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A Mathematical Model of Collagen Lattice Contraction

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Abstract

Two mathematical models for fibroblast-collagen interaction are proposed which reproduce qualitative features of fibroblast populated collagen lattice contraction in time. Both models are force based and model the cells as individual entities with discrete attachment sites however the collagen lattice is modeled differently for each model. In the collagen lattice model the lattice is more interconnected and formed by triangulating nodes to form the fibrous structure. In the collagen fiber model the nodes are not triangulated, are less interconnected, and the collagen fibers are modeled as a string of nodes. Both models suggest that the overall increase in stress of the lattice as it contracts is not the cause of the reduced rate of contraction. The reduced rate of contraction is due to an inactivation of the fibroblasts.

1 Introduction

In 1972 Elsdale and Bard first reported the contraction of collagen gels by fibroblasts [1]. Seven years later, Bell and coworkers introduced the fibroblast populated collagen lattice (FPCL) contraction model [2] with the goal of better understanding closure of an open wound by wound contraction. The FPCL system is intended to mimic cell matrix interactions which occur in wound granulation tissue. Although much has been learned from this system there are still many fundamental open questions. In this paper we develop two dimensional

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mathematical models designed to investigate the role of cellular forces in contracting lattices.

We describe two mathematical models which predict FPCL contraction for lattices with various cell densities and report the insights these models provide. In the next section we give necessary background including a description of stress-free and stressed lattices, a brief discussion of myofibroblasts and fibroblasts, and a review of some previous modeling efforts. Thereafter we present a section which describes the experimental methods and the numerical software used in the implementation of our model. The next section describes two separate mathematical models - one with a collagen lattice structure and one with a collagen string structure. This is followed by a section describing the results of our numerical simulations and we conclude with a discussion.

2 Background

Since their introduction several modifications have been made to FPCLs to model different types of biologically relevant lattices. The two main types of lattices frequently studied are stress-free lattices and stressed lattices. Stress-free lattices are typically free floating lattices, and the lattice can freely deform. Although there are local stresses in these lattices, they are imposed by the collagen structure within the lattice [3]. The stressed lattices, on the other hand, have external forces imposed on the lattice which constrain the shape of the lattice. In this manuscript we will restrict our study to stress free lattices. For a more complete review of FPCLs the reader is referred to [4].

The fibroblasts in FPCLs exhibit two phenotypes, the normal phenotype (referred to as fibroblast) and the myofibroblast phenotype. The myofibroblast phenotype is characterized by the expression of α-SMA, the presence of bundles of contractile microfilaments, and extensive cell-to-matrix attachments. Myofibroblasts appear to exert greater forces than fibroblasts, are more adhesive to the extracellular matrix and therefore less motile, and produce more extracellular matrix proteins [5, 6, 7]. The predominant phenotype in a lattice is dependent on experimental design [8].

There are several mathematical models of cell-extracellular matrix interactions which focus on the forces involved. Early models treated the cells and the extracellular matrix as continuum variables and used classical mechanical laws for continuum media to formulate the partial differential equations used in the models [3, 9, 10, 11, 12]. In an effort to better model the collagen network Baracos and coworkers [13, 14, 15] developed a hybrid method which considers the fibrous structure to determine forces in a volume averaging way and thus deduce the biomechanical properties of collagen tissue. On one scale they consider the extracellular matrix as a discrete fibrous structure but in solving the tissue properties they use a continuum description. Our models do not use a continuum description of the extracellular matrix or the cells. Other relevant models which are discrete in nature and not hybrids are the work of Stein and colleagues [16, 17]. They model three dimensional collagen structures with dis-
crete fibers. They consider these fibers as stiff rods which can twist at cross linking points to determine the deformation of the lattice. Schluter and coworkers modeled a single cell and discrete fiber interactions trying to understand how the extracellular matrix affects the migration of cells [18]. Their model takes a more phenomenological approach. They model a drag force on the cells through the matrix and realign the matrix in the direction the cell is moving. Reinhardt and coworkers used an agent-based model to simulate both complex extracellular matrix remodeling and durotaxis [19]. In their work as in the previous work they investigated the local structure of the lattice near one or two cells. Their model is the most similar to ours in that they model the force interactions between discrete fibers and cells, yet their model is based on the Fruchterman-Reingold algorithm [20] where the links between the cell and the binding site act as springs and the binding sites act as electrically charged particles. This model has the advantage of straightening collagen fibers. In this manuscript we model an entire lattice with many cells and focus on the contraction of the entire lattice. Finally, there are modeling efforts which use a discrete fiber formulation to derive a closed form for the strain energy [21]. The goals of this last type of work are quite different from ours.

3 Methods

3.1 Cell lines

Human dermal fibroblast cultures were derived from neonatal foreskin explants and maintained in complete DMEM, Dulbecco’s modification of Eagles medium, with 10% fetal bovine serum and 15 micrograms/ml of gentamicin. Fibroblasts were studied between their 8th and 12th passage. Dulbecco’s modification of Eagle’s medium (DMEM) and FBS were purchased from Life Technologies (Rockville, MD).

3.2 Casting Populated Collagen Lattices

Fibroblast PCL containing either 3,000, 10,000, 30,000, or 100,000 cells per ml of 1.25 mg of acid soluble rat tail tendon collagen in 1 mM HCl and complete DMEM. In a 60 mm Petri dish 0.2 ml drops of cell-collagen-medium mix were pipetted onto the surface of Petri dishes and allowed to polymerize before adding 2 ml of complete DMEM, which freed the lattices from the dish. Between 4 and 5 PCLs in each dish were maintained at 37°, with 5% CO₂ in a water saturated atmosphere incubator for 3 days without a change of medium. The diameter of the cell populated collagen lattices was measured initially and every 5 hours until 40 hours had elapsed.

3.3 Numerical methods

The numerical computations for our models utilized the following software packages. Initial triangulations for the collagen lattice model are created with the
software package Triangle [22]. The system of differential equations used to model the system is solved using SUNDIALS CVODE [23]. To determine the contraction of our simulated collagen we compare the area of the original lattice with the area of the contracted lattice. These areas are determined by taking the area of the convex hull of the node locations determined by qhull [24]. In order to find model parameters which would give results fitting the data, we used the optimization software gsl [25]. Finally we used MATLAB to visualize our results.

4 Model

Our mathematical model is force based and, as the biological system, has two main components – the cells and the collagen lattice. Although there is a significant amount of biochemical remodeling of the extracellular matrix by fibroblasts in both the wound environment and in collagen lattices, in the first 24 to 48 hours after casting a FPCL there is not much collagen deposition by the fibroblasts. The contraction of the FPCL therefore seems to be due to the forces generated by the fibroblasts.

Although the model is force based we do incorporate one important biochemical feature which is not a result of cell protein synthesis but is a cellular process resulting from cell forces. In our model we allow the collagen fibers to compact. The compaction of collagen is the combining of fibrils into longer and thicker collagen fibril. When the distance between two collagen fibrils is small enough the fibrils will combine into a larger collagen fiber. Eventually this merging process forms longer and thicker collagen fibril bundle. Evidence suggests this is the fundamental process which underlies wound contraction [26].

4.1 Cells

In our model we consider a collagen lattice with $N$ cells. A single cell is modeled as $K$ integrin based adhesion sites (I-sites) which exert force on a common location which can be thought of as the the center of mass (see Figure 1). We note that for the simulations shown in this manuscript we fix the value of $K$, the number of I-sites per cell, at 10. The I-sites exert forces on the center of mass according to Hooke’s law, i.e., the force is proportional to the distance from the center of mass. We may think of this as if the I-sites are attached to the center of mass with springs which have rest length $\ell$, set to be zero except in simulations with modification II and III (see section 5.1). The drag on the center of mass is modeled as a sphere in water with low Reynold’s number and is proportional to its velocity. The equation of motion for the center of mass of the $i$th cell is given by

$$ C\ddot{\mathbf{x}}_i = -\sum_{j=1}^{K} \alpha(||\mathbf{x}_i - \mathbf{u}_{p_{i,j}}|| - \ell) \frac{\mathbf{x}_i - \mathbf{u}_{p_{i,j}}}{||\mathbf{x}_i - \mathbf{u}_{p_{i,j}}||}, $$

(1)
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Figure 1: This figure depicts the way a cell is modeled mathematically. We consider a cell as a center of mass with attached springs. The other end of the springs are attached to I-sites which can interact with the extracellular matrix (membrane bound integrin based adhesion sites). In the simulations for this paper there are 10 I-sites per cell.

where $x_i$ is the location in $\mathbb{R}^2$ of the cell center, $i = 1, \ldots, N$. The spring constant which defines the cell force is $\alpha$, and the drag coefficient is $C$. The Reynold's number is low, thus because of the relative magnitudes of the coefficients, the acceleration term is set to zero.

The I-sites are constrained to be located at attachment sites on the lattice, called lattice nodes, whose locations are given by $u_k$ (see Sections 4.2 and 4.3). The set of indices $p_{i,1}, p_{i,2}, \cdots, p_{i,K}$ specify which lattice nodes are associated with the I-sites of cell $i$.

The I-sites remain attached to a node for a random amount of time, the attach time, taken from a Poisson distribution with mean 60 seconds (for simulations with modification I and III the time is extended, see section 5.1). Once the I-site detaches it immediately reattaches thus there are always $K$ attachment sites for each cell. To determine to which node the I-site attaches, a random angle chosen uniformly in the interval $-2$ to $2$ degrees is used to perturb the angle of the direction for the previous location of the I-site. In the new direction the I-site searches for the nearest node a random distance, chosen uniformly in the interval 0 to 115.726 microns, from the cell center. Each cell is arbitrarily assigned a front direction. If the previous I-site location was in the half plane normal to the front direction, the new I-site will be in the same half plane with probability 0.8. If the I-site was in the back half plane the new I-site will be in the back half plane with probability 0.2. For more information about the I-sites the reader is referred to a related model discussed in [27].

4.2 Collagen lattice

The collagen lattice is modeled by several nodes which are connected to form a network of spring-like connections (see Figure 2). To create the collagen lattice, $M$ nodes are placed in the domain and the connections between the nodes are determined through the use of a Delaunay triangulation [28]. Recall the cell
Figure 2: This figure depicts the way the collagen lattice is modeled mathematically. The collagen lattice is defined by nodes which are connected by spring-like elements. Although the nodes are regularly spaced in the figure, in most simulations they are randomly placed.

Figure 3: This figure depicts a model cell interacting with the collagen lattice. The lattice is depicted by grey lines and the “cell membrane” (the convex hull of the I-sites) is shown with black lines. The stars indicate I-sites. Notice the I-sites (stars) are all located at the intersection of grey lines i.e., lattice nodes. The forces acting on any lattice node come from the lattice connections (grey lines) associated with the node (terms in the first summation of equation 2) or the cell forces if a star is associated with the node (the terms in the second summation).

I-sites are constrained to be at node locations (as shown in Figure 3). The connections are spring-like in that if the connection is stretched the force is proportional to the stretching.

Forces acting on lattice nodes come from the lattice or the cells. The equation of motion for the lattice node \( k \) is

\[
\gamma \mathbf{u}_k(t) = \sum_{m=1, m \neq k}^{M} \mathbf{f}_{k,m}(t) + \sum_{i=1}^{N} \mathbf{c}_{i,k}(t) \quad \text{(2)}
\]

The first summation on the right hand side denotes the forces due to connections to other lattice nodes. Only the nodes directly connected to node \( k \) can have non-zero forces. The second summation on the right hand side gives the forces due to cells which have I-sites bound to node \( k \) (see Figure 3).

Lattice forces come from connections with other nodes which are classified into two types: normal or compacted. Having the second type of connection allows the compaction of the collagen. The forces due to both types of connections...
are spring-like in that if the connection is stretched the force is proportional to
the stretching. If a normal connection is compressed however there is no force
generated. This assumption is due to the nature of collagen. When the col-
lagen fibrils are pulled they resist the pulling due to their association with other
fibrils. Yet if a cell exerts forces at two points along the same fibril drawing
the two points closer, the fibril is not compressed but it becomes slack between
the two points similar to a rope. Compacted links act as true springs, that is
when a link is compressed a force is exerted. Thus the force due to a lattice
connection between node \(k\) and node \(m\) is defined as

\[
f_{k,m}(t) = \begin{cases} 0 & \text{if } \mathbf{u}_k \text{ and } \mathbf{u}_m \text{ are not linked}, \\ 0 & \text{if } d_{k,m} < \ell_{k,m} \text{ and the link is normal,} \\ -\beta(\ell_{k,m} - d_{k,m})(\mathbf{u}_k - \mathbf{u}_m)/d_{k,m} & \ell_{k,m} \leq d_{k,m} \text{ and the link is normal,} \\ -\beta^*(\ell_{k,m} - \ell_{k,m}^*)(\mathbf{u}_k - \mathbf{u}_m)/d_{k,m} & \text{if the link is compacted.} \end{cases}
\]

Here \(d_{k,m} = ||\mathbf{u}_k - \mathbf{u}_m||\), \(\ell_{k,m}\) is the rest length of the connection between
node \(k\) and node \(m\) and is set as the initial distance between the nodes at the
beginning of the simulation, \(\beta\) is the spring constant for normal links, \(\beta^* = d_\beta \beta\)
is the spring constant for compacted links, and \(\ell_{k,m}^* = d_\ell \ell_{k,m}\) is the rest length
for the spring connecting node \(k\) with node \(m\) when the link is compacted.
Initially all links are normal and become compacted if the distance between two
linked nodes becomes small enough (similar to the compaction process), that
is \(d_{k,m} < d_\ell \ell_{k,m}\). When links are compacted the rest length of the spring is
shortened \((d_\ell < 1)\), the spring constant is increased \((d_\beta > 1)\), and the link
resists compression.

The forces due to the cell \(i\) are defined by

\[
c_{i,k}(t) = \sum_{j=1}^{K} \alpha(||\mathbf{x}_i - \mathbf{u}_{p_{i,j}}|| - \ell) \frac{\mathbf{x}_i - \mathbf{u}_{p_{i,j}}}{||\mathbf{x}_i - \mathbf{u}_{p_{i,j}}||} \delta(p_{i,j} - k),
\]

where \(\delta(0) = 1\) and \(\delta(x) = 0\) for any non-zero \(x\) and indicates whether the \(j\)
I-site of cell \(i\) is interacting with node \(k\).

### 4.3 Collagen Strings

We now introduce an alternate model formulation for the collagen lattice. Rather
than place \(M\) nodes arbitrarily in a domain in \(\mathbb{R}^2\), and then interconnect them
using the Triangle program we instead create collagen strings. These strings
can be thought of as a chain of collagen nodes which are connected by normal
connections, that is the connections behave as springs when stretched but do
not resist compression (see description of normal connections 4.2). To create
these strings, we first fix a distant between the nodes of 100 microns and set a
string length by specifying the number of lattice nodes in the string. We have
the option of arranging the strings in an orderly fashion to mimic spun collagen
[29], however to better compare this model to the lattice model we instead al-
low them to arrange in a random fashion. This is done by randomly selecting a
starting position in our domain and randomly varying the angle formed between two segments of our string. Initially we restrict the angle between two segments of our string to be in the interval $[\pi/6, 11\pi/6]$ to avoid kinks. We then randomly place collagen strings in the domain until the desired density of collagen nodes is reached.

We now note a fundamental difference between the collagen string model and the collagen lattice model. In the lattice model all node linking is done before any simulations are run. In the collagen string model nodes are allowed to link at each time step if they are within 5 microns of each other. When nodes are linked in this scenario they form a compacted connection. (Recall compacted connections act like a spring under compression and extension.)

4.4 Model Parameters

There are many parameters in the model and three of the most important are $\alpha$ which represents the force the cells exert on the lattice and $\beta$ and $\beta^*$ which represent the Young’s modulus of collagen. These parameters have a large range of values reported in the literature.

The forces that fibroblasts exert on the extracellular matrix are measured in four different manners [7]. Each method gives very different forces which results in a range of reported fibroblast forces from 1nN to 2.65µN. The first method of measurement is by determining how much fibroblasts deform a silicone substrate [30, 31] which give the highest forces. The second is method is to measure how cells deform micro-machined devices [32, 33], and the third method is to measure forces on FPCLs and determined the average force exerted by a single fibroblast [34, 35]. The last two methods of measurements give forces in the range of 0.1 nN to 138 nN while the first method gives the higher force values. The fourth manner that fibroblast forces are calculated uses column buckling theory to determine the force of individual fibroblasts in fabricated lattices [36]. They find that fibroblast exert average forces ranging from 11-41 nN with an upper limit of 450 nN. We optimize the parameter $\alpha$ with others to fit the experimental data.

Young’s modulus for collagen gels (which depend on the makeup of the gel and whether the modulus is compressive or tensile) range from .004-24 kPa [37, 3, 38, 39, 40, 41]. Like the spring constant for the cells this parameter will be optimized in order to fit experimental data.

5 Results

The first goal of our work is the determination of appropriate numerical parameters for $\alpha$, $\beta$ and $\beta^*$ so that our numerical simulations will match the experimental observations. Our experimental method is detailed in Section 3 and the results for lattices with 3,750, 10,000, 30,000, and 100,000 cells per ml, gathered over a period of 40 hours, are shown in Figure 4. For the numerical
Figure 4: The lattice contraction of four different collagen lattice simulations are compared to the experimental data. In the top left panel the cell density is 3750 cells per ml, in the top right 10,000 cell per ml, in the bottom left 30,000 cells per ml and in the bottom right 100,000 cell per ml. The squares indicate the experimental values, the solid line indicates the simulations with modification III - stress dependent I-sites and cell contraction (incorporating both modification I and modification II), the dashed line indicates simulations with modification I - stress dependent I-sites (with I-sites which become permanent with high forces), the dots indicate the simulations with modification II - stress dependent cell contraction (stronger cells which resist compression), and the dotted line indicates simulations with standard cells. The parameter values for the simulations are $C_{\beta} = 0.193$ hours, $\alpha_{\beta} = 2.239$, $\gamma_{\beta} = 0.114$ hours, $d_{\ell} = 250.538$, $d_{p} = 0.365$, $d_{p} = 0.365$, $A_{f} = 0.172$, and $A_{l} = 59.954$ microns.

simulations, we assumed that only fibroblasts exist in the collagen lattice. We ran our numerical simulations for contraction with both collagen lattices and collagen strings with four different cell densities matching those in the experimental data.

5.1 Collagen Lattice Results

Scaling the equations by $\frac{1}{\beta}$ and using least squares to determine the best fit we varied the following values to try and find the best fit to the experimental observations: $C$, $C_{\beta}$ is the cell drag coefficient; $\gamma_{\beta}$, $\gamma$ is the collagen drag coefficient; $d_{\ell}$ the factor by which the collagen rest length is decreased when it is compacted; $d_{p}$ the factor which determines when the links compact; $\frac{\alpha_{\beta}}{\beta}$, $\alpha$ is the spring constant determining the force the cell exerts on the collagen; and $d_{\beta}$ the
factor which increases the spring constant of the collagen when it is compacted. Although we were able to find parameter values that gave a good fit to any single cell density we were unable to find parameter values that were valid for more than one cell density. These simulations are called standard simulations.

This inability to find a set of valid parameters for all the cell densities, motivated a closer study of the experimental results. In these results there are two main features: an initial fast decrease in size of the lattices and then a leveling off as the lattice size seems to stabilize and decrease less quickly. The main problem with our initial optimization seemed to be matching both these behaviors. In order to match both features we altered the model to allow the cells to have stress dependent I-sites and contraction.

We simulated three other cases: modification I - stress dependent I-sites, modification II - stress dependent cell contraction, and modification III - combining both modification I and modification II. The first step in changing the model was to allow the attach time of an I-site to depend on the force applied to the I-site, modification I. For simplicity, we set the attach time to be longer than the simulations effectively making the I-site permanently attached. To make this change we introduce two new parameters to the model. The first, $A_{l}$, is the minimum length the integrin must be stretched, and the second $A_{f}$ is a force factor. The specific rule used in modification I is: if the net force on the node (as determined by its velocity) is less than $A_{f}A_{l} \alpha$ and $||x_i - u_{p_{i,j}}|| > A_{l}$ where $i$ denotes the cell and $j$ denotes the I-site, then the I-site becomes permanently attached. The force the I-sites exert is spring like; thus, if the I-site is a long distance from the cell center the I-site should exert a large force on the collagen node. If the net force on the node is small this implies other lattice nodes are also exerting high forces and thus the lattice is under greater tension. If the I-site is not far from the cell center then the forces should be small. Making this change and optimizing on $A_{f}$ and $A_{l}$ in addition to the original parameters still did not give the desired results. Modification I, stress dependent I-sites, did not work any better than the standard simulations.

In the next set of simulations modification II - stress dependent cell contraction, we modified the standard simulations so the cells would become stronger when the force threshold was reached, using the same threshold as in modification I to fix the I-sites. In simulations with modification II the I-sites were not fixed but the cell’s contraction properties changed so they exerting greater forces and resisted compression. This was done by multiplying the force the cells exert ($\alpha$) by 10 and by changing the rest length of the I-site adhesion to be its length at the first time the rule indicated stronger forces. Modification II did not give satisfactory results. Simulations which did not fix the length of the I-sites to be the new length but had the greater force also failed.

Our final simulation combined modification I and modification II to create modification III. Optimizing the parameters where cells had both stress dependent I-sites and contraction properties gave results which matched the data (see Figure 4 solid lines). The figure also shows the effect of removing either or both of the new cell properties. The simulations in Figure 4 use the same parameter set which was optimized for the modification III simulations and not simulations.
where the parameters are optimized for each different set of simulations. These results suggest that allowing cells to resist compression and have permanently attached I-sites is important in matching the rate of cell contraction in the free floating lattices.

Having found simulations which matched the data, we examined the cell distribution at the conclusion of the simulations using modification III. Figure 5 shows the initial cell distribution in the left panels for each cell density and the final cell distribution in the right panels for each cell density. Figure 6 shows the results from a simulation with 500,000 cells per ml. After 40 hours the cells are much more concentrated near the boundary of the lattice with the cell density in the interior of the lattice being about 69,000 cells per cm squared and the cell density at the edge being about 210,000 cells per cm squared. The lattice is also compacted in a tight ring around the edge. Both of these features are seen experimentally [42, 3]. The experiments by Ehrlich et. al. use a cell density of 500,000 cells per ml and the experiments in Simons et. al. have a cell density of 50,000 cells per ml. To determine if the same trend held for the simulations with 100,000 cells per ml we averaged the interior cell density and the boundary cell density for 102 runs with the same initial lattice configuration but different random initial positions for the cells and different instantiations for the random behavior of the cell motion. The results confirm the same characteristic of the final cell distribution. The average cell density at the boundary of the lattice at 40 hours was 31,423 cells per cm squared and the average cell density for the interior was 20,074 cells per cm squared. Figure 7 is a magnification of the edge of the lattice shown in Figure 5 bottom right panel. Again a dense ring of collagen can be seen at the edge, just not as prominent as in the higher density case.

5.2 Collagen String Results

Once a set of valid parameters were found for the collagen lattice we wished to simulate the same four cell densities using the collagen string model. We began by simulating the standard case with the expectation that again a poor fit would be found to the experimental data. As can be seen in Figure 8 the standard case results in excessive contraction. It is worth noting though that when the collagen string case is compared to the collagen lattice case less contraction occurs for the string case. The reason for this decrease in contraction is due to the fact that the collagen strings are less interconnected initially. As the strings are contracted compaction occurs and the strings become interconnected, however there still exist collagen nodes that are connected to only one or two other nodes. This creates “collagen fingers” which trail the contraction occurring in the model. For a visualization of these fingers see Figure 10.

Knowing that modification III was critical to good experimental match for the collagen lattice model we ran simulations using modification III. Our goal was to minimize the number of parameters that must be changed in order to match the experimental data. We discovered that it was possible to closely match the experimental data by changing only the force factor $A_f$. Recall
Figure 5: The initial and final configuration of the lattices are shown for four different densities with the cell center marked as solid circles. The top row is the 3750 cells per ml, the second row 10,000 cells per ml, the third row is 30,000 cells per ml, and the fourth row is 100,000 cells per ml. The right panels show the initial configuration and the left panels show the configuration after 40 hours. The simulations shown here correspond to the simulations for the solid lines in figure 4.
Figure 6: The cell density for a simulation with 500,000 cells per ml is shown after 40 hours. In the left panel the light gray circle shows the boundary for the region where the interior density is calculated. The edge density is calculated using the convex hull of the lattice minus the circular region used for the interior density. The right panel shows the edge of the lattice magnified. Observe the highly organized collagen structure at the lattice edge. Other than the cell density the parameters are the same as the simulation shown in Figure 5. The cells centers outside of the lattice are due to the fixed spring size of the l-sites in modification III.

Figure 7: Magnification of the bottom right panel of Figure 5. Observe that the collagen structure near the edge is more dense than in the interior. The trend is not as prominent as the simulations with a higher cell density. The cell density for this simulation is 100,000 cells per ml and the lattice is shown after 40 hours.
that the force factor was used in the stress dependent rule to change the cell behavior, that is, if the net force on the node (as determined by its velocity) is less than $A_f A_i \alpha$ and $||x_i - u_{p_{i,j}}|| > A_l$ where $i$ denotes the cell and $j$ denotes the I-site, then the I-site becomes permanently attached. The values of $A_f$ used were 0.00286718, 0.005818, 0.026718 and 0.126718 for cell densities of 3750 cells per ml, 10,000 cell per ml, 30,000 cells per ml, and 100,000 cells per ml respectively. In order to maintain the closest possible match to the parameters used for the collagen lattice case we allowed for different values of $A_f$. The reason for the higher force factor for the higher concentration is that with so many cells pulling on collagen nodes a higher tension exists initially. The higher value of $A_f$ allows for cells to contract the collagen lattice for a short time until the tension surpasses a factor of the original tension. In the lower density cases the initial tension is lower so a lower force must be used to trigger the conversion to permanent I-sites and cells which resist compression and are stronger. It is worth noting that for the collagen lattice the force factor $A_f$ is 0.172 which is greater than any of the force factors used for the collagen strings indicating a higher initial tension. This is to be expected since the collagen lattice is more highly connected and moving one node will typically result in moving many nodes requiring a greater force. The greater connectivity of the collagen lattice distributes the cell and collagen forces over a larger region of the lattice.

We also wished to simulate modification I - stress dependent I-sites and the modification II - stress dependent cell contraction. For simulations with modification I, we decreased the motility of the cells once our stress rule was met by setting the attach time longer than the simulation. This case is shown by the dashed lines in Figure 8. As shown in the figure for the collagen strings the fixing of the integrins seems to be the more important property. To run simulations with modification II we strengthen the cell and allowed them to resist compression (but did not permanently attach the integrin) when the stress rule was met. The results of this simulation can be seen in Figure 8. Although not as important as the fixing of the integrins, strengthening the integrins and having the cell resist compression results in significant improvements in experimental matching, especially as the density of the cells increases. Figure 9 shows the initial and final configuration of the lattices for simulations with varying cell density.

6 Discussion

Both the force based mathematical models of fibroblast populated collagen lattices - the collagen lattice model and the collagen string model - presented here suggest that the conversion of fibroblasts from actively contacting the lattice to cells which are not actively contracting the lattice is important in explaining and mimicking the time course of contraction for lattices with different cell densities. While we cannot prove the necessity of the transition from active cells to inactive cells, the collagen lattice model indicates that it is difficult to explain the behavior of the collagen without allowing the cells to become
Figure 8: The contraction of four different collagen string simulations is compared to the experimental data. Everything is the same as in figure 4 except the value of $A_f$ is varied with each simulation and had values of 0.00286718, 0.005818, 0.026718 and 0.126718 for cell densities of 3750 cells per ml, 10,000 cell per ml, 30,000 cells per ml, and 100,000 cells per ml respectively.
Figure 9: This figure is the same as Figure 5 except the simulations are from the collagen string model.
Figure 10: The left panel is a magnification of the second to bottom right panel of Figure 9. Observe the collagen fingers that extend from the collagen mass. The right panel is a magnification of the bottom right panel of Figure 9. Observe that the collagen structure near the edge is more dense than in the interior. The trend is not as prominent as for the collagen lattice simulations with a similar cell density.

both immobile and stronger in resisting both stretching forces and compressional forces, whereas in the collagen string model the immobility seems to be the more important feature but the same general trends are seen. Experiments show that the first 5-10 hours for all but the lowest density lattices have the greatest rate of contraction. After this initial period the lattices contract at a slower rate. The models suggest that this is not a direct result of greater tension in the lattice but rather a result of the contractile cells becoming less active. The cells are strong enough to continue contracting the lattices to a much greater degree. Of course the greater local tension in the lattice could signal the cells to become less active in contracting the lattice, for example by becoming myofibroblasts or some other less actively contracting phenotype. This is consistent with the results of Stevenson et. al. who proposed that a cell in the lattice contracts only the collagen local to the cell and once nearby collagen is sufficiently compacted the cell becomes dormant [43]. Using these assumptions they were able to accurately model the cell mediated contraction of lattices (not time course data) with different collagen and cell densities. It is also consistent the modeling results of Reinhardt et. al. which suggest that the cells compact the matrix in the pericellular region to a greater extent than other areas [19]. We suggest that as fibroblasts contract the lattice the local collagen structure becomes more dense and less easily deformed. The cells in essences are in a stiffer less compliant part of the matrix (which they formed) and begin to become less mobile. The new behavior of the cells results in a slowing of the rate of contraction of the entire lattice. Since the myofibroblasts phenotype is more adhesive, less motile, and stronger than the normal fibroblast phenotype, one way to test this theory would be to collect time dependent data for the contraction of free floating lattices where the fibroblasts-myofibroblast transition is promoted (perhaps by adding TGFβ). We hypothesize that a free floating lattice populated predominantly by myofibroblasts would have a slower rate of contraction.

It is interesting to note the different results in the two model formulations.
The collagen lattice model gives an initial lattice which is more highly interconnected than the initial state of the collagen in the collagen string model. In the two higher density cases it is easy to see in the collagen string model that the rate of contraction slows down dramatically before 5 hours. It seems to be this change in rate which allows the good fit to the data. By either fixing the I-sites or giving them a non-zero rest length the contraction of the lattice slows down dramatically. The same dramatic changes is not seen in the collagen lattice simulations. Of course the forces are distributed over a larger regions in the collagen which should smooth out the transition.

The collagen lattice model not only matches the time course of free floating FPCL’s for several densities but also show density features observed in experimental lattices. The mathematical simulations with higher cell densities show that the final cell density is higher near the lattice boundary and the collagen is highly organized in a ring surrounding the lattice. The collagen string model seems to show the same trend. It appears that the free boundary is more readily contracted. The cells in the center are anchored by the surrounding cells and the surrounding collagen. In the center, cells are pulling on collagen nodes in each direction. However these collagen nodes are being pulled by other cells or other collagen nodes resulting in very little movement. Cells on the edges also pull on collagen nodes however those nodes on the edges are "free" from other cells and other collagen connections and contract into the cell. This results in a net movement of these cells towards the center. After a sufficient number of time steps this results in the bunching behavior we see. This theory can be supported by the bunching we see in the center of the collagen string. Here the collagen nodes are more free since they are less interconnected. Further evidence supporting this idea is the more highly stretched collagen in the very high cell density case right after the collagen ring on the edge (see Figure 6 right panel) as compared to the same region in the lower cell density case (see Figure 7). There are enough cells at the periphery of the high density case to cause the greater stretching of the interior lattice. If the free edge is the cause, an annulus shaped lattice should show the same cell density and collagen rings on both the outer edge and the inner edge.

In summary both the collagen lattice and the collagen spring model show promise in mathematically modeling the contraction of collagen. An important characteristic of these models is the ability to change behavior of the cells to become stress dependent. These results show the potential for simple mathematical models to provide insight into biological processes where cells interact with an extracellular matrix.

References


