’s viscoelastic response, and supports compressive loads (Chandran & Barocas, 2004). It was also suggested that interstitial fluid induces myofibroblast differentiation by providing a myofibroblast-activating signal through increasing matrix tension and autocrine production of TGFβ (Ng et al., 2005). Thus, it is important to
characterize the flow profile of the interstitial flow while investigating the micro-
mechanical environment in the ECM. While several previous finite element studies of
cellular mechanical environment used a biphasic theory (Guilak & Mow, 2000; Kim et
al., 2009), they assumed the ECM as a homogeneous object and neglected the
heterogeneity feature of the ECM. Others described the microstructure of the matrix as an
idealized network with uniform diameter collagen fibers, and the fluid phase was treated
as a void (Sander et al., 2009; Stein et al., 2010). Our models incorporated the collagen
fiber networks and the interstitial fluid, and provided detailed information regarding fiber
distribution and fiber/fluid interplay, which helps to fill in the gap between homogeneous
bi-phasic theory and solid discrete models.

In our models, we made several simplifying assumptions. The fiber-fiber
interactions were modeled as welded joints (physically cross-linked). Others have
modeled them as torsional springs (Stein et al., 2010), or with constitutive equation that
permits fibers to rotate but not bend or slide past each other (Sander et al., 2009). Better
description needs to be found to elucidate the details of these interactions. We also
assumed the fiber networks were comprised of uniform diameter and length collagen
fibers. Scanning electron microscopy of collagen gels revealed a heterogeneous
population of fiber diameters (Sander et al., 2009) while fiber lengths are relatively
constant in samples (Yang & Kaufman, 2009). Future work could account for the
heterogeneous feature of the collagen fiber network. Finally, we solved for a simulation
time of 0-200 seconds as the flow approached a steady state in several seconds for the 3D
models. Longer simulation may reveal more information on the fiber alignment by fluid
flow, but is significantly more computationally time consuming. And fiber collides or pass through might need to be considered in longer simulation.

In conclusion, this study highlights the interplay between collagen fibers and interstitial fluid. It demonstrates how collagen fiber might contribute to mechanical microenvironment in the ECM, and how interstitial fluid affected the movement of collagen fiber network. The findings could give insight into understanding physiological and pathological tissue remodeling as well as potential application in tissue engineering.
Chapter 4: Three-dimensional Computational Modeling of Cell Thickness Sensing in Fibrous Extracellular Matrix

4.1 Introduction

The elasticity of the substrate alters cell phenotype, cytoskeletal remodeling, gene expression, and its pattern of migration and can even direct stem cell fate (Engler et al., 2006; Halliday & Tomasek, 1995; Lo et al., 2000; Vernon et al., 1995; Wang & Pelham, 1997; Yeung et al., 2005). Cells probe the substrate elasticity by exerting traction forces on the substrate via its adhesion molecules (the focal adhesions), and generating a mechanical stress in the substrate. As cells probe the environment, their shape and adhesion, as well as cytoskeletal organization are affected and regulated by the stress at the cell/substrate interface. Stiff substrates generate higher reaction forces and support formation of rigid, stiff stress fibers. As a result, cells appear to have a well spread cell morphology. On the contrary, soft substrates with smaller reaction forces induce lesser polymerization in stress fibers, and cells appear to be round (Leong et al., 2010).

It was often assumed that substrate thickness, apart from the intrinsic substrate stiffness, played a negligible role in determining cellular microenvironment (Lin et al., 2010). This assumption was based on experimental results showing that the in-plane displacement of the matrix induced by cellular forces was limited to a very small region (~ 1 µm), much smaller than the substrate thickness of the matrix (Dembo & Wang,
1999; Yang et al., 2006). Consequently, the substrate thickness was widely considered to be a semi-infinite medium. But recently many studies have shown that the semi-infinite approximation fails when substrate thickness falls below a characteristic thickness. While cell response to the microenvironment on thick gels was found to be independent of gel thickness and guided by gel stiffness alone, thickness plays an increasingly important role as it approaches the characteristic depth scale (Lin et al., 2010; Maloney et al., 2008; Sen et al., 2009).

The interaction depth of cells with a compliant synthetic gel such as Polyacrylamide (PA) has been claimed to be in the range of a few to tens of microns (Buxboim et al., 2010; Maloney et al., 2008; Merkel et al., 2007; Sen et al., 2009). But it has been demonstrated that cells can sense over much larger length scales while interacting with biological polymers: over hundreds or even a thousand microns away. For example, a recent study using fibrin gel has shown up to 450 µm of lateral interaction distance with human mesenchymal stem cells (hMSCs) (Winer et al., 2009), while Leong et al. reported up to 1440 µm thickness sensing distance of hMSCs using type I collagen gels (Leong et al., 2010).

The ability of cells to remodel their environment enables them to sense not only the local (immediate at focal adhesions) but also global mechanical properties (deformed region at a distance away from focal adhesions) (Leong et al., 2010; Vogel & Sheetz, 2006). As a result, the thickness of the substrate comes into play with respect to cell behavior, and has been shown to be one of the determinants of the effective mechanical properties of which cells can sense. For example, with the same local Young’s modulus,
hMSCs developed actin cytoskeleton polymerization and neuronal lineage commitment on thin collagen gel (130 µm), while they maintained the quiescent cell state on thick collagen gel (1440 µm) (Leong et al., 2010). These experimental results imply that the cells sense the thin gel as having a higher effective stiffness than the thick gel, and in turn the cell fate was influenced.

The findings revealed the importance of considering substrate thicknesses when designing collagen-based substrates especially for soft tissue engineering applications. Identifying the mechanisms by which cells sense over one thousand microns of substrate thickness could help improve our understanding of above experimental observations and help guide the design of collagen-based substrates. The object of this study was to explore the potential role that collagen fibers play in thickness sensing of the cells based on idealized collagen fiber network geometries. As we discussed in chapter 2, we found that collagen fibers were the key factor that makes long-range stress transmission between cells possible. In this chapter we hypothesize that collagen fibers play a crucial role in cells’ long-distance thickness sensing. MATLAB (Natick, MA) programs were used to randomly generate idealized fiber network geometries, and 3-D finite element models were built based on the fiber network geometry using COMSOL 3.5a (Boston, MA) to explore force propagation and cell-ECM interaction.
4.2 Methods

4.2.1 Modeling of Collagen Fiber Gels

Custom MATLAB (MathWorks, Inc., Natick, MA) routines (listed in Appendix B) were used to randomly generate fiber networks. In brief, the routines generated anchor points on partial sine curves with starting points, phases, and amplitudes selected at random while the characteristic lengths were specified. An arc-length parameterization method was then used to create splines based on the anchor points. These splines served as the axes of cylindrical fibers that were oriented randomly or at specified angles, and the 3-D fibers were built using the LOFT function of COMSOL-MATLAB module. This method of generating fiber networks satisfies the intended purpose of creating a wide range of fiber networks of known structure, similar in appearance to those found in reconstituted gels.

To mimic cell-on-gel experiments (Fig. 4.1), the fiber networks were then imported into the COMSOL (COMSOL Inc., Boston, MA) finite element program as geometry objects (Fig. 4.2). 3-D continuum finite element models were built based on the fiber geometries with three components: (1) collagen fibers, (2) non-fiber material filling in inter-fiber spaces, and (3) a single cell on top of the gel matrix.
Figure 4.1  Confocal microscopy images of cell-on-gel experiments. (a) Confocal reflectance image showing fibers surrounding a cell. (b) DIC image showing the cell.
Figure 4.2 Idealized computational model: cell on a gel with a fixed base.
4.2.2 Material Properties

To examine the effect of stiff collagen fibers vs. a homogeneous matrix on long-range stress propagation through gel thickness, the ECM was modeled using 2 different sets of assumptions: 1) fibrous material with two subdomains: collagen fibers and non-fibrous material, 2) linearly-elastic homogeneous non-fibrous material.

For the first set of assumptions, the 3-D models of the collagen fiber networks had several characteristics:

(i) Each intersection of collagen fiber segments was assumed to be a cross-link (Chandran & Barocas, 2005), and modeled as a welded joint.

(ii) The model components were modeled with homogeneous hyperelastic Neo-Hookean materials (Kang et al., 2008; Kao et al., 2010) that are nonlinear and isotropic, and exhibit instantaneous elastic responses for large strains. The initial shear/bulk moduli were computed from the Young’s modulus and Poisson’s ratio based on the following equation:

\[ G = \frac{E}{2(1+\nu)} \]  (4.1)

where \( \nu \) is Poisson’s Ratio, E is the Young’s modulus, and \( G \) is the shear modulus.

(iii) The material constants were chosen from the literature for the collagen fibers (Bischofs & Schwarz, 2003; van der Rijt et al., 2006), non-fibrous material (Arevalo et al., 2006; Yang et al., 2009) and cells (Solon et al., 2007; Storm et al., 2005), see Table 4.1.
Young’s Modulus \( E \) (\( Pa \)) | Poisson’s Ratio \( \nu \)  
---|---
Collagen Fiber | \( 12 \times 10^6 \) | 0.45  
Non-fibrous Material | 120 | 0.45  
Fibroblasts | \( 12 \times 10^3 \) | 0.45  

Table 4.1  Material properties chosen for collagen fibers, non-fibrous material, and the cell when modeling fibrous gel.

For the second set of assumptions, that of a homogeneous ECM, simulations were based on the same images but the moduli of the fiber- and non-fiber extracellular regions were assumed to be equal (\( E=120 \) Pa) and linearly elastic (Table 4.2).

| Collagen Fiber | Young’s Modulus \( E \) (\( Pa \)) | Poisson’s Ratio \( \nu \)  
---|---|---
Collagen Fiber | 120 | 0.45  
Non-fibrous Material | 120 | 0.45  
Fibroblasts | \( 12 \times 10^3 \) | 0.45  

Table 4.2  Material properties chosen for collagen fibers, non-fibrous material, and the cell when modeling homogeneous gel.

4.2.3 Boundary conditions

To mimic cell-on-gel experiments shown in Fig. 4.3, a fixed boundary condition was imposed at the bottom surface of the matrix. Free boundary conditions were imposed
at the cell membrane and the lateral surface of the matrix. A uniform contraction pre-stress, 1e6 [N/m$^3$], was applied to the cell.

Figure 4.3 The cell-on-gel experiments in which cells are seeded on top of collagen gels and compact the gels.

4.2.4 Mesh and Solution Procedures

The 3-D solid objects were meshed using quadratic tetrahedron elements in COMSOL Multiphysics. The complex 3-D geometries required a large number of tetrahedron elements to generate good quality meshes due to the curvature and roughness of the contour of collagen fibers and cells.

The models were solved as static problems after load application using COMSOL3.5a. Post-analysis results for stress distribution were obtained using COMSOL post-processing features.

The percentage force transmitted from cell/ECM interface to the gel base was calculated by the following equation:
where $\sigma$ is the von Mises stress, $s_1$ is the gel top area, and $s_2$ is the gel base area.

4.3 Results

When contraction forces were applied on a cell on the top of idealized fibrous matrix, stresses were mostly concentrated in fibers (Fig. 4.4), and the close-up views of the top and the bottom of the matrix show that stresses propagated along fibers several tens of microns away from cell/ECM interfaces (Fig. 4.5). In contrast, for the same mesh with homogeneous ECM, stresses induced by cell contraction distributed locally around the cells and decayed to insignificant magnitudes within several microns of the site of cell-ECM interaction (Fig. 4.6, Fig. 4.7).
Figure 4.4  Plot of von Mises stress distribution in fibers. Non-fibrous material is not shown in the figure.
Figure 4.5  Close-up views of von Mises stress distributions in the top (a) and the bottom (b) parts of the fiber network in a fibrous ECM indicated by dashed box in Fig. 4.4. Non-fibrous material is not shown in the figure.
Figure 4.6  Plot of von Mises stress distribution in fibers if the fibers were modeled as compliant as non-fibrous material. Non-fibrous material is not shown in the figure.
Figure 4.7  Close-up views of von Mises stress distributions in the top (a) and the bottom (b) parts of the fiber network in a homogeneous ECM indicated by dashed box in Fig. 4.6. Non-fibrous material is not shown in the figure.
The slice plots of von Mises stress show the stress concentration feature of collagen fibers as well. With fibrous material, the stress was propagated through the thickness of the gel matrix (Fig. 4.8). With homogeneous material, the stress decayed quickly at the interface of cell/ECM (Fig. 4.9).

Figure 4.8  Slice plot of von Mises Stress for a model with fibrous ECM.
Figure 4.9  Slice plot of von Mises Stress for a model with homogeneous ECM.

Up to 90% of the forces on the cell/ECM interfaces were propagated along stiff fibers to the gel base that was 60 microns away. However with the homogeneous matrix, only 10% of the forces were transmitted to the gel base (Fig. 4.10).
4.4 Discussion

For both fundamental studies of cellular mechanotransduction and applied studies of gel substrates intended to recapitulate tissue environments, it is important to understand the extent to which substrata thickness affects the mechanical response of cells seeded on top of the substrates (Maloney et al., 2008). We developed finite element models to mimic the cell-on-top experiments, and compared the stress distribution and force transmission, induced by cell traction, in fibrous and homogeneous matrix.

These computational models with idealized collagen fiber networks quantitatively demonstrated that stiff fibers are critically important for cell thickness sensing in cell-on-top experiments. Force transmission was mainly through relatively stiff fibers, and forces were transmitted more in fibrous materials than in homogeneous ones. The presence of
fibers within the ECM may allow cells to mechanically sense the rigid base far beyond the length scale of cell-matrix adhesion contacts and cell dimensions.

Simulation results showed increased force transmission with stiff fibers in collagen gel than compared with homogeneous gel, which was consistent with the results of experiments that showed larger thickness sensing scale of cells on biological gel than on synthetic polymer: Leong et al. suggested that hMSCs have interaction depth between 130 to 1440 µm with collagen gel (Leong et al., 2010), and Winer et al. showed that hMSCs have up to 450 µm of lateral interaction distance (Winer et al., 2009); while Buxboim et al. reported that the interaction depth of MSCs with compliant synthetic gel, such as PA gel, starts at about 10 – 20 µm in thickness with a characteristic tactile length of less than about 5 µm (Buxboim et al., 2010). Identifying the role of collagen fibers in cells’ long-range thickness sensing can help us better understand these experimental findings, and help guide the design of collagen-based system for desired mechanical response of cells.

Careful attention to fiber interactions, including contact and friction (Stein et al., 2010; Sander et al., 2009), will likely be needed to fully understand the mechanics of stress transmission through gel thickness in collagen gels and other ECM. We assumed uniformly distributed contractile stress on cell body and continuous adhesions between the cell and the ECM because experiments showed the evidence of variation in stresses and adhesion on the subcellular length scale (McGarry et al., 2009). More detailed description of the stress state of the cell and adhesion between the cell and the ECM may improve our understanding of the stress transmission. We also assumed the fiber
networks were comprised of uniform diameter and length collagen fibers. The heterogeneous feature of the collagen fiber network has been revealed by many experimental studies (Sander et al., 2009; Yang & Kaufman, 2009). Future work could account for the heterogeneity of the network.

In conclusion, this study shows the difference in stress distribution and force transmission between cells on fibrous and homogeneous gels, and highlights the importance of collagen fibers in long-scale thickness sensing of the cells. The findings could give insight into understanding basic cellular mechanotransduction behavior as well as potential tissue engineering applications.
Chapter 5: Summary and Future Work

5.1 Summary

The objective of this study was to understand, simulate, and predict how cells and the surrounding collagen matrix interact mechanically. We hypothesize that the presence of collagen fibers significantly change the micromechanical environment of the matrix in which the cells are present. Different properties of the collagen matrix, including fiber number, cross-links and entanglements, and fiber length, all play important roles in determining mechanical properties of fiber networks, and we further hypothesize that cross-links are the key factors. Three specific aims were accomplished in this dissertation to achieve our goal of study. A summary of the conclusion for each aim was presented in this section.

**Aim 1. Develop a multi-phasic FE model to simulate the cell-matrix interaction in cell culture.**

This aim was achieved with image-based finite element models that compared the effect of fibrous, non-fibrous strain-hardening, and non-fibrous homogeneous ECM in lateral stress transmission between cells. As detailed in chapter 2, confocal reflectance microscopy images were processed to get the topological structures of fibers and cells distributing in cell culturing matrices. Then 2-D finite element models were built based on the topological structures and locations. Loading and boundary conditions were set to
match the experimental conditions. Non-observable features, e.g., stress, force propagation, and stress gradient, of the cell-matrix interaction were investigated and quantified.

The result showed that centripetal contraction of a cell with fibers that connect it to a second cell located ~100 microns away can focus stresses into the stiff fibers and lead to higher peak stresses and stress gradients observed on the surface of the second cells. Treating the ECM as a homogenous isotropic strain hardening material could not account for the increased stress transmission -- suggesting an important role for the fibers themselves.

**Aim 2. Determine the effect of different properties of collagen matrix on cell’s mechanical microenvironment.**

This aim was achieved by using fluid-structure interaction models. In chapter 3, a series of idealized 2-D and 3-D models in which a randomly generated fiber network floating in water represented the gel. While the waviness of each fiber in the networks was assigned randomly, fiber number, length, orientation, and cross-links were varied to determine the effect of each parameter on cell’s mechanical microenvironment.

The results demonstrated that maximum stresses developed in fibers that were located at the sites of cross-links. There were linear relationships found between fiber number, length and orientation and stresses, but a strong linear relationship between the number of cross-links and stresses. This suggested that cross-links play a key role determining the micro-mechanical environment. Also, the interplay between collagen fibers and interstitial fluid affects the fluid-flow development at the microscopic
‘cellular’ level, and the interstitial fluid tends to align collagen fibers in the direction of fluid extension.

**Aim 3. Examine the propagation of cell contraction induced force through collagen fiber networks.**

This aim was achieved by using idealized 3-D models with randomly generated fiber networks embedded in compliant non-fibrous material. Cell contraction induced force propagation was investigated. The result shows that with stiff fibers, up to 90% of force at the cell/ECM interface was transmitted to the gel base, while with homogeneous compliant matrix, only 10% was transmitted. The results demonstrated that collagen fibers play an important role in cell thickness sensing, and make possible long-range stress transmission through gel thickness possible.

The image-based finite element method we developed in this study used geometries of the collagen fibers and cells based on their *in vitro* morphologies. Simulation results showed that stiff fibers could always transmit more stress and large stress gradients than non-fibrous material. While the stress concentration feature of fibrous material is geometry independent, the magnitude of stress and stress gradients transmitted by fiber network are geometry dependent. This finding indicates that to precisely predict the stress and stress gradient distribution in the ECM and on the cells, accurate description of geometries of the fiber network and the cells is necessary. Several previous finite element studies of mechanical interactions between cells and substrates used homogeneous substrates and idealized cell geometries (Appelman et al., 2011; Krishnan et al., 2008). Stein et al. (Stein et al., 2010) used images of collagen fibers as
the basis for computational simulations of cell-free collagen gels, we believe that we are the first developed image-based finite element method to understand cell-matrix interactions.

In addition, previously developed mathematical models (Maini & Olsen, 2002; Ramtani, 2004; Zaman et al., 2005) treated the ECM as homogeneous media, and finite element models of cells and tissue treated the ECM as homogeneous biphasic porous material (Chandran & Barocas, 2004; Sander et al., 2009). However, these studies did not provide detailed information regarding fiber distribution and fiber/fluid interplay. The finite element method with solid-fluid interaction presented in Chapter 3 modeled interstitial fluids, and investigated the interplay between collagen fibers and the interstitial fluid. This study helps fill the gap between homogeneous mathematical models and biphasic porous finite element models.

5.2 Future Work

Based on the current research described in this dissertation, several future studies could be carried out to further our understanding of cell/ECM mechanical interaction. In chapter 2, the role of stiff collagen fibers in long-range lateral stress propagation was investigated and demonstrated by using image-based 2-D finite element models. In chapter 4, the role of collagen fibers in long-range stress transmission through thickness was examined using idealized 3-D finite element models. There have been efforts on developing image-based 3-D finite element models, and an example of such a mesh is shown in Fig. 5.1. In this initial mesh, vertically distributed collagen fibers were missing
because of the method of stacking 2-D microscopic images. Future work needs to account for the 3-D nature of the fibrous gel before realistic analyses can be performed. With high-resolution microscopic images, more accurate reconstruction of collagen fiber networks would be possible to incorporate trans-plane fibers. Also, developing image-based fluid-structure interaction finite element models would help us to better understand the micro-mechanical interaction between cells and the ECM.

Figure 5.1  A sample mesh for an image-based 3-D finite element model.
In chapter 3, we investigated the effect of interstitial fluid flow, induced by cell movement, on determining the cellular micro-mechanical environment. The cell movement was crudely modeled by imposing an idealized boundary condition on the fluid domain. To more accurately account for the cell movement and examine the effect of cell movement on collagen matrix organization, time-lapse microscopy could be used to follow the displacement of cells from the time cells are initially seeded, and the velocity of the cell could be calculated from the displacement field and applied as boundary conditions to the cell.


Appendix A: Strain Hardening Parameter Study Results

The content discussed in this appendix is in the supplemental material of the manuscript that presented in Chapter 2, and submitted to *Biophysics Journal*. The work related to exploring the effect of strain-hardening feature of the ECM was done by Maureen Weber.

In our computational studies that utilized microscopy-based images of cell morphology and a strain-hardening material model based on published data in collagen gels (Fig 2.1), we observed a miniscule effect of strain hardening on the stress field produced during contraction (Fig. 2.12) and no effect on the stress transmission efficiency (Fig. 2.29) relative to those of a homogeneous material. To further investigate how strain hardening material properties might influence stress fields and force propagation, we have explored a range of strain hardening materials using an idealized finite element model (Fig. 2.4), where the homogeneous material was replaced by an isotropic strain hardening material and the second cell was set to have a Young’s modulus of 1000 Pa, to test parameter variations of variables $a$, $b$, and $c$ from equation 2.2.

Parameters $a$ and $b$ were varied one order of magnitude higher and lower than the collagen strain hardening the baseline values while parameter $c$ was varied two orders of magnitude higher and lower than the baseline condition. Each parameter had a total of
five levels assigned to it. Figure A.1 (top row) shows the range of strain hardening functions tested when varying parameters a, b, and c independently. Figure A.1 (second row) shows the x-direction stress tensor along a line directly between the two cell centroids for the highest, lowest, and middle level of each. Figure A.1 (third row) shows the mean normal stress on the second cell boundary. As seen by the third row in Figure A.1, none of the parameters varied independently resulted in increased stress transmission as compared to the homogeneous case represented by the horizontal dotted line.
Figure A.1  For parameters a, b, and c varying independently the top row is the range of strain hardening functions tested showing the change of parameters. Second row shows the stress in the x-direction along a line going between the two cell centroids. The third row shows the mean normal stress on the boundary of the second cell where the dotted line is the value for a material with no change in modulus (i.e. homogeneous).
Appendix B: Stiffness Difference Parameter Study Results

In our computational studies that utilized microscopy-based images of cell morphology and a fibrous material model, we observed higher stress gradients and higher peak stresses on the boundary of the unloaded cell relative to those of a homogeneous material. To further investigate how different the fibrous and non-fibrous materials have to be to get the higher peak stress on the boundary of the second cell, we explored a range of non-fibrous materials using an idealized finite element model (Fig. B.1). The model contained a block of homogeneous material with a 20 µm void representing the first (contracting) cell and a 20 µm diameter cell located 150 µm away in the c-c direction. The model also contained a thin domain connecting the two cells. Contraction of the first cell was simulated by applying a 1 Pa inward normal stress, and fixed boundary conditions were applied on the block edges. The connection between the two cells was modeled as stiff fibrous material (300 MPa), and the stiffness of the non-fibrous material varied from that of a homogeneous material (42.6 Pa) to fibrous material (300 MPa). The second cell was set to have a Young’s modulus of 1000 Pa.
Figure B.1  Idealized finite element model - geometry and boundary conditions.

The results showed that as the stiffness of non-fibrous material got closer and closer to the stiffness of fibrous material (from 42.6 Pa to 300 MPa), the peak normal stress on the boundary of the unloaded cell decreased. When the stiffness of non-fibrous material increased to 1 order smaller than that of fibrous material, peak normal stress on the boundary of the unloaded cell dropped to less than 1% of the applied stress. We concluded that the stiffness mismatch between fibrous and non-fibrous materials is important for stress transmission, and it has to be at least 1 order difference between two materials.
Figure B.2  Peak normal stress at the boundary of the second cell decreases as the mismatch of fibrous and non-fibrous material decreases.
Appendix C: MATLAB M-file Functions and Subroutines

This appendix contains the user-defined functions to generated randomly distributed 2-D and 3-D collagen fibers used in chapter 3 and chapter 4. Table C.1 displays a list of the user-defined MATLAB functions and a brief description of each.

<table>
<thead>
<tr>
<th>Function Name</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>randomfiber</td>
<td>Generate 2-D fiber networks with randomly distributed fibers</td>
</tr>
<tr>
<td>getFiberProp</td>
<td>Assign fiber length, number, and orientation</td>
</tr>
<tr>
<td>threeDGeom</td>
<td>Generate 3-D fiber networks with randomly distributed fibers</td>
</tr>
<tr>
<td>getOneFiber</td>
<td>Generates a randomly oriented curve that serves the axis of a 3-D collagen fiber</td>
</tr>
</tbody>
</table>

Table C.1 List of user-defined MATLAB functions.

C.1 M-file Script: randomfiber.m

The m-file script randomfiber.m generates a 2-D fiber network that contains randomly distributed fibers. It reads the value of fiber length, number and orientation from m-file script getFiberProp.m.
%======================================================
% PROGRAM RANDOMFIBER
% GENERATE RANDOMLY DISTRIBUTED FIBERS IN 2D
% FIBER NUMBER, DIAMETER, LENGTH, ORIENTATION CAN BE VARIED
% ONLY GENERATE THE GEOMETRY
% SI UNIT SYSTEM
% XIAOYUE MA
%======================================================

clear all;
clf

% COMSOL version
clear vrsn
vrsn.name = 'COMSOL 3.5';
vrsn.ext = 'a RCI';
vrsn.major = 0;
vrsn.build = 595;
vrsn.rcs = '$Name: $';
vrsn.date = '$Date: 2008/11/24 17:02:12 $';
fem.version = vrsn;

% Geometry
g1=rect2('8*1.2','.8','base','corner','pos',{'-.4','-.4'},'rot','0');
racell=10e-6;
xcorcell=0;
ycorcell=0;
g2=circ2(racell,'base','center','pos',{xcorcell,ycorcell},'rot','0');
geomplot(g1,'pointmode','off','edgecolor','r');
hold on;

****************************************************************************
[fibernum, thickness, fiberLength, randomangle]=getFiberProp();
domainlength=0.8;
rand('twister', 5489);
coords=cell(fibernum,1);
for kk=1:1:fibernum
    format long g;
    rand('twister', sum(kk*clock));
    lenpoint=pi;
    startscale=domainlength/fiberLength;
    startpoint=(rand+rand+rand+rand-3)*startscale*pi;
    ystartpoint=(rand+rand-1)*startscale*pi;
    %too many points results in out of memory problem
    tempx=linspace(startpoint,startpoint+lenpoint,10);
    tempy=ystartpoint+rand*sin(tempx);
    sinscale=fiberLength/lenpoint;
    si=length(tempx);

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scalePPx=zeros(si,1);
scalePPy=zeros(si,1);
for jj=1:si
    scalePPx(jj)=tempx(jj)*sinscale;
    scalePPy(jj)=tempy(jj)*sinscale;
end

% then use arclength parameterization
slen=zeros(si,1);
slen(1)=0;
slen(2:si)=sqrt(diff(scalePPx).^2+diff(scalePPy).^2);
slen=cumsum(slen);
% interpolation again
cs=spline(slen,[scalePPx';scalePPy']);
ss=linspace(slen(1),slen(si),si);
ds=(slen(si)-slen(1))/si;
curve=pvval(cs,ss);
curve=curve';
xx=curve(:,1);
yy=curve(:,2);

maxxx=max(xx);
minxx=min(xx);
maxyy=max(yy);
minyy=min(yy);
if (maxxx <= -0.4)
    xx = xx + 1.4*abs(minxx + 0.4);
end
if (minxx >= 0.4)
    xx = xx - 1.4*abs(maxxx - 0.4);
end
if (maxyy <= -0.4)
    yy = yy + 1.4*abs(minyy + 0.4);
end
if (minyy >= 0.4)
    yy = yy - 1.4*abs(maxyy - 0.4);
end

clear minxx;
clear minyy;
clear maxxx;
clear maxyy;
minxx=min(xx);
minyy=min(yy);
maxyy=max(yy);
maxxx=max(xx);
if (minxx < -0.4)
    xx = xx + 1.1*abs(+0.4+minxx);
end
minxx=min(xx);
if (maxxx > 0.4)
    xx = xx - 1.1*abs(maxxx-0.4);
end
maxxx=max(xx);
if (minyy < -0.4)
    yy = yy + 1.1*abs(+0.4+minyy);
end

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end
minyy=min(yy);
if (maxyy > 0.4)
  yy = yy - 1.1*abs(maxyy-0.4);
end
maxyy=max(yy);

% now we obtain unit speed tangent vector \mathbf{v}
\mathbf{tangent}=zeros(si,2);
\mathbf{tangent}(2:si,1)=diff(curve)./ds;
\mathbf{tangent}(1,1)=-\mathbf{tangent}(si,1);
% and normal vector
\mathbf{normal}=zeros(si,2);
\mathbf{normal}(2:si,1)=diff(\mathbf{tangent})./ds;
\mathbf{normal}(1,1)=\mathbf{normal}(si,1);
\mathbf{scale}=zeros(si,1);
\mathbf{scaledX}=zeros(si,1);
\mathbf{scaledY}=zeros(si,1);
for jj=1:si
  \mathbf{scale}(jj)=\text{thickness}/(\text{normal}(jj,1)^2+\text{normal}(jj,2)^2)^{0.5};
  \mathbf{crosprod}=\text{crosprod}([\text{\mathbf{tangent}}(jj,1),\text{\mathbf{tangent}}(jj,2),0],\text{\mathbf{normal}}(jj,1),...)
  \text{\mathbf{normal}}(jj,2),0));
  if crosprod(3)<0
    \text{\mathbf{scaledX}(jj)}=\text{\mathbf{scale}(jj)}*\text{\mathbf{normal}(jj,1)}+\text{\mathbf{xx}(jj)};
    \text{\mathbf{scaledY}(jj)}=\text{\mathbf{scale}(jj)}*\text{\mathbf{normal}(jj,2)}+\text{\mathbf{yy}(jj)};
  else
    \text{\mathbf{scaledX}(jj)}=-\text{\mathbf{scale}(jj)}*\text{\mathbf{normal}(jj,1)}+\text{\mathbf{xx}(jj)};
    \text{\mathbf{scaledY}(jj)}=-\text{\mathbf{scale}(jj)}*\text{\mathbf{normal}(jj,2)}+\text{\mathbf{yy}(jj)};
  end
end

\text{pp}=[\text{\mathbf{xx}(3:si)}, \text{\mathbf{scaledX}(si-1:3)}, \text{\mathbf{yy}(3:si)}, \text{\mathbf{scaledY}(si-1:3)}];
eval(\{'\text{c}' num2str(kk) ...
  'spline', 'splinestyle', 'centripetal', 'closed', 'on')\}});
C.2 M-file Script: getFiberProp.m

The m-file script `getFiberProp.m` assign fiber number, length and orientations.
C.3 M-file Script: threeDGeom.m

The m-file script *threeDGeom.m* generates a 3-D fiber network that contains randomly distributed fibers. It reads the value of fiber length, number and orientation from m-file script *getFiberProp.m*. It also reads coordinates of the curved axis for one collagen fiber from m-file script *getOneFiber.m*.
PROGRAM THREEGEGM
GENERATE RANDOMLY DISTRIBUTED FIBERS IN 3D
FIBER NUMBER, DIAMETER, LENGTH, ORIENTATION CAN BE VARIED
ONLY GENERATE THE GEOMETRY
SI UNIT SYSTEM
XIAOYUE MA

fllclear fem;
% COMSOL version
clear vrsn
vrsn.name = 'COMSOL 3.5';
vrsn.ext = 'a';
vrsn.major = 0;
vrsn.build = 663;
vrsn.rcs = '$Name: $';
vrsn.date = '$Date: 2008/12/03 17:02:19 $';
fem.version = vrsn;
format long g;
axis equal;
[fiberum, radius, fiberLength, randomangle, pointsonline, extra, ...
 layers]=getFiberProp();
lq = cell(l,fbernum*layers);
coords=cell(fbernum*layers,1);
bl=block3('0.8*1.2', '0.8', (2*radius+extra)*layers, ... 
 'base', 'corner', 'pos', ('-4', '-4', '0'), 'axis', ('0', '0', '1'), 'rot', '0');
bl=scale(bl,0.001,0.001,0.001,0,0,0);
intersec=0;
% Geometry
for la=1:l1:layers
    rand('twister', sum(clock*rand*3));
    domainlength=0.8e-3;
    lenpoint=pi;
si=pointsonline;
    for kk=1:l1:fbernum
        format long g;
        [curvex,curvey,curvez]=getOneFiber(fiberLength,pointsonline,radius,extra);
        fiberindex=(la-1)*fibernum+kk;
        clear tll;
        tll = cell(1,pointsonline);
        clear cc;
        cc=cell(1,pointsonline);
        clear cr;
        cr=cell(1,pointsonline);
        clear el;
el=cell(1, pointsonline);

for ii=1:pointsonline
  centerx=curvev(1,ii);
  centery=curvey(1,ii);
  centerz=curvez(1,ii)+(la-1)*(2*radius+extra);
  cr(ii)=circo2(radius, 'base', 'center', 'pos', ...
    {centery,centerz}, 'rot', '0');
  cc(ii)=geomcoefce('solid', cr(ii));

  el(ii)=1;
end

dvr=curvev(1,2:pointsonline)=curvev(1,1:pointsonline-1);

% Use the LOFT function in COMSOL Script to get the 3D geometry
lg{fiberindex} = loft(cc,'loftedge',el,'loftsecpos',(dvr,[],[]));

if(randomangle > 180)
  rand('twister', sum(clock*rand+3));
  angleReal=rand*2*pi;
else
  angleReal=(randomangle-90)*pi/180;
end

angleFirst=pi/2;
angleSec=-pi/2;
movescale=(-1+rand*3)*0.2;
randpoint=round(rand*si);
if(randpoint=0)
  randpoint=1;
end

lg{fiberindex}=rotate(lg{fiberindex},angleFirst,[1,0,0],[0,0,0]);
lg{fiberindex}=move(lg{fiberindex},[0,movescale,0]);

[xx] = geominfo(lg{fiberindex}, 'out', '{xx}', ...
  'par', {1});

lg{fiberindex}=rotate(lg{fiberindex},angleReal,[0,0,1],...
  [0.9*xx{1,1}{1,1},0.9*xx{1,1}{1,1},0.9*xx{1,1}{1,1}]);
lg{fiberindex}=scale(lg{fiberindex},0.001,0.001,0.001,0,0,0);

clear finalcoor;
finalcoor=get(lg{fiberindex}, 'vertex');
end
end
disp(intersec);

axis equal;

% Geometry objects
clear =
objStr='s.objs={bl}';
nameStr='s.name=``bl''';
tagStr='s.tags=``bl''';

for kkl=1:layers*fibernum-1
  objStr=[objStr ',lg(num2str(kkl))'' ];
  nameStr=[nameStr ',``lg'' num2str(kkl)'' ];
  tagStr=[tagStr ',``lg'' num2str(kkl)'' ];
end

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C.4 M-file Script: getOneFiber.m

The m-file script `getOneFiber.m` generates a randomly oriented curve that later serves as the axis of one collagen fiber. It outputs the coordinates of the curved axis of a collagen fiber.

```matlab
% MATLAB script to generate a random fiber axis

objStr = 'lg{ num2str(layers*fibernum) '});
nameStr = 'lg' num2str(layers*fibernum) '

fem.draw-struct('s',s);
clear units;
units.basesystem = 'SI';
fem.units = units;
```
% PROGRAM THREEDGEON
% GENERATE RANDOMLY DISTRIBUTED FIBERS IN 3D
% FIBER NUMBER, DIAMETER, LENGTH, ORIENTATION CAN BE VARIED
% ONLY GENERATE THE GEOMETRY
% SI UNIT SYSTEM
% XIAOYUE MA

% Purpose: This script generates a randomly oriented curve that later
% serves as the axis of a collagen fiber
% Output: coordinates of the curved axis of a collagen fiber

function [curvex,curvey,curvez]=getOneFiber(fiberLength,pointsOnLine,radialRange,extraRadius)

domainLength=0.6;
lenpoint=pi;
startScale=domainLength/fiberLength;
startPoint=(rand+rand+rand+rand-3)*startScale*pi;
yzPoint=(rand+rand-1)*startScale*pi;
tempX=linspace(startPoint,startPoint+lenPoint,pointsOnLine);
tempY=yzPoint+rand*sin(tempX);
sinScale=fiberLength/lenPoint;
si=length(tempX);
scalePxFx=zeros(si,1);
scalePFPy=zeros(si,1);
for jj=1:si
    scalePxFx(jj)=tempX(jj)*sinScale;
    scalePFPy(jj)=tempY(jj)*sinScale;
end

% then use arclength parameterization
sLen=zeros(1,1);
sLen(1)=0;
sLen(2:si)=sqrt(diff(scalePxFx).^2+diff(scalePFPy).^2);
sLen=cumsum(sLen);
% interpolation again
cs=spline(sLen,[scalePxFx;scalePFPy]);
ss=linspace(sLen(1),sLen(si),si);
del=sLen(si)-sLen(1))/si;
curve=ppval(cs,ss);
curves=curve';
xx=curve(1,1);
yy=curve(1,2);

maxxx=max(xx);
minxx=min(xx);
maxyy=max(yy);
minyy=min(yy);
if (maxxx <= 0)
    xx=xx+1.1*abs(minxx);
end
if (minxx >= 0.1)
    xx=xx-1.1*abs(maxxx-0.1);
end

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\begin{verbatim}
if (maxyy <= 0)
    yy = yy + 1.2 * abs(minyy);
end

if (minyy >= 0.06)
    yy = yy - 1.2 * abs(maxyy - 0.06);
end

t = ones(1, pointsonline) * radius + extra / 2;
Q = [xx'; yy'; t];
curvx = Q(1, :);
curvy = Q(2, :);
curvez = Q(3, :);
\end{verbatim}