Published in final edited form as:
Int J Non Linear Mech. 2012 March 1; 47(2): 173–178. doi:10.1016/j.ijnonlinmec.2011.04.029.

On a Class of Admissible Constitutive Behaviors in Free-Floating Engineered Tissues

D.D. Simon and **J.D. Humphrey**
Department of Biomedical Engineering, Yale University, New Haven, CT, USA

Abstract

A commonly used assay for studying cell - matrix interactions is the free-floating fibroblast populated collagen lattice, which was introduced in 1979. Briefly, fibroblasts are seeded within an initially thin, amorphous, untethered, circular gel consisting of reconstituted fibrillar collagen. Although the gel remains traction free and circular, the cells typically contract the gel to less than 50% of its original diameter within hours to days. Cellular mechanotransduction mechanisms are fundamental to this contraction, but there has not been a careful study of the associated mechanics. In this paper, we model the initial contraction of a circular gel by assuming a homogeneous, axisymmetric finite deformation while allowing possible radial variations in material properties, including material symmetry. We show that trivial solutions alone (i.e., no deformation, no contraction) are admitted by equilibrium and boundary conditions unless radial variations exist in the material behavior, including cell contraction. Although more complete data are needed to model better this initial-boundary value problem, the present results are consistent with both the salient features of the gel assay and recent observations reported in the literature that cells often introduce regional variations in tissue properties in vivo in an attempt to achieve, maintain, or restore mechanical homeostasis.

Keywords

stress; collagen gel; fibroblasts; contraction; mechanotransduction

INTRODUCTION

Tissue engineering is a multi-disciplinary field committed to developing living tissue constructs that can be used to repair or replace native tissue following injury or disease (cf. Guilak et al., 2003). Despite remarkable advances in tissue engineering over just a few decades, many fundamental questions remain regarding how cells interact with both native extracellular matrix and synthetic polymer scaffolds as they evolve the engineered construct. One of the first, and simplest, tissue-engineered constructs that remains useful for studying basic cell - matrix interactions is the so-called free-floating fibroblast populated collagen lattice (Bell et al., 1979). Typically, reconstituted fibrillar collagen is allowed form a thin “gel” within a circular mold and fibroblasts suspended in an appropriate cell culture media are seeded within the gel at prescribed densities (e.g., on the order of 10^5 cells per ml).

Address for Correspondence: J.D. Humphrey, Ph.D., Department of Biomedical Engineering, Malone Engineering Center, Yale University, New Haven, CT 06520-8260, (P) +1-203-432-6428, (F) +1-203-432-0030, jay.humphrey@yale.edu.

When the circular gel is released from the mold, and not otherwise tethered, the cells tend to contract the gel over a period of days, often to less than 50% of the original diameter. Many studies document effects on the rates and extents of contraction as a function of collagen type (e.g., I, II, or III) and density, cell type and density, the presence of exogenous growth factors (e.g., transforming growth factor – beta), and various drug treatments designed to alter cell receptor binding or cytoskeletal integrity. For a nice review of many of these studies, see Dallon and Ehrlich (2008).

Amongst the many findings, it appears that fibroblasts use multiple but related methods to contract the gel. In particular, it has been suggested that cells can compact and align collagen fibers as they (1) spread out on and attach to the gel, (2) draw in the collagen fibers via cell extensions and integrin-mediated tractions, or (3) exhibit overall contraction (Harris et al., 1980; Dallon and Ehrlich, 2008). Regardless of the means of contracting the gel, linear momentum balance must be satisfied in this traction free initial-boundary value problem. In this paper, we use a simple homogeneous finite deformation to model the initiation of contraction of an initially thin, amorphous, untethered, circular collagen gel and show that non-trivial solutions can be obtained only when the material properties are allowed to vary radially. We submit that these, and similar future, solutions can increase our ability to interpret the important free-floating collagen gel assay.

METHODS

The collagen gel initially consists of an amorphous mixture of collagen fibers, cells, and water. Consistent with formulations in growth and remodeling biomechanics (e.g., Humphrey and Rajagopal, 2002), we use a rule-of-mixtures relation for the Cauchy stress response. For example, conceptually we let the Cauchy stress $\mathbf{t} = -\tilde{p}\mathbf{I} + \phi^s\tilde{\mathbf{t}}^s + \phi^f\tilde{\mathbf{t}}^f$, where $\tilde{p}$ is a Lagrange multiplier enforcing intrinsic incompressibility of the solid constituents or transient motions at a fixed level of contraction, ϕ^s is the mass fraction of the solid, $\tilde{\mathbf{t}}^s$ is the Cauchy stress response of the solid, ϕ^f (= $1-\phi^s$) is the mass fraction of the fluid, and $\tilde{\mathbf{t}}^f$ is the Cauchy stress response of the fluid. Although many report that water is exuded during contraction of the gel, we assume that the associated momentum exchanges (from the perspective of mixture theory) are small because of the slowness of the process. Moreover, consistent with a quasi-static assumption, we let the contribution of the fluid to the overall stress be a hydrostatic pressure and thus lump its contribution together with the arbitrary Lagrange multiplier. Hence, we write $\mathbf{t} = -p\mathbf{I} + \mathbf{t}^s$ where the mass fraction of the solid is embedded in its stress response.

Focusing on early contraction of the gel, we consider homogeneous axisymmetric finite deformations whereby material particles initially at (R, Θ, Z) are mapped to (r, ϑ, z) according to

$$r=\lambda R, \quad \vartheta=\Theta, \quad z=\Lambda Z \quad \forall r \in [0, r_o], \vartheta \in [0, 2\pi], z \in [-h/2, h/2], \tag{1}$$

where r_o is the deformed outer radius, h the deformed thickness, and λ and Λ are stretch ratios (where $\lambda<1$ for contraction; see Figure 1). Hence, physical components of the deformation gradient are (Humphrey, 2002)

$$\mathbf{F}=\begin{bmatrix} \frac{\partial r}{\partial R} & \frac{\partial r}{R\partial\Theta} & \frac{\partial r}{\partial Z} \\ \frac{r\partial\vartheta}{\partial R} & \frac{r\partial\vartheta}{R\partial\Theta} & \frac{r\partial\vartheta}{\partial Z} \\ \frac{\partial z}{\partial R} & \frac{\partial z}{R\partial\Theta} & \frac{\partial z}{\partial Z} \end{bmatrix} = \begin{bmatrix} \lambda & 0 & 0 \\ 0 & \lambda & 0 \\ 0 & 0 & \Lambda \end{bmatrix}. \tag{2}$$

Despite reports that fluid is exuded from the gel as it contracts, there are no data on the rate or degree of water loss. Because of this lack of data, and our focus on initial gel contraction, we first assume overall incompressibility. Consequently, $\det \mathbf{F} = 1$ and $\Lambda = 1/\lambda^2$.

Below, we allow the material properties to vary with radial location, but assume complete axisymmetry and no variations in the z-direction because of the thinness of the gel. Assuming the inertial effects are negligible, we thus enforce equilibrium in this plane stress problem as (Humphrey, 2002)

$$\frac{\partial t_{rr}}{\partial r}+\frac{1}{r}(t_{rr}-t_{\vartheta\vartheta})=0,\ \frac{\partial t_{\vartheta\vartheta}}{\partial \vartheta}=0,\ \frac{\partial t_{zz}}{\partial z}=0. \tag{3}$$

The last two of these equations reveal that the Lagrange multiplier may depend on the radial direction at most; hence, we must only satisfy radial equilibrium. Our traction-free boundary conditions are: $t_{zz}(z = \pm h/2) = 0$ and $t_{rr}(r = r_o) = 0$.

RESULTS

Homogeneous Properties

Let us now contrast results for multiple potential descriptors of the constitutive behavior of the solid, which consists of hydrated collagen and cells. Given that the gels are initially very compliant, and the collagen fibers initially distributed randomly, let us begin by considering a neo-Hookean (isotropic) behavior for collagen, namely $\mathbf{t} = -p\mathbf{I} + 2\mu\mathbf{B}$, where μ is a mass averaged shear modulus (i.e., the mass fraction is absorbed within this parameter) and $\mathbf{B} = \mathbf{F}\mathbf{F}^T$ is the left Cauchy-Green tensor. That is, let us first consider the case when the cells do not generate any (contractile) stress, for which

$$t_{rr}=-p+2\mu\lambda^2,\quad t_{\vartheta\vartheta}=-p+2\mu\lambda^2,\quad t_{zz}=-p+2\mu\frac{1}{\lambda^4}. \tag{4}$$

Enforcing the traction-free condition on the upper and lower faces requires $0 = -p + 2\mu / \lambda^4$, whereby

$$t_{rr}=2\mu\left(\lambda^2-\frac{1}{\lambda^4}\right),\quad t_{\vartheta\vartheta}=2\mu\left(\lambda^2-\frac{1}{\lambda^4}\right). \tag{5}$$

Notice that this constant equibiaxial state of plane stress satisfies radial equilibrium (equation 3_1) identically, but the traction-free condition at the outer radius $t_{rr}(r = r_o) = 0$ requires further that $\lambda \equiv 1$, the trivial solution. That is, a thin, traction-free, circular specimen endowed with a neo-Hookean behavior cannot deform via equation 1.

Without repeating the mathematical details, one arrives at the same conclusion for a mixture of isotropic collagen (described by a neo-Hookean constitutive relation) and cells that exhibit an isotropic active (i.e., contractile) stress $t_a\mathbf{I}$. That is, if $\mathbf{t} = -p\mathbf{I} + 2\mu\mathbf{B} + t_a\mathbf{I}$, then the traction-free upper and lower surfaces require $0 = -p + 2\mu / \lambda^4 + t_a$ and the stress state is identical to that in equation 5; in other words, the only solution possible for this traction-free problem is again the trivial solution, $\lambda \equiv 1$ with no isotropic cell contraction.

Among others, Costa et al. (2003) noted that cells tend to align parallel to traction-free surfaces. Hence, let us consider the stress response for an isotropic collagen matrix plus circumferentially oriented active cells, namely $\mathbf{t} = -p\mathbf{I} + 2\mu\mathbf{B} + t_c\mathbf{e}_\vartheta \otimes \mathbf{e}_\vartheta$, where t_c denotes an anisotropic cell contraction. The traction-free condition on the upper and lower surfaces again requires $0 = -p + 2\mu/ \lambda^4$, hence the stress field is

$$t_{rr}=2\mu\left(\lambda^2-\frac{1}{\lambda^4}\right),\ t_{\vartheta\vartheta}=2\mu\left(\lambda^2-\frac{1}{\lambda^4}\right)+t_c. \tag{6}$$

Again, however, the traction-free boundary condition at the outer radius requires $\lambda \equiv 1$. Moreover, these two conditions leave $\mathbf{t} = t_c\mathbf{e}_\vartheta \otimes \mathbf{e}_\vartheta$, which satisfies radial equilibrium if and only if $t_c = 0$, thus yielding the trivial solution. In other words, a thin, traction-free, circular specimen endowed with a uniform neo-Hookean response by the collagen and a uniform uniaxial cell contraction cannot deform via equation 1.

It is also known, of course, that collagen fibers often align parallel to the cells that maintain them (Costa et al., 2003; Dallon and Ehrlich, 2008), which motivates inclusion of a transversely-isotropic term for the collagen fibers as well (with the circumferential direction preferred). For example, consider a stored energy function for collagen of the form $W(I_C, IV_C)$ where $I_C = tr\mathbf{C}$ and $IV_C = \mathbf{M}\cdot\mathbf{CM}$, with $\mathbf{C} = \mathbf{F}^T\mathbf{F}$ the right Cauchy-Green tensor and $\mathbf{M}$ a unit vector denoting the original orientation of a locally parallel family of fibers (which give rise to a transverse-isotropy). In this case, the associated Cauchy stress for the collagen is given by $\mathbf{t} = -p\mathbf{I} + 2\mathbf{W}_1\mathbf{B} + 2W_4\alpha^2\mathbf{m}\otimes\mathbf{m}$, where $W_1 = \partial W/\partial I_C$, $W_4 = \partial W/\partial IV_C$, and α is the stretch of a collagen fiber that was originally oriented in direction $\mathbf{M}$ but after deformation is oriented at direction $\mathbf{m}$. Because of the initial low stiffness of these collagen gels, we let $W(I_C, IV_C) = \mu(I_C - 3) + c(IV_C - 1)^2$ where μ and c are material parameters; the first term captures the same neo-Hookean (isotropic) contribution used above. If we let the direction $\mathbf{M}$ be circumferential, then $\alpha \equiv \lambda$ and the material exhibits transverse-isotropy with the preferred direction being circumferential. Assuming this combined isotropic – transversely isotropic form of the stored energy for the collagen plus both an isotropic ($t_a\mathbf{I}$) and a transversely isotropic ($t_c\mathbf{e}_\vartheta \otimes \mathbf{e}_\vartheta$) contribution to the Cauchy stresses by cell contraction, the associated stresses are

$$t_{rr}=-p+2\mu\lambda^2+t_a, \tag{7}$$

$$t_{\vartheta\vartheta}=-p+2\mu\lambda^2+t_a+4c(\lambda^4-\lambda^2)+t_c, \tag{8}$$

$$t_{zz}=-p+2\mu\lambda^2+t_a. \tag{9}$$

The traction-free top and bottom surfaces again require $0 = -p + 2\mu\lambda^2 + t_a$, whereby the state of plane stress reduces to

$$t_{rr}=2\mu\left(\lambda^2-\frac{1}{\lambda^4}\right),\ t_{\vartheta\vartheta}=2\mu\left(\lambda^2-\frac{1}{\lambda^4}\right)+4c(\lambda^4-\lambda^2)+t_c. \tag{10}$$

Note again that the isotropic contractile stress is not admitted in the final stress field. Moreover, it is easy to see that the traction-free condition at the outer radius again does not admit a contraction of the gel (i.e., $\lambda \equiv 1$), both with and without isotropic or transversely isotropic cell activation. Finally, because the two boundary conditions leave $\mathbf{t} = t_c\mathbf{e}_\vartheta \otimes \mathbf{e}_\vartheta$, radial equilibrium requires that $t_c = 0$, thus yielding the overall trivial solution yet again.

It appears, therefore, that a homogeneous, axisymmetric contraction of a fully traction-free, thin, circular specimen under plane stress does not admit a uniform isotropic or transversely isotropic (circumferential) response for either the collagen or the cells.

Heterogeneous Properties

Next, consider radially varying material properties, which can be modeled via a rule-of-mixtures relation for the stress with appropriate mass fractions ϕ_j varying with radial location. Consistent with the above, we can assume forms of the stored energy function for collagen to be $W_{iso} = \mu(I_C - 3)$ and $W_{aniso} = c(IV_C - 1)^2$ and similarly let the contractile cells exhibit either isotropic ($t_a\mathbf{I}$) or transversely isotropic ($t_c\mathbf{m} \otimes \mathbf{m}$) contractile responses (such an additive split into isotropic and anisotropic contributions is consistent with that advocated by Holzapfel and Ogden in other biomechanical applications; Holzapfel and Ogden, 2006). Hence, we consider the following general form for the stress response,

$$\mathbf{t} = -p\mathbf{I} + \phi_{iso}(r)(2\mu\mathbf{B} + t_a\mathbf{I}) + \phi_{aniso}(r)\left(4c\lambda^2(\lambda^2 - 1)\mathbf{m} \otimes \mathbf{m} + t_c\mathbf{m} \otimes \mathbf{m}\right), \tag{11}$$

where we let $\mathbf{m} \equiv \mathbf{e}_\vartheta$ herein. For the deformation in equation 1, therefore, the Cauchy stress response is

$$t_{rr} = -p + \phi_{iso}(r)\left(2\mu\lambda^2 + t_a\right), \tag{12}$$

$$t_{\vartheta\vartheta} = -p + \phi_{iso}(r)\left(2\mu\lambda^2 + t_a\right) + \phi_{aniso}(r)\left(4c(\lambda^4 - \lambda^2) + t_c\right), \tag{13}$$

$$t_{zz} = -p + \phi_{iso}(r)\left(2\mu/\lambda^4 + t_a\right), \tag{14}$$

where the mass fractions must sum to unity, namely $\phi_{iso} + \phi_{aniso} = 1$ at each point.

Traction-free upper and lower surfaces require $0 = -p + \phi_{iso}(r)\,(2\mu / \lambda^4 + t_a)$, which reveals that the Lagrange multiplier can now vary with radial location. The state of plane stress thus reduces to

$$t_{rr} = 2\mu\phi_{iso}(r)\left(\lambda^2 - \frac{1}{\lambda^4}\right), \tag{15}$$

$$t_{\vartheta\vartheta} = 2\mu\phi_{iso}(r)\left(\lambda^2 - \frac{1}{\lambda^4}\right) + \phi_{aniso}(r)\left(4c(\lambda^4 - \lambda^2) + t_c\right). \tag{16}$$

Note, again, that a possible isotropic cell contraction cannot contribute to the stress field. Next, consider two specific cases of the regional variations.

First, consider a linear variation in material properties, that is, $\phi_{aniso} = r / r_o$ and thus $\phi_{iso} = 1 - r / r_o$. In other words, we let the material exhibit an isotropic response at the center ($r = 0$) but a strongly transversely isotropic response at the outer edge ($r = r_o$); at radial positions between the center and edge we have an increasingly stronger transverse isotropy (Figure 2). Notice from equation 15 that the traction-free condition is satisfied identically at $r = r_o$ because $\phi_{iso}(r = r_o) = 0$. Radial equilibrium (equation 3) thus requires,

$$2\mu\left(\lambda^2 - \frac{1}{\lambda^4}\right)\left(\frac{-1}{r_o}\right) - \left(\frac{1}{r_o}\right)\left(4c(\lambda^4 - \lambda^2) + t_c\right) = 0 \quad \forall r. \tag{17}$$

Consequently, if there is no anisotropic contractile contribution by the cells (i.e., $t_c \equiv 0$), then $2\mu(1/\lambda^4 - \lambda^2) = 4c(\lambda^4 - \lambda^2)$. For arbitrary values of the material parameters μ and c, it can be shown numerically that the only solution is the trivial one, $\lambda \equiv 1$. In contrast,

solving equation 17 for a non-zero transversely isotropic active stress, $t_c = -2\mu(\lambda^2 - 1/\lambda^4) - 4c\lambda^2(\lambda^2 - 1)$, with $\lambda<1$ for contraction of the gel, the final state of plane stress becomes

$$t_{rr}=2\mu\left(\lambda^2-\frac{1}{\lambda^4}\right)\left(1-\frac{r}{r_o}\right),\quad t_{\vartheta\vartheta}=2\mu\left(\lambda^2-\frac{1}{\lambda^4}\right)\left(1-\frac{2r}{r_o}\right). \tag{18}$$

Note again that the radial traction-free boundary condition is satisfied automatically at the outer edge ($r = r_o$). Figure 3 shows associated distributions of stress, normalized with respect to the material parameter μ, for multiple degrees of contraction ($\lambda<1$). As would be expected in this traction-free problem, one obtains a "residual-type" stress distribution of stresses wherein regions of compression balance those of tension for the circumferential stress.

Second, and finally, consider an exponential regional variation in properties, namely $\phi_{iso} = 1 - \exp[n(r/r_o - 1)]$ and therefore $\phi_{aniso} = \exp[n(r/r_o - 1)]$. For large enough values of n, this distribution again yields essentially an isotropic response at the center ($r = 0$) but a strongly transversely isotropic response at the outer edge ($r = r_o$); at radial positions between the center and edge we have an increasingly stronger transverse isotropy (Figure 2). Albeit involving slightly more algebra, one finds a result similar to that for the linear radial variation in properties. Absence of an anisotropic cell mediated contraction again yields the trivial solution. In contrast, enforcing radial equilibrium for a non-zero t_c yields a non-trivial solution. In particular, $t_c = -\mu(\lambda^2 - 1/\lambda^4)(nr/r_o) - 4c\lambda^2(\lambda^2 - 1)$. Hence, the final state of plane stress becomes

$$t_{rr}=2\mu(1-\mathrm{e}^{n(r/r_o-1)})\left(\lambda^2-\frac{1}{\lambda^4}\right), \tag{19}$$

$$t_{\vartheta\vartheta}=2\mu\left(1-(1+\frac{nr}{r_o})\mathrm{e}^{n(r/r_o-1)}\right)\left(\lambda^2-\frac{1}{\lambda^4}\right). \tag{20}$$

Figure 4 shows illustrative results for $n = 10$ and multiple levels of contraction ($\lambda<1$), again normalized with respect to the material parameter μ. The results are qualitatively similar to those for the case of linear variations in material properties, but as expected, focusing the anisotropy near the outer edge creates stronger gradients in the radial distribution of stress.

DISCUSSION

Prior studies of fibroblast populated collagen lattices have focused on what cells do when various experimental conditions are altered, but they have not addressed the more fundamental question, Why do cells contract the gels and thus compact the collagen? We do not claim to resolve this issue, but consider the following. Although the are traction free when in suspension, and thus when introduced into the traction-free gel, once they contact the gel they appear to spread out and begin to pull on the extracellular matrix; indeed, they continue to pull on the matrix for long periods. By pulling on the matrix, which in turn pulls back, the cells can establish a stressed environment in which to reside under stress themselves. Note, therefore, that diverse observations consistently suggest that cells seek to establish and maintain a "tensional homeostasis" in connective tissues (Brown et al., 1998; Tomasek et al., 2002), that is, a target state of mechanical loading (Humphrey, 2008). We suggest that the contraction of the gel may be an attempt by the cells to establish such a preferred, or at least a favorable, mechanical environment that is fundamental for optimal cell function. Similar processes appear to be operative in diverse clinical settings, including lens epithelial cell responses following cataract surgery (Pedrigi et al., 2009).

The present analysis revealed that cells within a thin, circular, traction-free gel cannot satisfy both equilibrium and boundary conditions by uniformly contracting isotropically. Indeed, they similarly cannot satisfy these fundamental mechanical requirements by uniformly contracting transversely-isotropically or by uniformly organizing the collagen circumferentially. Rather, it appears that one way to satisfy both equilibrium and boundary conditions, while avoiding a non-trivial situation (i.e., no deformation, no contraction), is to generate a radially varying contractile response that becomes increasingly transversely isotropic with respect to the circumferential direction with increasing radius (i.e., moving toward the edge of the gel). This simple consequence of the mechanics is consistent with observations that myofibroblasts (which generate greater contractile stresses) tend to be found only near the outer edge of free-floating collagen gels (Ehrlich, 1988) and that the collagen similarly tends to align only near the outer edge in moderate cell density free-floating collagen gels (Ehrlich and Rittenberg, 2000). Indeed, this consequence of the mechanics is also consistent with clever in vitro observations in tethered collagen gels reported by Costa et al. (2003) wherein they found that fibroblasts tend to align parallel to a traction-free surface. Based on their observations, Costa and colleagues further suggested that “Similar to Saint-Venant’s principle in linear elasticity theory, it appears that the influence of boundary conditions decreases with increasing distance from the boundary. Therefore, heterogeneity of cell alignment naturally arises.” Our theoretical results support their fundamental claim. The values of the transversely-isotropic active stresses, at a given level of contraction, were also required by equilibrium and boundary conditions to increase in proportion to the matrix stiffness (gel parameters μ and c). This finding also appears to be consistent with some reports that contractility is higher on or in stiffer matrices (Discher et al., 2005).

In addition to in vitro experimental findings, it is becoming increasingly evident in vivo that cells often deposit and/or organize matrix in a regionally heterogeneous manner to offset complexities in geometry and applied loads that would otherwise result in a nonhomogeneous state of stress and, by definition, a mechanical environment that is not equally optimal for all cells in different regions. Examples of such regional heterogeneities can be found both in health (e.g., the native lens capsule; Pedrigi et al., 2007) and in disease (e.g., cerebral aneurysms; Ryan and Humphrey, 1999), and also appear to be consistent with the existence of residual stresses in arteries that help homogenize the transmural distribution of stress (Cardamone et al., 2009).

The free-floating collagen lattice preparation has proven convenient for studying many aspects of cell-matrix interaction (cf. Dallon and Ehrlich, 2008), yet the present analysis reveals that it imposes highly restrictive mechanical constraints on the cells. Because the outer edge and the upper/lower surfaces remain traction free, the gross state of stress must either be zero (trivial case) or residual (with self-equilibrating compressive and tensile circumferential stresses), neither of which can satisfy the potential cellular goal of establishing a uniform, non-zero state of mechanical loading that is homeostatic. Indeed, it may be for this reason that the fibroblasts tend to enter the cell death cycle prematurely (i.e., undergo apoptosis) in free-floating but not in constrained collagen gels (Grinnell, 2003). We emphasize, however, that the present analysis reveals that such an unfavorable fate for the cells need not arise because the cells and matrix remain stress free, as suggested by many (e.g., Grinnell, 1994; Tomasek et al., 2002; Bride et al., 2004; John et al., 2009). Rather, the unfavorable fate may result from the residual-type stress field that is admitted by the mechanics, but is nevertheless not optimal biologically. We note, however, that a residual-type stress field does allows tensile circumferential stresses at and near the outer traction-free edge (cf. Figures 3 and 4), hence the collagen fibers could align in the direction of the maximum tensile stress consistent with many observations (cf. Baaijens et al., 2010) and not be forced to behave differently at a traction-free surface (cf. Costa et al., 2003). Indeed,

allowing strong gradients in the material properties and hence stresses (cf. Figures 2 and 4), may allow the majority of the collagen fibers to remain nearly isotropically distributed (in the central region) as reported by some (cf. Grinnell, 2003). In other words, the simple solution offered herein yields results consistent with many seemingly confusing experimental findings (cf. Dallon and Ehrlich, 2008). It is interesting in this regard that Winer et al. (2009) note the importance of accounting for the nonlinear elasticity under finite deformations as we did. They write "local strain stiffening [due to nonlinear elasticity] allows an initially isotropic matrix to reinforce cell-applied mechanical anisotropy and transmit forces between cells up to half a millimeter apart. In this way isolated cells can create far-reaching mechanical gradients and produce a global pattern." In other words, consistent with the findings herein, cells may, under particular conditions, create gradients in matrix stress (and thus stiffness) that enable cooperative behaviors, perhaps as they seek together to establish or maintain a mechanical homeostasis.

To the best of our knowledge, there has not been another mechanical analysis of this experimental preparation. Among others, Barocas and colleagues (e.g., Ohsumi et al., 2008) have considered cell-driven compaction of collagen and fibrin gels that are tethered to fixtures, but this is a very different situation. Independent of differences in the basic initial-boundary value problems considered, the mechanical model used by Barocas and colleagues is also very different from that used herein. For example, they model the collagen matrix as a homogeneous Maxwell fluid to endow the highly hydrated gel with viscoelastic behavior. It is not clear that viscoelasticity plays a strong role in the cell-mediated contraction of the free-floating gel considered herein. John et al. (2009) modeled the collagen as linearly elastic, homogeneous, and isotropic. The associated contractile response was simulated using an equivalent "thermal contraction," which necessarily resulted in a uniform zero stress field.

Theory should always guide experiments. The present analysis suggests that regional material properties and cell behaviors may be needed to satisfy equilibrium and boundary conditions in the free-floating collagen gel assay, which gives rise to a nonuniform (residual-type) stress field. We suggest, therefore, that there is a need to measure carefully potential radial gradients in collagen orientation, cell phenotype, and cell status (e.g., contractile or apoptotic). In other words, because of the expected gradients in the stress field – with radial gradients in the always compressive radial stresses and similarly radial gradients in the self-equilibrating tensile and compressive circumferential stresses – and the mechanosensitivity of fibroblasts (e.g., Laurent et al., 2007), there is a need to determine if similar gradients exist in cell response. Indeed, if different cell responses exist at different radial locations, then more complete correlations of cell response with mechanical stress may be possible within a single experiment. We emphasize, therefore, that although the present study can provide both insight into prior reports of evolving tissue equivalents and additional guidance for new experiments, there is clearly a need for more data to refine the theoretical framework. Even in the first report by Bell et al. (1979), it was suggested that "When cells are incorporated into hydrated collagen lattices, the lattice is contracted and water is squeezed out." Whereas the time-course of changing gel diameter, or area, has been measured and used as a primary indicator of cell activity, complete information on the amount and rate of fluid loss is not available. There is clearly a need for such data and an associated refinement of the analysis to exploit poroelastic or mixture descriptions of the behavior of the gel. Regardless, continued use of a rule-of-mixtures approach, wherein radial gradients in properties can be introduced via mass fractions rather than by allowing material parameters to vary regionally (cf. equations 18–20), appears to confer some utility. It has also been reported that when contracting the gel, the fibroblasts repress their synthetic capability (Payne et al., 1987). For this reason, we did not consider potential growth and remodeling of the gel (cf. Humphrey and Rajagopal, 2002). Ultimately, however, the desire

to understand the initial mechanics of a tissue engineered construct is to understand the stimuli that eventually begin to drive growth and remodeling. There will be a need, therefore, to extend the present studies to include subsequent evolution of the construct as additional matrix is produced and removed (cf. Niklason et al., 2010).

Acknowledgments

This work was supported, in part, by NIH grant R01 EB-008836 (L. Niklason and J.D. Humphrey, MPIs). We very much appreciate the invitation by Professors J. Merodio and L. Dorfman to contribute to this issue honoring Prof. Ray Odgen, a true pioneer in nonlinear mechanics and biomechanics.

REFERENCES

Baaijens F, Bouten C, Driessen N. Modeling collagen remodeling. J Biomech. 2010; 43:166–175. [PubMed: 19818962]

Bell E, Ivarsson G, Merrill C. Production of tissue-like structure by contraction of collagen lattices by human fibroblasts of different proliferative potential in vitro. Proc Nat Acad Sci. 1979; 76:1274–1278. [PubMed: 286310]

Brown RA, Prajapati R, McGrouther DA, Yannas IV, Eastwood M. Tensional homeostasis in dermal fibroblasts: Mechanical responses to mechanical loading in three-dimensional substrates. J Cell Physiol. 1998; 175:323–332. [PubMed: 9572477]

Bride J, Viennet C, Lucarz-Bietry, Humbert P. Indication of fibroblast apoptosis during the maturation of disc-shaped mechanically stressed collagen lattices. Arch Dermatol Res. 2004; 295:312–317. [PubMed: 14652775]

Cardamone L, Valentin A, Eberth JF, Humphrey JD. Origin of axial prestress and residual stress in arteries. Biomech Model Mechanobiol. 2009; 8:431–446.

Costa KD, Lee EJ, Holmes JW. Creating alignment and anisotropy in engineered heart tissue: Role of boundary conditions in a model three-dimensional culture system. Tissue Engr. 2003; 9:567–577.

Dallon JC, Ehrlich HP. A review of fibroblast-populated collagen lattices. Wound Rep Regen. 2008; 16:472–479.

Discher DE, Janmey P, Wang Y-L. Tissue cells feel and respond to the stiffness of their substrate. Science. 2005; 310:1139–1143. [PubMed: 16293750]

Ehrlich HP. Wound closure: Evidence of cooperation between fibroblasts and collagen matrix. Eye. 1988; 2:149–157. [PubMed: 3058521]

Ehrlich HP, Rittenberg T. Differences in the mechanism for high- versus moderate-density fibroblast-populated collagen lattice contraction. J Cell Physiol. 2000; 185:432–439. [PubMed: 11056014]

Guilak, F.; Butler, DL.; Goldstein, SA.; Mooney, DJ. Functional Tissue Engineering. NY: Springer; 2003.

Grinnell F. Fibroblasts, myofibroblasts, and wound contraction. J Cell Biol. 1994; 124:401–404. [PubMed: 8106541]

Grinnell F. Fibroblast biology in three-dimensional collagen matrices. Trends Cell Biol. 2003; 13:264–269. [PubMed: 12742170]

Harris AK, Wild P, Stopak D. Silicone rubber substrata: A new wrinkle in the study of cell locomotion. Science. 1980; 208:177–179. [PubMed: 6987736]

Holzapfel, GA.; Ogden, RW. Mechanics of Biological Tissue. NY: Springer; 2006.

Humphrey, JD. Cardiovascular Solid Mechanics: Cells, Tissues, and Organs. NY: Springer; 2002.

Humphrey JD, Rajagopal KR. A constrained mixture model for growth and remodeling of soft tissues. Math Model Meth Appl Sci. 2002; 12:407–430.

Humphrey JD. Vascular adaptation and mechanical homeostasis at tissue, cellular, and sub-cellular levels. Cell Biochem Biophys. 2008; 50:3–78.

John J, Quinlan AT, Silvestri C, Billiar K. Boundary stiffness regulates fibroblast behavior in collagen gels. Annl Biomed Engr. 2009

Laurent GJ, Chambers RC, Hill MR, McAnulty RJ. Regulation of matrix turnover: fibroblasts, forces, factors and fibrosis. Biochem Soc Trans. 2007; 35:647–651. [PubMed: 17635112]

Niklason LE, Yeh AT, Calle EA, Bai Y, Valentin A, Humphrey JD. Enabling tools for engineering collagenous tissues integrating bioreactors, intravital imaging, and biomechanical modeling. Proc Nat Acad Sci. 2010; 107:3335–3339. [PubMed: 19955446]

Ohsumi, Tk; Flaherty, JE.; Evans, MC.; Barocas, VH. Three-dimensional simulation of anisotropic cell-driven collagen gel compaction. Biomech Model Mechanobiol. 2008; 7:53–62. [PubMed: 17354006]

Payne M, Nusgens BV, Lapiere CM. Modulation of cellular biosynthetic activity in the retracting collagen lattice. Eur J Cell Biol. 1987; 45:44–50. [PubMed: 3443111]

Pedrigi RM, David G, Dziezyc J, Humphrey JD. Regional mechanical properties and stress analysis of the human anterior lens capsule. Vis Res. 2007; 47:1781–1789. [PubMed: 17467027]

Pedrigi RM, Dziezyc J, Kalodimos HA, Humphrey JD. Ex vivo quantification of the time-course of contractile loading of the porcine lens capsule after cataract surgery. Exp Eye Res. 2009; 89:869–875. [PubMed: 19638277]

Ryan JM, Humphrey JD. Finite element based predictions of preferred material symmetries in saccular aneurysms. Ann Biomed Engr. 1999; 27:641–647.

Winer JP, Oake S, Janmey PA. Non-linear elasticity of extracellular matrices enables contractile cells to communicate local position and orientation. PLoS One. 2009; 4:e6382. [PubMed: 19629190]

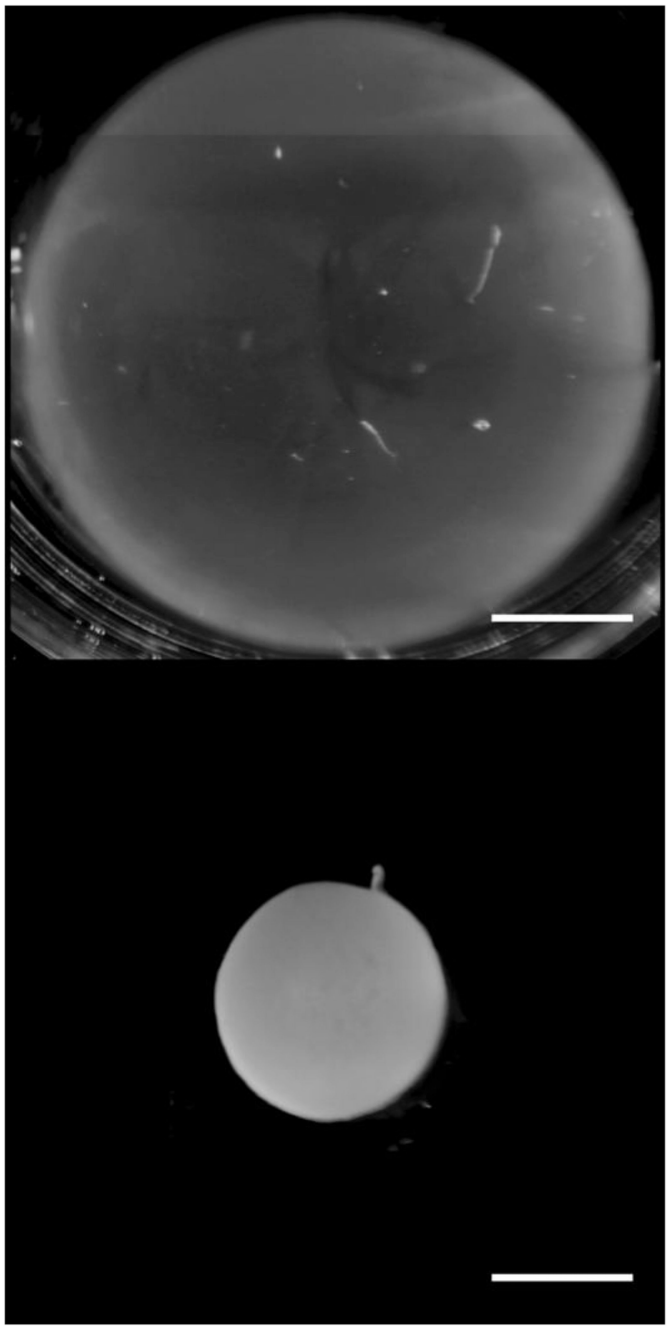

Figure 1.
Representative contraction of an initially circular collagen gel by fibroblasts: initial gel (top) and gel 5 days after seeding with fibroblasts (bottom). Note the retained circularity. Scale bars are 5mm.

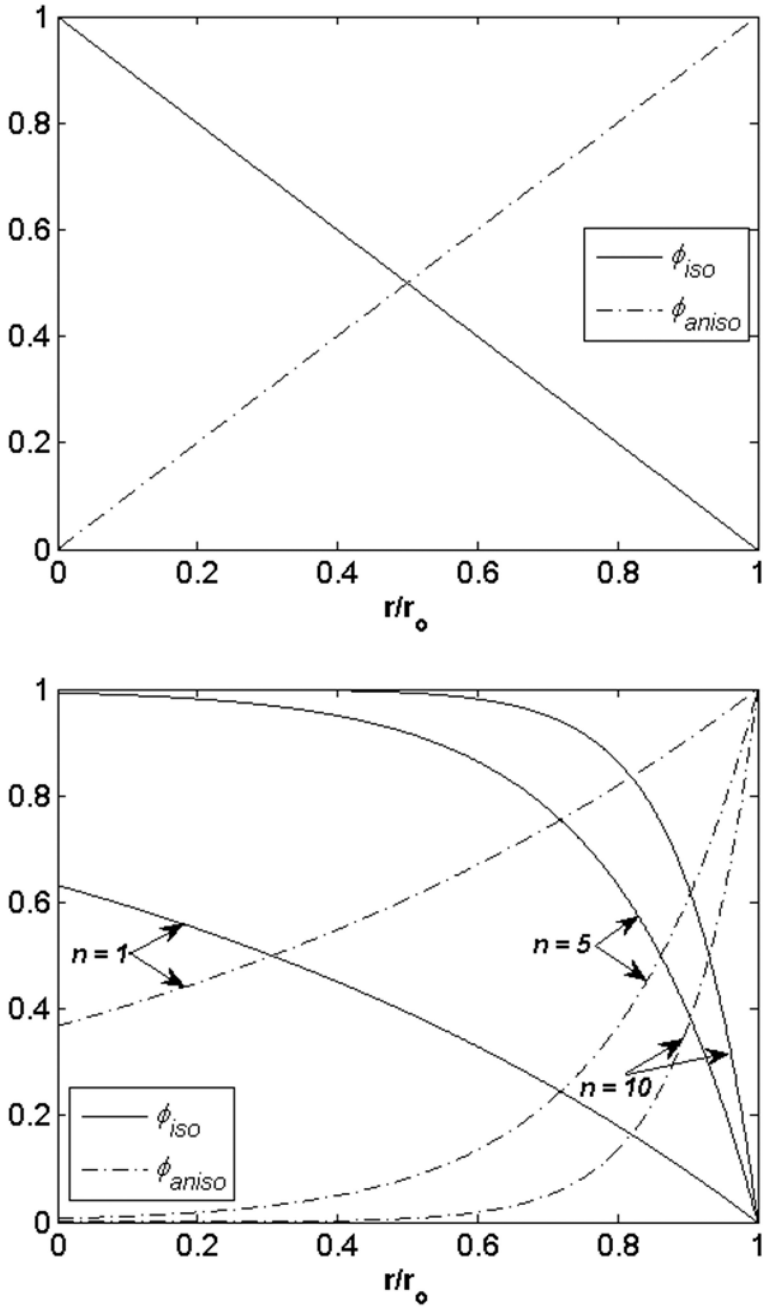

Figure 2.

Radial distributions of the mass fractions ϕ_{iso} and ϕ_{aniso} for an assumed linear (top) or exponential (bottom) variation. The exponential case is shown for the parameter $n = 1$, 5, and 10, which reveals that for n large enough one can model a material that transitions from purely isotropic at the center to strongly transversely isotropic at the edge similar to that for the linear variation.

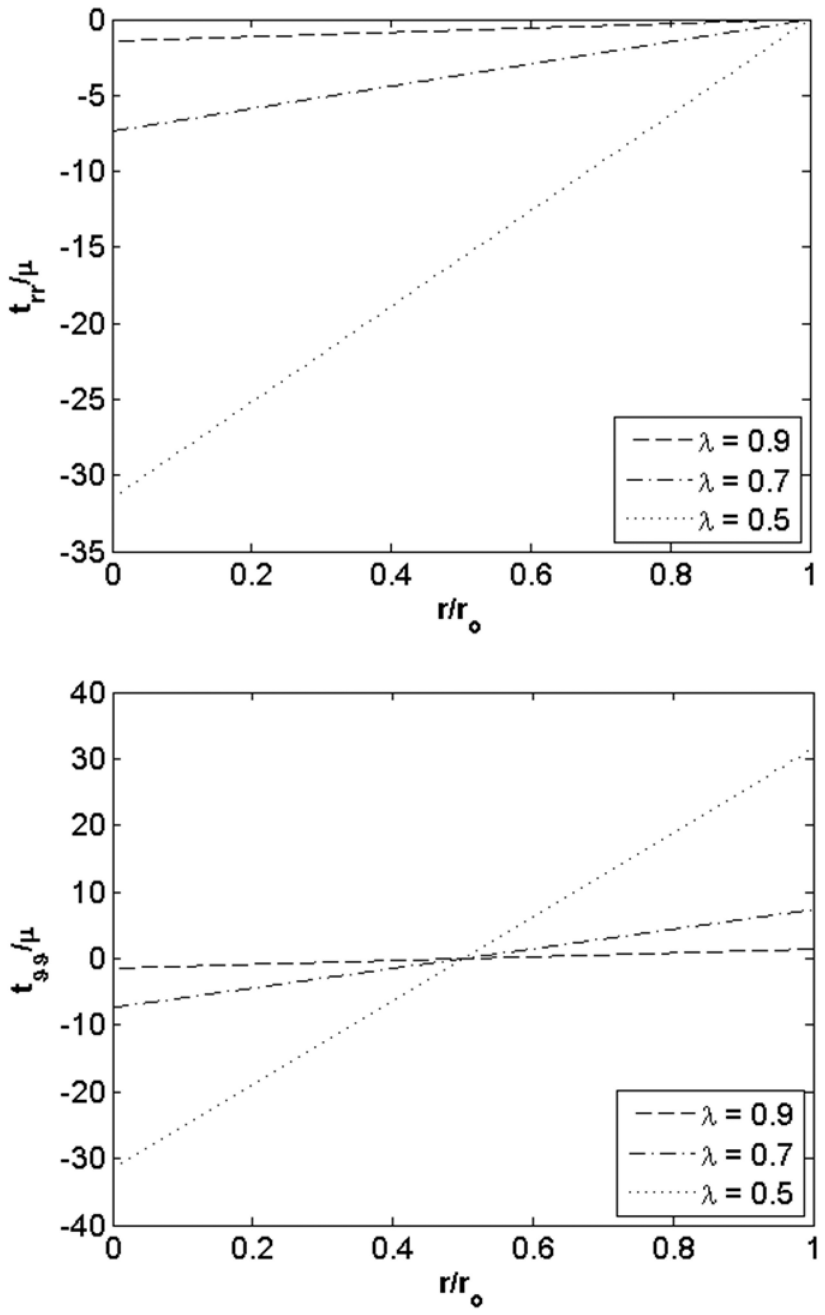

Figure 3.
Predicted distributions of radial (top) and circumferential (bottom) stress in a model fibroblast seeded collagen gel for a linearly changing radial heterogeneity in material properties ($\phi_{aniso} = r/r_o$ and $\phi_{iso} = 1 - r/r_o$) and different degrees of contraction ($\lambda = 0.9$, 0.7, and 0.5). Note that stress is non-dimensionalized using the material parameter μ.

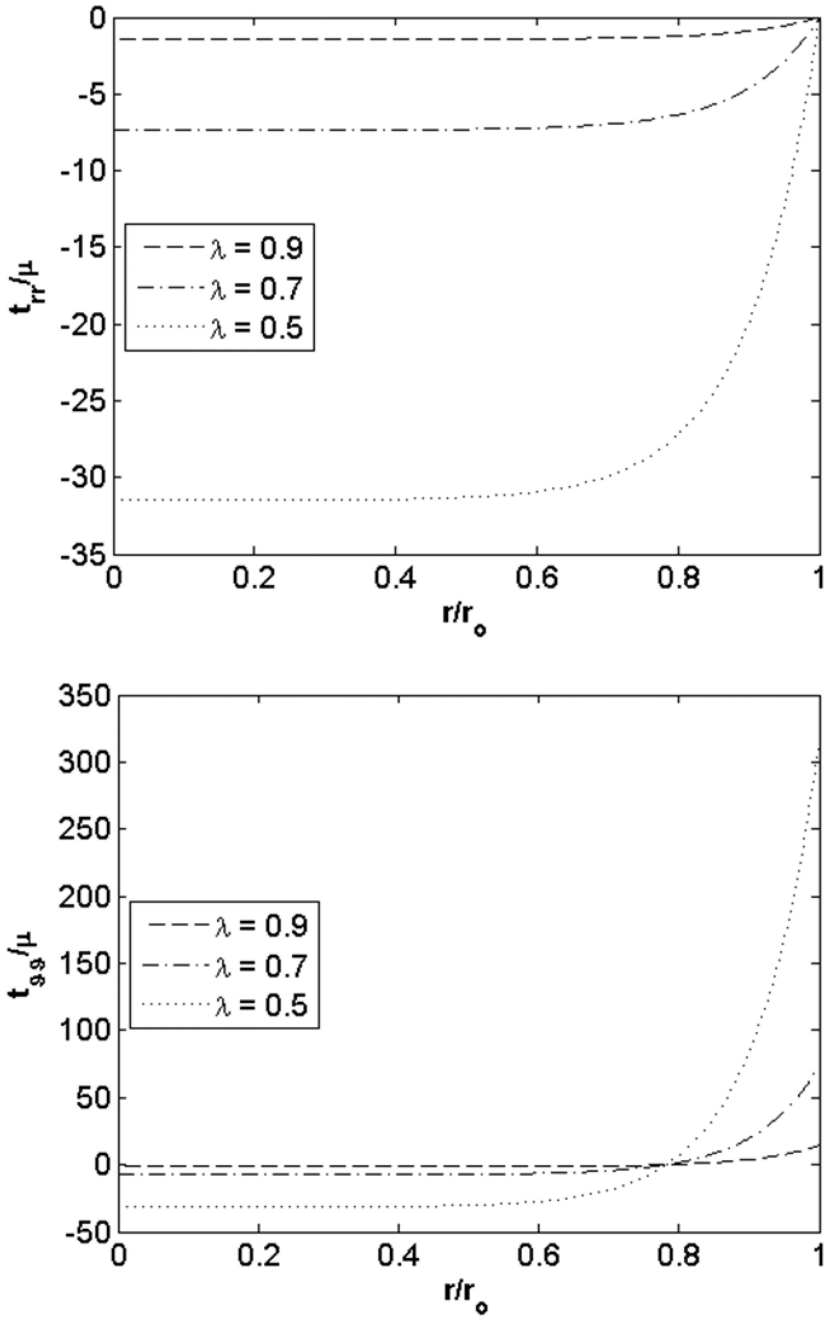

Figure 4.

Predicted distributions of radial (top) and circumferential (bottom) stress in a model fibroblast seeded collagen gel for an exponentially changing radial heterogeneity in material properties ($\phi_{iso} = 1 - \exp[n(r/r_o - 1)]$ and $\phi_{aniso} = \exp[n(r/r_o - 1)]$ with $n = 10$) and different degrees of contraction ($\lambda = 0.9$, 0.7, and 0.5). Albeit not shown, results for other values of n large were similar.