A tissue adaptation model based on strain-dependent collagen degradation and contact-guided cell traction

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\textbf{A B S T R A C T}

Soft biological tissues adapt their collagen network to the mechanical environment. Collagen remodeling and cell traction are both involved in this process. The present study presents a collagen adaptation model which includes strain-dependent collagen degradation and contact-guided cell traction. Cell traction is determined by the prevailing collagen structure and is assumed to strive for tensional homeostasis. In addition, collagen is assumed to mechanically fail if it is over-strained. Care is taken to use principally measurable and physiologically meaningful relationships. This model is implemented in a fibril-reinforced biphasic finite element model for soft hydrated tissues.

The versatility and limitations of the model are demonstrated by corroborating the predicted transient and equilibrium collagen adaptation under distinct mechanical constraints against experimental observations from the literature. These experiments include overloading of pericardium explants until failure, static uniaxial and biaxial loading of cell-seeded gels in vitro and shortening of periostium explants. In addition, remodeling under hypothetical conditions is explored to demonstrate how collagen might adapt to small differences in constraints.

Typical aspects of all essentially different experimental conditions are captured quantitatively or qualitatively. Differences between predictions and experiments as well as new insights that emerge from the present simulations are discussed. This model is anticipated to evolve into a mechanistic description of collagen adaptation, which may assist in developing load-regimes for functional tissue engineered constructs, or may be employed to improve our understanding of the mechanisms behind physiological and pathological collagen remodeling.

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\textbf{1. Introduction}

This work presents a numerical model that aims to study the adaptive behavior of soft tissues to mechanical loading. A thorough understanding of this adaptation may provide insight in pathological processes, it may assist in optimizing surgical reconstructions of load-bearing soft tissues, and it may serve the tissue engineering of such tissues through optimized mechanical conditioning during culture. In addition to these practical purposes, it is the beauty and the robustness of soft tissue adaptation that is intriguing. The resulting variety in macroscopic structural organizations of tissues, which are fully adapted to their particular function, yet with similar constitution at the microscopic level, fascinates scientists. This fascination for self-regulating biological processes has been key to Huiskes’ successful career. Studying fundamental aspects of human joint replacement (Huiskes, 1979), he understood that bone adaptation is one of the governing factors that determine implant failure in the long term. By challenging himself with questions and hypotheses on bone adaptation, he went from the tissue level down to cell level mechanics. The title of one of his many publications is: “If bone is the answer, then what is the question?” (Huiskes, 2000). By posing ourselves such questions for various processes and tissues, we develop fundamental understanding of tissue structure and adaptive processes. The present paper is the result of efforts to understand soft fibrous tissue adaptation in general. It shows how the interplay between external loading, tissue properties and cell activity governs such adaptation process, very similar to bone adaptation theories developed by Huiskes et al. (2000b).

Tissues adapt to changing mechanical conditions by continuous remodeling of their collagen network through cell-mediated and matrix-associated mechanisms. In the short term, cell alignment depends on topological patterns (‘contact guidance’) and the maximal principal strain direction (‘strain avoidance’) (Foolen et al., 2012; Mudera et al., 2000; Wang and Grood, 2000). Cell

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traction to the extracellular matrix (ECM) depends on cell shape, size and focal adhesions (Rape et al., 2011). Cells monitor their mechanical state and adjust traction forces depending on the environmental conditions to reach ‘tensional homeostasis’ (Brown et al., 1998; Saez et al., 2005; Ghibaudo et al., 2008; Petroll et al., 2004). Cell traction results in alignment of the collagen network (Stopak and Harris, 1982), which in turn becomes a topological trigger for cells to orient. The result is a continuously adapting tissue with an anisotropic angular distribution of cell orientation, coinciding with the anisotropic angular collagen fibril distribution (Guido and Tranquillo, 1993). Constraints such as silicone posts spatially restrict tissue contraction, leading to dense anisotropic tissue arrays (Legant et al., 2009a, 2009b; Foolen et al., 2012).

In the long term, collagen turnover influences the collagen structure (Wang et al., 2007). Coll-I, MMP-1 and MMP-2 genes are upregulated with cyclic strain in fibroblasts (Breen, 2000; Yang et al., 2004; Butt and Bishop, 1997; Shelton and Rada, 2007; Yang et al., 2005; He et al., 2004), while coll-II, MMP-3, -9 and -13 expression is enhanced in dynamically loaded chondrocytes (Fitzgerald et al., 2006). Thus, mechanical stimulation increases both collagen catabolism and anabolism, resulting in an increased remodeling rate. Collagen orientation is also mechanoregulated. Tensile strain in collagen fibrils modulates their susceptibility to enzymatic degradation (Nabeshima et al., 1996; Ruberti and Hallab, 2005; Bhole et al., 2008; Wyatt et al., 2009; Flynn et al., 2010; Zareian et al., 2010; Camp et al., 2011), likely through a switch in the molecular state that makes it enzyme-resistant when strained (Wyatt et al., 2009; Camp et al., 2011). This mechanism preserves collagen fibrils that are loaded in an appropriate strain range, and allows degradation of under-stained fibrils.

Based on the above experimental findings, various hypotheses were formulated with regard to cell activity, cell-matrix interaction and cell-independent collagen fiber remodeling. Even though experimental data were quantified, the resulting hypotheses are qualitative in nature. To explore whether the individual hypotheses, or the combination thereof, would indeed result in quantitative tissue adaptation seen under a wide variety of well-defined conditions, requires theoretical approaches. Such theoretical models show that cell traction and contact guidance can qualitatively predict cell alignment and collagen gel compaction in vitro (Barocas and Tranquillo, 1997), while adaptive cell traction may control stress and strain homeostasis (Humphrey, 1999). By assuming that collagen aligns in between the two principal strain directions, equilibrium collagen structures in various tissues are predicted (Boerboom et al., 2003; Driessen et al., 2003; Wilson et al., 2006), and similar results are obtained starting from the mechanical description of individual collagen fibrils (Kuhl and Holzapfel, 2007). After predicting the final tissue structure, the next step is to predict the transient adaptation process during the time of tissue development. Such prediction could for instance be used to develop loading conditions that maximize the rate at which load-bearing tissues develop their structure, in addition to optimizing their final mechanical properties at the end of a tissue engineering study. To predict such transient adaptation process, more physically motivated constitutive models have been developed. These include tissue adaptation based on strain-dependent collagen degeneration (Van Donkelaar and Wilson, 2007) as well as effects of cell contraction on collagen alignment (Nagel and Kelly, 2012; Soares et al., 2012), or combinations thereof (Loerakker et al., 2013; Van Donkelaar et al., 2013).

The present study demonstrates the versatility and limitations of a model that combines strain-dependent collagen degradation, mechanosensitive collagen turnover and contact-guided cell traction. Predictions for transient and equilibrium collagen adaptation under distinct mechanical constraints are corroborated against experimental observations from the literature.

2. Materials and methods

Collagen remodeling is implemented in a fibril-reinforced finite element (FE) model as two repeating sequential steps. First, tissue deformation and collagen strains are calculated as the result of both cell traction and externally applied forces. Second, collagen content and distribution change as a result of strain-dependent collagen degradation and collagen turnover.

2.1. Constitutive model

In a fibril-reinforced biphasic FE model for soft hydrated tissues (Wilson et al., 2004, 2005), the solid contains a fibrillar part representing the collagen network and a non-fibrillar part representing the ground substance. The total stress \( \sigma_{\text{tot}} \) is given by

\[
\sigma_{\text{tot}} = -p \mathbf{I} - p_t \mathbf{I} + \sigma_s
\]

with \( \sigma_s \) the effective solid stress, \( p \) the hydrostatic pressure, \( \mathbf{I} \) the unit tensor and \( n_t \) and \( n_f \) the solid and fluid volume fractions, respectively. The mechanical behavior of the non-fibrillar part is described with a compressible neo-Hookean model:

\[
\sigma = K \mathbf{I} + \frac{2}{3} \mathbf{G} (\mathbf{I} - \mathbf{J}^{1/2})
\]

with \( \mathbf{G} \) the Cauchy stress tensor, \( \mathbf{I} \) the left Cauchy Green deformation tensor, \( K \) the bulk modulus, \( G \) the shear modulus and \( J \) the volumetric deformation. The mechanical behavior of the fibrillar part is defined as

\[
P_1 = \begin{cases} 1, & \varepsilon_{\text{fibril}} > 0 \\ 0, & \varepsilon_{\text{fibril}} \leq 0 \end{cases}
\]

with \( P_1 \) the fibril stress, \( \varepsilon_{\text{fibril}} \) the logarithmic fibril strain and \( E_1 \) and \( k_1 \) positive material constants. This behavior is applied to each fibril, which represents a group of fibrils in the tissue and is defined by a density, orientation, initial and current length. Although collagen is viscoelastic, it is modeled as an elastic material because the process of collagen remodeling occurs over a much longer time-scale (weeks-months) than the viscoelastic effects (min).

This material model is implemented in ABAQUS v6.11 (Hibbitt, Karlsson & Sorensen, Inc., Pawtucket, RI, USA) using the UMAT subroutine. Per integration point of a 2-dimensional mesh, 30 collagen fibrils are included. One fibril obtains a random orientation, which defines the orientations of the others with interfibrillar angles of 6°. Increasing the number of fibrils did not change the result of the simulations and the minimum angular resolution of fiber orientations that can be computed with 30 fiber suffices for comparison with experimental data.

2.2. Implementation

The general concept is that collagen content and distribution are changed by strain-dependent collagen degradation, collagen production and mechanical failure. Collagen turnover rate is regulated by mechanosensitive cell metabolism. Fibris strain is changed by contact-guided cell traction, while cells continuously adapt their traction forces to reach tensional homeostasis.

2.2.1. Collagen turnover

It is assumed that mechanical stimulation increases both collagen production and degradation rate (Wang et al., 2007), and that cell metabolism \( (J_{\text{met}}) \) increases with cell deformation. Given that cells attach to their environment, deformation of the cell is assumed to be equal to deformation of the ECM, and is calculated as the ratio between the length of the longest \( (l_{\text{max}}) \) and the shortest \( (l_{\text{min}}) \) fibril:

\[
f_{\text{cell}} = \frac{l_{\text{max}}}{l_{\text{min}}}
\]

The change in collagen density \( (d\rho) \) over time is calculated depending on the metabolic rate and the difference between the rates of collagen degradation \( (d\rho_{\text{coll}}/dt) \) and production \( (d\rho_{\text{coll}}/dt') \):

\[
d\rho = f_{\text{cell}} \left( \frac{d\rho_{\text{cell}}}{dt} - \frac{d\rho_{\text{coll}}}{dt'} \right)
\]

in which \( f_s \) is a density factor, which ensures that collagen self-assembly and degradation are more pronounced when there are more fibrils to interact with:

\[
f_s = \frac{\rho_{\text{cell}}}{\rho_{\text{cell}}}
\]

as defined by collagen density \( (\rho_{\text{cell}}) \) and the number of collagen fibrils \( (\text{tot}) \) in integration point \( ip \).
Whether collagen degradation or synthesis prevails (Eq. (5)) depends on the fiber strain (εf), because enzymatic collagen degradation is strain dependent (Wyatt et al., 2009). A transition value (εtransmission) represents the strain at which degradation and synthesis occur at equal rates. This is assumed to equal the average between maximal (dtotfmax/dt) and minimal (dtotfmin/dt) degradation rates for under-strained and highly strained collagen fibrils, respectively:

$$\frac{d\rho_{prod}}{dt} = \frac{d\rho_{max}/dt + d\rho_{min}/dt}{2}$$

(7)

Strain-dependent enzymatic collagen degradation is implemented by decreasing collagen density according to Wyatt et al. (2009):

$$\frac{d\rho_{degr}}{dt} = \frac{d\rho_{max}/dt - d\rho_{min}/dt}{2}$$

(8)

where smoothing factor B accommodates for the fact that one collagen fibril in the model represents a population of fibrils within ±3° and −3° angles and with various strain levels.

In addition, excessive strains may induce fibril damage. Because each fibril direction represents a population of fibrils, a gradual transition between no damage and full fibril rupture is defined by two strain thresholds (εmax, εmin) and a strain constant (εf):

$$f_{np} = \begin{cases} 
0, & \text{if } \epsilon_f < \epsilon_{max} \\
\frac{\epsilon_f - \epsilon_{min}}{\epsilon_{max} - \epsilon_{min}} f_{max, np} & \text{if } \epsilon_{min} \leq \epsilon_f \leq \epsilon_{max} \\
1 & \text{if } \epsilon_f > \epsilon_{max}
\end{cases}$$

(9)

Finally, this mechanical damage is effective through an effect on the local collagen density (ρnp):

$$\rho_{np} = (1 - f_{np})\rho_p$$

(10)

2.2.2. Cell traction

Fibril strain is calculated from the deformation tensor F of the solid matrix. However, cell traction may cause additional fibril strain. This is implemented by pre-stretching the fibrils in the reference configuration, i.e. by changing the initial fibril length. Cell traction is a function of cell alignment (Rape et al., 2011), implemented as an ellipsoidal function with Tmin and Tmax, the traction perpendicular and parallel to cell alignment. It is assumed that cells may align to any fibril (f) at each location (p), yet that the number of aligning cells per fibril direction (α) scales with the fibril density. These contributions combined give fibril traction Tf:

$$T_f = \sum_p \rho_f (T_{min} \cos (\alpha - \alpha_f)^2 + T_{max} \sin (\alpha - \alpha_f)^2)$$

(11)

with

$$T_{min} = 1 - \frac{f_{cell} - 1}{f_{cell} + 1}$$

(12)

$$T_{max} = 1 + \frac{f_{cell} - 1}{f_{cell} + 1}$$

The traction value of each fibril direction is normalized (εf) to maintain a constant cell traction force per integration point:

$$\epsilon_f = \frac{T_f}{\sum f_{cell} T_f}$$

(13)

Fibril strain (εf) depends on current fibril length (l) as calculated from tissue deformation, and on cell traction direction which increases with cell density (fcell) and relates inversely to fibril density (ρp):

$$\epsilon_f = \frac{100\% (l_f - 1) + (\epsilon_f^0 f_{cell} + 1)}{\epsilon_f^0 \rho_p}$$

(14)

with εf0 the fibril strain induced by cell traction if εf = fcell and ρp equal 1. To implement this cell traction effect, a new initial fibril length (l0) is determined from the current fibril length (l) and the calculated fibril stretch:

$$l_0 = \frac{l}{1 + \epsilon_f / 100\%}$$

(15)

2.2.3. Cell traction adaptation towards tensional homeostasis

Cells adjust their traction forces to maintain tensional homeostasis (Brown et al., 1998; Saez et al., 2005; Ghibaudi et al., 2008; Petrot et al., 2004). This is achieved by adjusting the maximum cell traction per fibril with an adaptation factor (fad), such that fibril strain is remodeled towards the strain at which synthesis and degradation are in equilibrium (εtransmission in Eq. (7)). First, the target traction value (ftransmission) at which fibril strain is equal to εtransmission is calculated by rewriting the previous equations to:

$$f_{transmission} = \frac{\epsilon_{transmission} - 100\% (l_f - 1)}{\epsilon_f^0 f_{cell} + 1}$$

The adaptation factor at current time (t) then becomes

$$f_{ad}(t) = \begin{cases} 
\frac{\epsilon_f(t) - f_{transmission}}{\epsilon_{max} - f_{transmission}} f_{max, np} & \text{if } \epsilon_{min} \leq \epsilon_f(t) \leq \epsilon_{max} \\
\frac{\epsilon_f(t) - \epsilon_{max}}{\epsilon_{max} - \epsilon_{min}} f_{max, np} & \text{if } \epsilon_f(t) > \epsilon_{max}
\end{cases}$$

(17)

with fmax, np the adaptation rate, which may range between 1 (complete adaptation towards ftransmission) and 0 (no adaptation) within each time step (dt). The adaptation factor is limited between maximum value ftransmission and 0 to prevent fibril compaction by cell traction.

2.3. Simulations

To evaluate the versatility of the model, predictions are corroborated against distinct in vitro experiments: tissue overloading until failure, static uniaxial and biaxial loading, and tissue shortening. In addition, remodeling in some hypothetical cases is predicted to demonstrate how collagen might adapt to small differences in constraints.

In all simulations, two-dimensional FE meshes with 4-node plane strain pore pressure elements (CPE4P) are used. Because the tissues in the experiments are thin, water transport to reach equilibrium is relatively fast (min) compared to the time constant of the collagen remodeling process (days), as well as to the duration of the experiments (hours to days). For this reason, water transport is assumed infinitively fast and this is implemented by prescribing zero pore pressure throughout the mesh. Unless indicated otherwise, initial collagen density equals one for all fibrils. One hour experimental time equals one time step with dt = 100. All parameters are provided in Table 1.

2.3.1. Tissue overloading

Axially loaded decellularized bovine pericardium strips were subjected to collagenase, showing that local stresses accelerate collagen fibril degradation (Elsmere et al., 1999). Simulations with and without collagen fibril rupturing (Eq. (9)) were performed on a rectangular FE mesh (10 × 4.5 mm; 180 elements). While it is constrained along the top edge, constant pressure of 3.4, 36 or 200 kPa was applied to the bottom edge using a coupling constraint, representing weights of 1, 10 or 60 g, respectively. Initial collagen distribution in pericardium was approximated from Billica and Sacks (1997). The values for onset (εtransmission) and total collagen damage (εtransmission = 30%) (Eq. (9); Table 1) are selectively used for simulating this tissue overloading experiment. Literature reports a wide range of collagen failure strains. The maximum value was chosen based on the strains at which individual collagen fibrils yield and break 20% and 30–50%, respectively (Shen et al., 2008). Yet, damage starts before those strains are reached. Human patella tendons fail at 16% strain (Butler et al., 1978), and in some cases microscopic collagen damage was already observed at 4% strain (Wang, 2006). Therefore, a lower threshold for collagen was chosen in this range.

2.3.2. Uniaxial loading

Collagen adaptation over 72 h in uniaxially constrained fibroblast-populated collagen gels (Lee et al., 2008) was simulated by fully constraining top and bottom nodes and coupling the horizontal displacement of nodes along the left and right edges, separately, to simulate polyethylene sidebars. Mean angle and vector length of the fibril distribution are calculated for each integration point using a Matlab (Mathworks Inc) toolbox for circular statistics (Beraez, 2009), taking into account that fibril angles are only unique within 180°.

2.3.3. Biaxial loading

The arms of fibroblast-seeded cruciform collagen gels were subjected to 20% static stretch (Hu et al., 2009). At day 0, 3 and 6 the angular collagen distribution was measured in the center (CT), long arm (LA) and short arm (SA) of the gel. An alignment index (AI), defined as the normalized fraction of fibrils within 20° of the predominant direction, was calculated to compare time points and experiments. In simulations, the gel was allowed to remodel for 24 h during which the arms were constrained. Subsequently, the short arms were stretched to 1.01 and the long arms to either 1.04 or 1.20 times their initial length for six days.

2.3.4. Tissue shortening and lengthening

Embrionic periostem was able to restore its preferred mechanical strain state within 72 h of culture, when it was shortened by 10% or lengthened by 5% (Foolen et al., 2010). It could not fully recover when shortened by 15%. Simulations were performed for length-changes between −15% and +5%, including only cell traction, only collagen degradation and production, or with a combination thereof. A 50 × 50 mm square FE mesh (527 elements) was used, representing a central area of the idealized cylindrical periostem. Nodal displacements were constrained in
all directions at the top and bottom edge and in horizontal direction at the left and right edge. After 72 h of initial remodeling, tissue length was changed by displacement of the top edge and the tissue remodeled for another 72 h after which a force-stretch curve was determined. For comparison with experiments, a transition stretch was defined as the stretch at the reaction force equal to the one reached in the unstrained tissue at 0.96 times the in vivo length. Note that the term ‘transition stretch’ is copied for this particular simulation from the original experimental paper (Foolen et al., 2010), and should not be confused with the transition strain parameter used in the model (ε_{transition} in Eq. (16); Table 1).

2.3.5. Hypothetical remodeling cases

Four hypothetical remodeling cases illustrated possible effects of small changes applied to a common TE setup. A collagen gel (20 x 20 mm, 400 elements, top and bottom edges constrained) was allowed to remodel for 72 h, after which the top surface was sheared, asymmetrically elongated or asymmetrical shortened during another 72 h. In a fourth simulation the collagen gel was attached to poles on its four corners for 72 h.

3. Results

3.1. Tissue overloading

A two-phase tissue elongation pattern occurs over time for all simulations (Fig. 1). Only when fibril rupturing is included, time to failure decreases with increasing applied load to the tissue, similar to the experimental observations.

3.2. Uniaxial loading

Collagen aligns with the loading direction over time, with typical distributions of density and degree of alignment near the constrained sites (Fig. 2).

Table 1

<table>
<thead>
<tr>
<th>Material</th>
<th>Overloading (Ellsmere et al., 1999)</th>
<th>Uniaxial constraint (Lee et al., 2008)</th>
<th>Biaxial constraint (Hu et al., 2009)</th>
<th>Shortening &amp; lengthening (Foolen et al., 2010)</th>
<th>Collagen gel **</th>
</tr>
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<tr>
<td>E1 (Pa)</td>
<td>3.0 x 10^7</td>
<td>2.5 x 10^7</td>
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<td>5</td>
<td>5.5</td>
<td>25</td>
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<tr>
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</table>

* Material parameters fitted to biaxial loading experiment (Langdon et al., 1999).
** Material parameters fitted to biaxial tensile tests after 72 h of culture under uniaxial constraint (Lee et al., 2008).
*** Material parameters manually adjusted and parameter study is added to evaluate effects (see text).
* Derived from Zareian et al. (2010).
** Derived from Wyatt et al. (2009).
# Adjusted per simulation to match differences in experimental time, tissue type, cell number and collagen density.

Fig. 1. Elongation-time curves of statically loaded pericardium strips exposed to collagenase, loaded with 1, 10 and 60 g. Experimental curves (dotted; Ellsmere et al., 1999) and results of simulations without (A) and with (B) strain-dependent collagen rupturing.
3.3. Biaxial loading

For the 4% strain ($\lambda_{LA} = 1.04$) simulation, the AI in the central (CT) region remains low and the LA and SA regions increase to levels comparable with the experiment (Fig. 3). Only the AI in the LA does not stabilize after 3 days. However, simulations with 20% strain show a different pattern, with increased AI in the CT region and low AI in the LA.

3.4. Tissue shortening and lengthening

During 3 days of remodeling after lengthening or shortening, the transition stretch shifts towards the new tissue length and the stiffness of the tissue changes. With only collagen remodeling and no cell traction (Fig. 4B), the shift in transition stretch is not as apparent as with cell traction included. This importance of cell traction is in agreement with the experimental data (Foolen et al., 2010). Changes in tissue stiffness also concur with experiments. This is the consequence of collagen remodeling (Fig. 4A) in the simulations, and is strongly influenced by whether or not cell traction occurs in parallel (Fig. 4E). In simulations, the change in stiffness is associated with changes in collagen content, but collagen loss is not observed experimentally.

3.5. Hypothetical remodeling cases

After the initial remodeling phase, collagen density is highest in the corners and lowest at the free edges, while fibrils align in the constrained direction and towards the corners (Fig. 5A). When subsequently shear is applied, the fibrils rotate towards the longest axis of the gel, with a modest increase in collagen density along that line (Fig. 5B). Asymmetric lengthening of the gel increases collagen density and stimulates alignment at the modulated side of the gel (Fig. 5C), while asymmetric shortening decreases density and reduces alignment at the modulated side (Fig. 5D). When a gel is constrained at 4 corners, collagen aligns along the edges and towards the corners, with highest collagen density in the corners. The collagen distribution is isotropic in the center (Fig. 5E).

4. Discussion

This study demonstrates the versatility of a description of collagen remodeling including strain-dependent collagen degradation, collagen production, contact guided cell traction and mechanical collagen rupture. Typical aspects of essentially different experimental conditions and with both explants and cell-populated gels are captured qualitatively. These include the typical two-phase elongation curves in the overloading-to-failure experiments on mature pericardium by Ellsmere et al. (1999). The characteristic shift of the transition point where failure starts was only predicted correctly when mechanical rupture of collagen was included in addition to enzymatic degradation, suggesting that the experimental data cannot be explained by enzymatic collagen degradation alone. In periosteum explants, the peculiar shift in the transition point of the force–stretch curve in response
to tissue shortening or lengthening is similar between predictions and experiments (Foolen et al., 2010). Interestingly, tissue fails to shift the transition point beyond 10% shortening in both experiments and simulations. Also, cell contraction was the most efficient to induce this shift, which agrees with the observation that Cytochalasin-D, which disrupts the actin cytoskeleton, blocks this effect (Foolen et al., 2010). In TE constructs, collagen adaptation under uniaxial loading concurs quantitatively with the experiments (Lee et al., 2008), both for collagen density as a function of angular distribution and for the changes over time. In addition, lateral compaction occurred, which is also apparent on a photograph after remodeling (Fig. 1 in Lee et al., 2008). Collagen alignment in the direction between the applied constraints also concurs with other observations in both gels (e.g. Legant et al., 2009a, 2009b) and tissues (e.g. Foolen et al., 2012), and high qualitative resemblance with experiments is observed for the pinned gel simulation (Fig. 5E; experimental data by Legant et al., 2009a, 2009b not shown). Similar inhomogeneous effects seem to occur after casting TE gels; in the axisymmetric setup of Hu et al. (2009) anisotropy develops even before stretch is applied to the arms, resulting in a discrepancy between simulations (Al = 1) and the experiment (Al = 1.25; data not shown). Such variations in fiber populations are not incorporated into the model, although some effects are accounted for by using gradual transitions in for instance the equations representing fibril rupture (Eq. (9)). Second, the non-fibrillar matrix, representing the ground substance, does not remodel. Pressures associated with compression of the non-fibrillar matrix in the model may explain why tissues seem to compact less in simulations than in for instance the experiments of Lee et al. (2008). Third, tissue growth is not included. Although the tissue is able to adapt appropriately to 5% elongation (Fig. 4B and C), this is not due to tissue growth. The model assumes that new collagen molecules attach to existing fibrils and automatically attain the prevailing fibril strain. However, there are also differences that require discussion. First, the collagen network in explants likely contains variations in fibril direction and pre-strains. Also crosslinking may be important, more so in explants than in TE constructs. Such effects may affect experimental results such as the failure patterns in the tissue overloading experiments (Ellsmere et al., 1999; Fig. 1) or the adaptation to tissue shortening (Foolen et al., 2012; Fig. 4). Similar inhomogeneous effects seem to occur after casting TE gels: in the axisymmetric setup of Hu et al. (2009) anisotropy develops even before stretch is applied to the arms, resulting in a discrepancy between simulations (Al = 1) and the experiment (Al = 1.25; data not shown). Such variations in fiber populations are not incorporated into the model, although some effects are accounted for by using gradual transitions in for instance the equations representing fibril rupture (Eq. (9)). Second, the non-fibrillar matrix, representing the ground substance, does not remodel. Pressures associated with compression of the non-fibrillar matrix in the model may explain why tissues seem to compact less in simulations than in for instance the experiments of Lee et al. (2008). Third, tissue growth is not included. Although the tissue is able to adapt appropriately to 5% elongation (Fig. 4B and C), this is not due to tissue growth. The model assumes that new collagen molecules attach to existing fibrils and automatically attain the prevailing fibril strain. However, this will result in continuous increase of collagen density if a constant strain is applied that exceeds the transition strain ($\varepsilon_{\text{transition}}$ in Eq. (8)) (Eq. (5)). To exemplify this effect, simulations with 20% strain in the biaxial condition overpredict collagen density in the center and do not stabilize over time as experimentally observed (Fig. 3D), whereas a physiologically more realistic response prevails in simulations where the applied static strain remains lower (Fig. 3C). This unnatural response may be solved by combining the present model with a tissue growth approach in which newly synthesized collagen assemblies to form an unstrained fibril within the present...
geometry, i.e. within the deformed matrix (Van Donkelaar and Wilson, 2007, 2012; Khoshgoftar et al., 2014). Fourth, because cells attach to the ECM, cell deformation is assumed to follow ECM strain. This is functional, because they sense these strains and because maximum cell contraction force relates to cell shape. However, assuming that cell attachment is a static condition is a simplification. Focal adhesions are dynamic and changes in focal adhesion points may change cell strain. Such adaptive behavior has not yet been accounted for and may be part of future model extension. Fifth, selecting parameters is difficult. Optimizing collagen remodeling and cell traction parameters per study is arguable because each study uses different tissues, gels and cell sources. Yet, in order to allow for comparison between the different cases in this study, parameter variations were kept rather small (Table 1). To explore the effect of these choices for cell-traction related parameters, a small parameter study is performed. This study reveals that $\varepsilon_{\text{transition}}$ (Eq. (8)), $\varepsilon_{f0}$ (Eq. (14)) and $f_{\text{nad,max}}$ (Eq. (17)) together determine tissue compaction, but they do not influence the time to reach equilibrium. Thus, the residual force that is produced under a given strain condition is hardly changed by any of these parameters, but the stress that is generated may change significantly. This may be relevant when comparing simulations with experimentally determined stress rather than force. The time that it takes to reach equilibrium is dominated by $k_{\text{ad}}$ (Eq. (17)). Another effect involving numerical-experimental comparison relates to methods for calculation of parameters. For instance, the transition stretch in the shortening study (Fig. 4) was for practical reasons determined by Foolen et al. (2010) as the tissue length where 1/8th of the tissue stiffness was obtained. Because stress–strain curves are non-linear, this definition cannot be copied exactly in simulations. The present study uses an alternative strain-based definition of the transition-stretch,

Fig. 4. Reaction force–stretch curves (A, C and E) and transition stretch against applied stretch (B, D and F) after 72 h of remodeling at the applied stretch level, for sets of simulations with only collagen remodeling (A and B), both collagen remodeling and cell traction adaptation (C and D), and only cell traction adaptation included (E and F). For comparison, the experimental data (Foolen et al., 2010) are indicated in grey diamonds (B, D and F).
boundary conditions may differ between experiments and simulations, for instance due to experimental inaccuracies. The set of simulations with small changes in constraints shows that this may have significant effects on the collagen network (Fig. 5). This insight is important for the design of TE experiments and the interpretation of variability in experimental datasets.

Some of the presented simulations provide new scientific insight and hypotheses that may be explored in the future. For instance, tissue response to mechanical overloading without fibril rupturing resulted in curves with characteristics that were not seen experimentally (Fig. 1). Thus, fibril rupturing is postulated to occur throughout lengthening, which suggests significant variability in fibril pre-strain. Also, simulations propose that the shift in transition stretch after tissue shortening results from cell-traction, while the observed change in tissue stiffness develops due to collagen remodeling (Fig. 4). From these simulations it is further hypothesized that failure to fully adapt to 15% shortening may be caused by the maximal cell-traction force.

In conclusion, this manuscript presents the versatility of a model that describes the collagen remodeling process by strain-dependent collagen degradation, collagen production, contact-guided cell traction and mechanical fibril rupture. Care was taken to use principally measurable parameters in physiologically meaningful equations. This is expected to make translation of experimental data to model input easier, stimulating further development towards a mechanistic model of collagen adaptation. The model is anticipated to improve our understanding of the mechanisms behind physiological and pathological collagen remodeling, and to assist in developing load-bearing TE constructs.

References


whereas other numerical studies used force- or stress-based parameters (Loerakker et al., 2013; Nagel and Kelly, 2012). This may explain why the results of the present study compare better to the experimental data than the result of other studies. Finally, whereas other numerical studies used force- or stress-based parameters (Loerakker et al., 2013; Nagel and Kelly, 2012). This may explain why the results of the present study compare better to the experimental data than the result of other studies. Finally, whereas other numerical studies used force- or stress-based parameters (Loerakker et al., 2013; Nagel and Kelly, 2012). This may explain why the results of the present study compare better to the experimental data than the result of other studies.

Fig. 5. Collagen density distribution (left; density values range linearly from 2/3 (dark blue) to 2 times (red) the initial collagen density) and fibril orientation (right; values for vector length indicated in insert (E)) after the initial remodeling phase (A) and after the second remodeling phase in which tissue was loaded by shearing the top surface (B), elongating (C) or shortening (D) the right side, or pinning at the corners (E). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)


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