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Citation for published version (APA):

DOI:
10.1016/j.jmbbm.2015.10.001

Document status and date:
Published: 01/05/2016

Document Version:
Publisher’s PDF, also known as Version of Record (includes final page, issue and volume numbers)

Please check the document version of this publication:

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A computational analysis of cell-mediated compaction and collagen remodeling in tissue-engineered heart valves

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\textbf{Abstract}

One of the most critical problems in heart valve tissue engineering is the progressive development of valvular insufficiency due to leaflet retraction. Understanding the underlying mechanisms of this process is crucial for developing tissue-engineered heart valves (TEHVs) that maintain their functionality in the long term. In the present study, we adopted a computational approach to predict the remodeling process in TEHVs subjected to dynamic pulmonary and aortic pressure conditions, and to assess the risk of valvular insufficiency. In addition, we investigated the importance of the intrinsic cell contractility on the final outcome of the remodeling process. For valves implanted in the aortic position, the model predictions suggest that valvular insufficiency is not likely to occur as the blood pressure is high enough to prevent the development of leaflet retraction. In addition, the collagen network was always predicted to remodel towards a circumferentially aligned network, which is corresponding to the native situation. In contrast, for valves implanted in the pulmonary position, our model predicted that there is a high risk for the development of valvular insufficiency, unless the cell contractility is very low. Conversely, the development of a circumferential collagen network was only predicted at these pressure conditions when cell contractility was high. Overall, these results, therefore, suggest that tissue remodeling at aortic pressure conditions is much more stable and favorable compared to tissue remodeling at pulmonary pressure conditions.

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1. Introduction

Heart valve disease remains a major health problem worldwide, with approximately 300,000 heart valve replacements being performed on an annual basis due to congenital defects or acquired valve disease (Pibarot and Dumesnil, 2009; Takkenberg et al., 2008). Although the currently used mechanical and biological valve prostheses are life-saving devices, they do exhibit some significant drawbacks due to the need for anticoagulation therapy in case of mechanical valves, and the limited durability in case of bioprosthetic valves (Kidane et al., 2009; Pibarot and Dumesnil, 2009). Furthermore, their lack of growth and remodeling capacity presents a serious limitation for pediatric patients, who need multiple re-operations due to somatic growth or valve-related morbidity (Ackermann et al., 2007; Lee et al., 2011). As a result, young recipients of a valve replacements have a significantly reduced life expectancy compared to healthy age-matched individuals (Puvimanasinghe et al., 2001).

Tissue engineering may provide a solution for particularly this group of patients, as it allows for creating heart valves with an intrinsic growth and remodeling capacity. The traditional method for creating tissue-engineered heart valves (TEHVs) consists of an in vitro approach, where cell-seeded scaffolds are mechanically and biochemically stimulated to transform into functional living valves inside bioreactor systems (Flanagan et al., 2009; Gottlieb et al., 2010; Hoorstrup et al., 2009; Mol et al., 2005; Neidert and Tranquillo, 2006). Due to the complexity and logistical issues associated with this approach, in situ tissue engineering is emerging as a promising alternative approach that allows for creating TEHVs with off-the-shelf availability (Dijkman et al., 2012; Driessen-Mol et al., 2014; Weber et al., 2013). The short-term functionality of TEHVs was demonstrated to be excellent in many pre-clinical studies (Driessen-Mol et al., 2014; Flanagan et al., 2009; Gottlieb et al., 2010; Hoorstrup et al., 2009; Schmidt et al., 2010). Medium-term follow-up, however, often revealed the progressive development of valvular insufficiency due to leaflet retraction. Understanding the underlying mechanisms of this process is crucial for developing TEHVs that maintain their functionality in the long term.

Leaflet retraction is the result of tissue growth and remodeling, which are hypothesized to occur to obtain and maintain a homeostatic mechanical state (Ambrosi et al., 2011; Ateshian and Humphrey, 2012; Humphrey et al., 2014). There are many factors that contribute to growth and remodeling, which are dependent on and interact with the mechanical state of the tissue. For example, cellular traction forces are partly, and directly, responsible for the shrinkage of the leaflets (Van Vlimmeren et al., 2012). Other important factors are cell-mediated contraction or alignment of extra-cellular matrix components such as collagen (McLeod et al., 2013; Meshel et al., 2005), and remodeling of the collagen network due to the mechanical stimuli imposed on the valve (Bhole et al., 2009; Ruberti and Hallab, 2005). Contraction of the matrix components directly contributes to leaflet retraction, while changes in the collagen architecture indirectly contribute to valvular insufficiency via changes in the anisotropic behavior of the tissue which affects the mechanical performance of the valve (Fan et al., 2013; Loerakker et al., 2013; Sacks et al., 2009). In addition, the diastolic pressure difference over the valve has the potential to counteract leaflet retraction, and as such can also play an important role in the development of valvular insufficiency (Van Loosdregt et al., 2014).

All together, there are many factors that contribute to the progressive development of valvular insufficiency in vivo, and their exact contributions to this process are not fully understood. The main reason for this is the dynamic interplay between all these factors and the mechanical load, which makes it difficult to predict the final outcome of the growth and remodeling process. Computational models are, therefore, necessary to increase our understanding of the underlying mechanisms, and predict when there will be a risk for the development of valvular insufficiency, which may, for example, be different for valves implanted in the pulmonary and the aortic position, and dependent on the initial scaffold properties. Many tissue growth and remodeling algorithms have already been developed that predict remodeling of the collagen network in response to mechanical stimuli (Baek et al., 2006; Driessen et al., 2003, 2004, 2008; Kuhl and Holzapfel, 2007; Martufi and Gasser, 2012; Valentin et al., 2013; Wan et al., 2016; Watton and Hill, 2009), as this is the main load-bearing component of most biological tissues. In addition, many advances have been made in predicting cellular alignment and traction forces due to mechanical cues, either focused on the behavior of cells only (Deshpande et al., 2006; Obbink-Huizer et al., 2014; Pathak et al., 2008; Vernerey and Farsad, 2011; Vigliotti et al., 2015) or on cell behavior in combination with collagen remodeling (Heck et al., 2015; Loerakker et al., 2014). Ultimately, computational models can be used to predict the in vivo development of engineered tissues starting from polymeric scaffolds (Khosravi et al., 2015; Miller et al., 2014, 2015; Niklason et al., 2010).

In a recent study (Loerakker et al., 2014), we developed a computational framework to predict cell-mediated tissue compaction and collagen remodeling by combining a computational model to predict cell traction and alignment (Obbink-Huizer et al., 2014) with laws for collagen contraction and remodeling. To verify the model and avoid excessive computational costs, we previously used this model to predict tissue remodeling in static tissues as observed in many experimental studies. In the present study, we used this computational framework to predict the remodeling process in TEHVs subjected to dynamic pressure conditions as experienced in vivo. To be able to simulate remodeling due to dynamic loading conditions, we first had to develop an analytical approximation of the stress fiber remodeling process to prevent the excessive computational costs associated with including all the dynamic variations in mechanical loads that occur during remodeling periods of weeks (Ristori et al., 2015). With this improved framework, we investigated the development of valvular insufficiency and remodeling of the collagen architecture in TEHVs to address the following research questions: (1) are there differences in tissue remodeling between valves exposed to pulmonary and aortic pressure conditions? (2) what is the effect of different cell phenotypes...
The material behavior was implemented in the commercial finite element package Abaqus (Dassault Systèmes Simulia Corp., Providence, RI), using the user-defined subroutine UMAT.

2. Methods

To describe tissue remodeling in dynamically loaded TEHVs, we used our previously developed computational framework (Loerrakker et al., 2014) and adapted it to account for dynamic loading conditions. These adaptations consisted of using an analytical approximation for the stress fiber remodeling algorithm to reduce computational costs (Ristori et al., 2015), and including both the loaded and unloaded configuration in the assessment of the degree of collagen contraction and strain-induced collagen degradation. As described in more detail in Loerrakker et al. (2014), the engineered tissue was modeled as a mixture of cells, collagen fibers, and isotropic matrix constituents (Fig. 1), where the total Cauchy stress consists of three parts

\[ \mathbf{\sigma} = \sigma_{sf} + \sigma_{cf} + \sigma_{mc} \]  

(1)

Here, \( \sigma_{sf} \) represents the anisotropic contractile contribution of the cells due to the presence of actin stress fibers, \( \sigma_{cf} \) equals the stress exerted by the collagen fibers, and \( \sigma_{mc} \) describes the stress due to the isotropic matrix components and the passive contribution of the cells. Actin stress fibers and collagen fibers were modeled using angular distributions with a resolution of 6°, where the original fiber directions \( \mathbf{e}^i_{f0} \) were defined using two orthogonal vectors \( \mathbf{u}_1 \) and \( \mathbf{u}_2 \), and the angle \( \phi^i \) of each direction \( i \) with respect to \( \mathbf{u}_1 \)

\[ \mathbf{e}^i_{f0} = \cos(\phi^i) \mathbf{u}_1 + \sin(\phi^i) \mathbf{u}_2 \]  

(2)

The material behavior was implemented in the commercial finite element package Abaqus (Dassault Systèmes Simulia Corp., Providence, RI), using the user-defined subroutine UMAT.

2.1. Actin stress fibers

Remodeling of the actin stress fibers in response to mechanical stimuli was described using the model of Obbink-Huizer et al. (2014). Briefly, the total amount of actin \( \varphi_i \) consists of a monomeric volume fraction \( \varphi_m \) and fractions of actin \( \varphi_{sf}^{i}/N \) that have polymerized into actin stress fibers in \( N \) directions

\[ \varphi_i = \varphi_m + \frac{1}{N} \sum_{i=1}^{N} \varphi_{sf}^{i} \]  

(3)

The stress \( \sigma_{sf}^{i} \) exerted in each direction is dependent on the global Green–Lagrange strain \( \varepsilon_i \), strain rate \( \dot{\varepsilon}_i \), and the maximum cell traction \( \sigma_{max} \), which depends on the cell phenotype

\[ \sigma_{sf}^{i} = \sigma_{max} f_i(\varepsilon_i, \dot{\varepsilon}_i) \]  

(4)

where the Green–Lagrange strain is defined as \( \varepsilon = \sqrt{\varepsilon_{ij} \varepsilon_{ij} - \frac{1}{3} (\varepsilon^2_1 - 1) \}

The deformation gradient tensor. The strain-dependent part \( f_\varphi \) is composed of an active part \( f_\varphi \) representing acto-myosin contraction, and a passive part \( f_{\varphi p} \) representing the strain-hardening response in extension

\[ f_i = f_{\varphi a} + f_{\varphi p} \]  

(5)

\[ f_{\varphi a}(\varepsilon) = \exp\left(-\left(\varepsilon/\varepsilon_0\right)^2\right) \]  

(6)

\[ f_{\varphi p}(\varepsilon) = \begin{cases} (\varepsilon/\varepsilon_1)^2, & \varepsilon \geq 0 \\ 0, & \varepsilon < 0 \end{cases} \]  

(7)

with \( \varepsilon_0 \) and \( \varepsilon_1 \) constants describing the reduction in active contraction when the strain differs from zero and the degree of passive strain hardening, respectively. The strain rate dependent part \( f_\varphi \) is given by

\[ f_\varphi(\varepsilon) = \frac{1}{1 + 2/\sqrt{5} \left( 1 + \frac{k_\varphi \varepsilon^2 + 2}{\sqrt{(k_\varphi \varepsilon^2 + 2)^2 + 1}} \right)} \]  

(8)

where \( k_\varphi \) defines the reduction in stress as the rate of shortening increases. Remodeling of the stress fibers is included in the model via changes in the volume fraction of polymerized actin in each fiber direction, which is governed by the stress that each fiber can exert

\[ \frac{d\varphi_{sf}^{i}}{dt} = \left( k_0 + k_1 \sigma_{max} f_i \right) \varphi_m - k_d \varphi_{sf}^{i} \]  

(9)

with \( k_0 \) and \( k_1 \) the constants describing stress fiber formation, and \( k_d \) the constant determining fiber dissociation. Finally, the total stress fiber stress is given by

\[ \sigma_{sf} = \frac{1}{N} \sum_{i=1}^{N} \varphi_{sf}^{i} \sigma_{sf}^{i} \mathbf{e}^i_{f} \mathbf{e}^i_{f} \]  

(10)

with \( \mathbf{e}^i_{f} \) the fiber orientation in the current configuration.

2.1.1. Analytical approximation of the stress fiber remodeling process

As shown in the previous section, stress fiber remodeling in the model of Obbink-Huizer et al. (2014) depends on the deformation state of the tissue, and direct numerical integration of Eq. (9) requires the application of the complete load history onto the tissue. To predict the tissue remodeling process in TEHVs due to in vivo loading conditions, it would therefore be necessary to include all the dynamic variations in mechanical stimuli that occur over the period of each heartbeat, which will lead to excessive computational costs when the remodeling process takes days to weeks. For this reason, we propose to use an analytical approximation of the stress

Fig. 1 - Overview of the different tissue components in the computational model (picture by Anthal Smits).
fiber remodeling process that approximates the volume fractions of the stress fibers for $t \to \infty$ for a given (periodic) mechanical state (Ristori et al., 2015). In this way, the periodic changes in mechanical stimuli that occur during the period of a heart beat can be accounted for in the approximation, and it is no longer necessary to actually apply the dynamic changes in mechanical stimuli in the model during most of the remodeling process.

As shown by Ristori et al. (2015), the stress fiber volume fractions in case of static mechanical loads evolve towards

$$
\psi_{sf, f}^{t} = \lim_{t \to \infty} \psi_{sf} = \frac{a_i}{\sum_{i=1}^{N} a_j + k_d} \phi_a
$$

with

$$
a_i = k_f + \frac{k_f' \sigma_{max}}{1 + 2/\sqrt{5}} \left(1 + \frac{k_b \sigma_{1}^2 + 2}{\sqrt{(k_b \sigma_{1}^2 + 2)^2 + 1}} \exp \left(-\left(\epsilon'/\epsilon_{0}\right)^2\right) \right)
$$

which is the exact solution of the system of ordinary differential equations given by Eq. (9), and we assume that each stress fiber evolves towards this preferred value $\psi_{sf, f}^{t}$. For periodic loads, which is the case for the in vivo conditions that TEHVs are exposed to, the stress fiber volume fraction in each direction will tend towards a function $\psi_{sf}^{t}$ with small oscillations with period $T$ around a constant value (Ristori et al., 2015). In this case, we can approximate the volume fraction for $t \to \infty$ with the mean value of this periodic function

$$
\psi_{sf, f}^{t} = \frac{1}{T} \int_{0}^{T} \psi_{sf}^{t} \, dt \approx \frac{\sum_{i=1}^{N} a_j + k_d}{a_i} \phi_a
$$

with

$$
\mathbf{a} = \frac{1}{T} \int_{0}^{T} \left[ k_f + \frac{k_f' \sigma_{max}}{1 + 2/\sqrt{5}} \left(1 + \frac{k_b \sigma_{1}^2 + 2}{\sqrt{(k_b \sigma_{1}^2 + 2)^2 + 1}} \exp \left(-\left(\epsilon'/\epsilon_{0}\right)^2\right) \right) \right] \, dt
$$

The evolution of the stress fiber volume fraction is then determined by the difference between the actual and preferred volume fraction, and the time constant of the remodeling process $t_{sf}^{f}$

$$
\frac{d\psi_{sf}^{f}}{dt} = \frac{1}{t_{sf}^{f}} \left(\psi_{sf, f}^{t} - \psi_{sf}^{t}\right)
$$

For more details regarding the derivation and quality of this approximation, the reader is referred to Ristori et al. (2015). By using this approximation (Eqs. (13)–(15)), we can include the dynamic load variations that occur during the period of a heart beat by means of Eq. (14), without the necessity of including the complete load history in the model.

### 2.2. Collagen fibers

The collagen fibers in the tissue are subject to cell-mediated contraction and collagen remodeling. Cells can contract collagen fibers (McLeod et al., 2013; Meshel et al., 2005) and thereby induce shrinkage (negative growth) of the tissue. Therefore, as in Loerakker et al. (2014), we split the total fiber stretch $\lambda_{sf}^{f}$ in each fiber direction into an elastic part $\lambda_{sf}^{e}$ and a growth part $\lambda_{sf}^{g}$, in analogy with the general approach to model tissue growth (Ambrosi et al., 2011; Menzel and Kuhl, 2012; Rodriguez et al., 1994).

$$
\lambda_{sf}^{f} = \lambda_{sf}^{e} \lambda_{sf}^{g}
$$

where only the elastic stretch leads to stress in the collagen fibers. The magnitude of this stress in each fiber direction is given by

$$
\sigma_{i}^{f} = \begin{cases} 
\nu_{i}^{f} \nu_{i}^{f} \left(1 - \frac{\nu_{i}^{f}}{\nu_{i}^{f} - 1}\right), & \lambda_{sf}^{e} \geq 1 \\
\nu_{i}^{f} \nu_{i}^{f} \left(1 - \frac{\nu_{i}^{f}}{\nu_{i}^{f} - 1}\right), & \lambda_{sf}^{e} < 1
\end{cases}
$$

The total stress in the collagen network then depends on the stress exerted by the fibers, the volume fraction $\phi_{sf}^{f}$ of collagen in each direction, and the fiber orientation in the current configuration

$$
\sigma_{i}^{f} = \sum_{j=1}^{N} \phi_{sf}^{f} \frac{d \lambda_{i}^{j}}{d t} \lambda_{i}^{j} \epsilon_{i}^{j} \epsilon_{i}^{j} \quad \text{with} \quad \sum_{j=1}^{N} \phi_{sf}^{f} = \phi_{sf}^{f}
$$

#### 2.2.1. Cell-mediated collagen contraction

As in Loerakker et al. (2014), we assume that cells contract the collagen fibers until there is a stress equilibrium between the stress fibers and collagen fibers. The preferred collagen stress is therefore given by

$$
\sigma_{sf}^{f} = \sigma_{sf}^{f}
$$

The preferred growth stretch $\lambda_{sp}^{f}$ can then be determined from $\sigma_{sf}^{f}$ and the current fiber stretch $\lambda_{sf}^{f}$. As the loads in the present study are periodic, $\lambda_{sf}^{f}$ and $\lambda_{sp}^{f}$ are both dependent on the time point in the load cycle. To account for this, we determined the value of $\lambda_{sp}^{f}$ for both the maximally and minimally loaded configuration, and used the average value as the preferred growth stretch $\lambda_{sp}^{f}$, with a restriction to values $\leq 1$ as we only considered collagen crimp. Finally, we used a first-order rate equation with time constant $t_{c}$ to describe the evolution of $\lambda_{sf}^{f}$

$$
\frac{d \lambda_{sf}^{f}}{dt} = \frac{1}{t_{c}} \left(\lambda_{sp}^{f} - \lambda_{sf}^{f}\right)
$$

#### 2.2.2. Collagen remodeling

For each fiber direction, the change in collagen volume fraction is determined by production and degradation

$$
\frac{d \phi_{sf}^{f}}{dt} = \frac{d \phi_{sf}^{f, prod}}{dt} - \frac{d \phi_{sf}^{f, deg}}{dt}
$$

In correspondence with many experimental observations (Ruberti and Hallab, 2005; Bhole et al., 2009; Wyatt et al., 2009), we assumed that strain protects collagen fibers from degradation and used a sigmoid function to describe the rate of degradation as a function of the elastic strain $\epsilon_{s} = 0.5(\lambda_{sf}^{e} - 1)$ in the collagen fibers (Heck et al., 2015; Loerakker et al., 2014; Wyatt et al., 2009)

$$
\frac{d \phi_{sf}^{f, deg}}{dt} = \left(\frac{D_{min} + D_{max} - D_{min}}{1 + 10^{2000(\epsilon_{trans} - \epsilon_{s})}}\right) \phi_{sf}^{f}
$$

where $D_{min}$ and $D_{max}$ determine the minimum and maximum degree of degradation, $\epsilon_{trans}$ is the transition strain, and $t_{sf}^{f}$ the time constant associated with collagen remodeling. Due to the presence of periodic loads, we used the average elastic fiber
strain in the load cycle to determine the rate of collagen degradation. Collagen production was assumed to be proportional to the alignment of the cells, and therefore the volume fraction of stress fibers (Loerakker et al., 2014; Wang et al., 2003)

\[
\frac{d\phi_{\text{cf,prod}}}{dt} = \frac{\phi_{\text{cf}}}{\sum_{i=1}^{N} \phi_{\text{cf,deg}}^{i}} \sum_{k=1}^{N} \frac{d\phi_{\text{cf,deg}}^{i}}{dt}
\]  

(23)

The production of collagen in each fiber direction was scaled with the total amount of degradation, as we assumed that the total volume fraction of collagen remains constant over time (Loerakker et al., 2014).

2.3. Isotropic tissue components

The isotropic part of the tissue (isotropic matrix constituents and passive contribution of the cells) was modeled as a compressible Neo-Hookean material

\[
\sigma_{\text{mc}} = \phi_{\text{mc}} \left( \frac{\ln(J)}{J} I + \frac{G}{J} \left( B - J^{-2/3} I \right) \right)
\]

(24)

with \( \phi_{\text{mc}} \) the volume fraction of the isotropic part, \( J = \det(F) \), \( B = F^T F \), and \( G \) and \( k \) the shear and compression modulus, respectively.

2.4. Simulations of tissue remodeling in TEHVs

2.4.1. Valve geometry

The leaflet geometry was modeled using the lower half of a spherical surface with the same radius as the valve itself, which was set to 13.5 mm. The center of this surface was located at the outer edge of the valve (radius 13.5 mm, with the center of the valve representing the origin of the coordinate system), and the section where \( r \leq 13.5 \text{ mm} \) was considered as the leaflet surface. Finally, the part of the leaflet that would cross the vertical plane that separates the leaflets was adjusted to follow this vertical plane, and as such represented the initial coaptation region (area of the leaflet that is in contact with the other leaflet(s) of the valve (Fig. 2a). The thickness of the leaflets was set to 0.5 mm. Due to symmetry, we only modeled one half of one leaflet and divided this into 163 quadratic brick elements with full integration (type C3D20), and included a vertical contact surface at the interface between the leaflets to simulate the contact between the different leaflets (Loerakker et al., 2013). For each element, the orthogonal vectors \( \mathbf{v}_1 \) and \( \mathbf{v}_2 \) were defined perpendicular to the outer normal of the element \( \mathbf{n} \), and in the circumferential and radial direction, respectively (Loerakker et al., 2013).

2.4.2. Boundary conditions

The outer edge of the valve was fixed to simulate the connection of the valve with its surroundings. Furthermore, normal displacements were suppressed at the symmetry edge of the leaflet. For the dynamic loading conditions that the valves are exposed to, we assumed that the pressure on the arterial side of the leaflet would be maximal during the diastolic phase, and equal to zero during the systolic phase. In addition, we assumed that the pressure would vary linearly in time between the minimum and maximum pressure on the leaflet during each heart beat. Since collagen contraction and degradation were assumed to depend only on the average strain in each heart beat, and the variation in strain and strain rate over the load cycle were indirectly included in the stress fiber remodeling algorithm via Eq. (14), it was not necessary to include all the dynamic variations in pressure occurring during the complete remodeling period. It appeared to be sufficient to apply the maximum pressure during most of the loading period to assess the temporal changes in the strains in the maximally loaded configuration, and only add a relatively small number of complete load cycles to update the strains in the minimally loaded configuration as well (which are unequal to zero due to the active cellular contraction) (Fig. 2b). A variation of the number of complete load cycles during the remodeling period (data not shown) revealed that including a complete load cycle every 8.4 h during a total remodeling period of 14 days was sufficient for obtaining stable results.

Using this loading profile, we investigated the remodeling process in TEHVs subjected to three different loading conditions. First of all, we simulated tissue remodeling in valves not subjected to any pressure at all, to verify the model results using experimental observations and to have a worst-case scenario of what may happen when the valve becomes insufficient, as this will lead to a tremendous drop in transvalvular pressure. Subsequently, we simulated the remodeling process in valves exposed to dynamic pulmonary and aortic pressure conditions, where the maximum pressure was set to 2 kPa and 10 kPa, respectively. The time period of each heart beat was set at 0.85 s, corresponding with a frequency of ~70 beats per minute.

Fig. 2 – (a) Geometry of the tissue-engineered heart valve (TEHV). The outer edge of the valve was fixed, and a pressure was applied to the arterial side of the valve. (b) Schematic representation of the complete loading history experienced by heart valves over time, and the actual load applied in the computational model.
2.4.3. Material parameters

For the stress fiber remodeling algorithm, we used the same parameter values as in Obbink-Huizer et al. (2014), which mostly resembles the myofibroblast phenotype (see Table 1). However, since we used an analytical approximation to calculate the preferred stress fiber volume fraction, we had to define a time constant for the evolution of the volume fractions (Eq. (15)). As several experimental observations have shown that stress fiber remodeling in response to cyclic stretching becomes stable within 30 min (Hayakawa et al., 2001; Hsu et al., 2010; Na et al., 2007), we set $\tau_{cf}$ equal to 5 min, to reach an equilibrium situation after a similar period of time.

The stiffness parameters $k_1$ and $k_2$ of the collagen fibers were chosen in accordance with Driessen et al. (2007), and an arbitrary large value was chosen for $k_r$ to prevent numerical difficulties during the iterative solution procedure associated with discontinuities in the derivative of the fiber stress (Loerakker et al., 2014). Regarding the collagen prestretch, Van Vlimmeren et al. (2012) reported that the retraction of engineered tissue strips becomes stable within a period of 6 h, which is due to both direct cell traction and collagen contraction. For this reason, we assumed the time constant of collagen contraction $\tau_i$ to be 1 h. Determining an accurate estimation for the time constant of collagen remodeling $\tau_{cf}$ was more difficult, as most experimental studies mainly focus on the changes in absolute collagen content, which are not taken into account in the current model. Since it is clear that collagen remodeling occurs over a larger time scale than the other two processes, we set $\tau_{cf}$ to 12 h, as a result of which collagen remodeling in the model stabilizes within a few days. For the remodeling algorithm itself, all parameters were derived from our previous study (Loerakker et al., 2014), except for the minimum degradation rate $D_{min}$, which was decreased from 0.5 to 0.1 to increase the effect of strain-dependent collagen degradation in the model. We considered this decrease to be reasonable given the large spread in the experimental data from which this parameter value was obtained (Wyatt et al., 2009).

For the isotropic component of the tissue, all parameters were derived from our previous study (Loerakker et al., 2014), except for the Young’s modulus which was increased from 10 kPa to 50 kPa to prevent numerical instabilities.

At the start of each simulation, the volume fractions of the stress fibers were set to 0 in all directions ($\phi_a = \phi_{fi}$), and the initial stress fiber stress was set to $\sigma_{max}$ due to the absence of strain. The collagen network was assumed to be initially isotropic ($\phi_{cf} = \phi_{g}$), and collagen contraction was assumed to be absent in all directions ($\lambda_i = 1$). For all the cases of tissue remodeling in TEHVs due to different loading conditions (no pressure, pulmonary pressure conditions, aortic pressure conditions), we simulated 14 days of remodeling after which the remodeling had reached a stable configuration. Since we were not only interested in the effect of the pressure magnitude on the remodeling process, but also in the effect of different cell phenotypes, we investigated the effect of changing the maximum cell contractility $\sigma_{max}$ from 100% of

<table>
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<th>Table 1 – Parameter values used in the simulations.</th>
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<td>Model component</td>
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$^a$ Corresponding with 100% cell contractility.
the original value to 50 and 10% of this for each loading condition as well.

3. Results

3.1. Comparison with experimental data

When no pressure was applied to the valve in combination with the original degree of cell contractility ($s_{\text{max}}$ equals the original value proposed by Obbink-Huizer et al. (2014) (Table 1)), the leaflets remodeled from an initially spherical surface into almost straight leaflets (Fig. 3a). A comparison of the model predictions for these conditions with experimental data of a valve created using ovine vascular-derived cells shows that the general morphological changes due to the remodeling process, e.g. straightening of the leaflets in combination with a triangular opening of the valve, were indeed captured by the computational model (Fig. 3b).

3.2. Leaflet retraction after tissue remodeling

The strains in the unloaded configurations of the valves after remodeling represent the amount of tissue compaction (Fig. 4). A comparison of the circumferential (Fig. 4a) and radial strains (Fig. 4b) shows that the retraction of the leaflets is mainly due to tissue compaction in the radial direction, as the radial strains were lower than the circumferential strains in all simulations. This may be attributed to the fact that there were more restrictions to compact in the circumferential direction compared to the radial direction due to the presence of the free edge. Without any pressure applied to the valve (left columns in Fig. 4a and b), a reduction of 50% in cell contractility hardly had any effect on the compaction of the tissue. Significant changes in the shape of the valve, e.g. less straightening of the leaflets and loss of the triangular opening of the valve, could only be obtained when the contractility was reduced to 10% of the original value. Remodeling at pulmonary pressure conditions (middle...
columns in Fig. 4a and b) resulted in less straightening of the valves compared to the simulations without any pressure application. Furthermore, the opening of the valve was less severe in all cases and decreased considerably when the cell contractility was decreased. At aortic pressure conditions (right columns in Fig. 4a and b), the amount of leaflet retraction was so low, that the original geometry of the valve (Fig. 2a) was preserved almost completely. For 50% and 10% cell contractility, tissue compaction was only present in the coaptation area, and therefore hardly influenced the spherical leaflet shape in the belly of the valve.

Without any pressure applied to the valve, a closer look at the strains in the center of the belly of the valve reveals that mainly the radial compaction was affected by a change in cell contractility (Fig. 5). In fact, a 90% reduction in cell contractility resulted in a 51% decrease in radial compaction, whereas the decrease in circumferential compaction was limited to only 29%. Conversely, for pulmonary pressure conditions, the decrease in compaction when the cell contractility was decreased with 50% was particularly evident in the circumferential direction (38% reduction in circumferential strain as opposed to 19% reduction in radial strain). Most importantly, a 90% decrease in cell contractility under these pressure conditions resulted in a complete resolution of the circumferential compaction, and only a small amount of radial compaction. Strikingly, an absence of circumferential compaction for all degrees of contractility and radial compaction at 50% and 10% contractility was observed when aortic pressure conditions were applied in the model, confirming that the pressure was sufficiently high to counteract most of the retraction.

3.3. Development of valvular insufficiency

Whether or not tissue remodeling could result in the development of valvular insufficiency was determined by investigating the loaded configuration of the valves after remodeling (Fig. 6). Clearly, the valves without any pressure applied to the arterial side were all insufficient, as there was no external

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**Fig. 5** - Circumferential (a) and radial (b) strain in the center of the belly of the valves in their unloaded configuration after remodeling due to different pressure conditions and intrinsic cell contractilities.

**Fig. 6** - Circumferential (a) and radial (b) strain distributions in the loaded configurations of the valves after remodeling due to different pressure conditions and intrinsic cell contractilities. **Note:** The results for 0 kPa are the same as in Fig. 4, as the maximum pressure is the same as the minimum pressure.
load to counteract the cell-mediated retraction of the leaflets (left columns in Fig. 6a and b). The simulations with pulmonary pressure conditions demonstrate that valve closure could not be obtained for all degrees of cell contractility (middle columns in Fig. 6a and b). In fact, valvular insufficiency could only be prevented when the contractility of the cells was reduced with 90%. Apparently, the pulmonary pressure was not able to significantly counteract tissue retraction by stretching the tissue, which is confirmed by the small differences in strain magnitude between the loaded (Fig. 6) and unloaded configuration (Fig. 4). In contrast, valve closure under aortic pressure conditions was evident for all degrees of cell contractility (right columns in Fig. 6a and b). The strains in the radial direction were relatively unaffected in these simulations, but the circumferential strains increased considerably upon pressure application.

To compare the presence of valvular insufficiency and the quality of valve closure between the different simulations, we calculated the valve opening area in the loaded configuration as a percentage of the total orifice area (Fig. 7a), and the coaptation area (calculated per half of the leaflet) during pressurization (Fig. 7b). Without any pressure applied to the valve, the valve opening was 46% at maximum cell contractility, and 24% when cell contractility was reduced with 90%. For pulmonary conditions, the valve opening dropped significantly to 9.8% in case of maximum cell contractility. At 50% cell contractility, the additional decrease in valve opening to 2.7% resulted in the presence of a limited coaptation area (7.6 mm²). Only in case of 10% contractility, valve closure was almost perfect under these loading conditions (valve opening of <0.2%), in combination with a solid coaptation area of 41 mm². At aortic pressure conditions, valve closure was present in all cases (valve opening was <0.4% in all cases), with a coaptation area ranging between 35 mm² at 100% cell contractility and 69 mm² at 10% contractility.

3.4. Tissue architecture after remodeling

Stress fiber and collagen remodeling in response to the applied pressure conditions transformed the initially absent stress fiber networks and isotropic collagen networks into networks with different dispersities and main directions of alignment, depending on the magnitude of the applied maximum pressure and the intrinsic cell contractility. Along the fixed edge of the valve, the stress fibers generally followed the curvature of this edge in all simulations (Fig. 8a), which can be explained by the fact that tissue deformation was restricted along this direction. Along the free edge, a similar pattern was observed for most cases as well. In the belly region, more distinct differences were present, which are shown in more detail in Fig. 8b. Without any pressure on the valve, stress fiber remodeling is completely determined by the static strains in the tissue, which consisted mainly of compression in the radial direction (Figs. 4 and 6). As a result, the stress fibers primarily aligned in the circumferential direction to avoid the compression in the radial direction. This circumferential alignment was most prominent when cell traction was maximal (left column of Fig. 8b), as the differences between the circumferential and radial strains increased with the degree of cell traction (Figs. 4 and 5).

For the dynamic pulmonary and aortic pressure conditions, the stress fibers not only responded to the static strains in the tissue, but also to the strain rates which were maximal in the circumferential direction. At pulmonary pressure conditions and maximum cell contractility, the static strains were still dominant in the deformation profile, as a result of which the stress fibers mainly aligned in the circumferential direction (middle column in Fig. 8b). In contrast, at 10% contractility the strain rates were the dominant factor due to the decrease in the absolute strain values. Consequently, the model predicted the development of a radially aligned stress fiber network under these circumstances. Finally, for the aortic pressure conditions, the strain rates had a large effect in all simulations due to the large temporal variations in pressure during the load cycle. In addition, the strain magnitudes in the radial direction were relatively small due to the limited amount of tissue compaction. As a result of these two phenomena, a radial orientation of the stress fiber network was predicted at 50% and 10% contractility (right column in Fig. 8b), which increased when cell contractility was decreased due to the decrease in compressive radial strains.
Similar to the stress fiber distributions, the collagen fibers were aligned along the free and fixed edge in most simulations (Fig. 9a), while the distribution of the collagen fibers in the center of the belly differed between the different simulations (Fig. 9b). Collagen remodeling is determined by oriented production in correspondence with the alignment of the stress fibers, and strain-dependent degradation which is determined by both cell-mediated collagen prestretch and the strains induced by external loads. Without any pressure applied to the valve or at pulmonary pressure conditions (left and middle column in Fig. 9b), the strains induced by the pressure were absent or small, and collagen remodeling was determined completely by the organization of the stress fiber network. Therefore, the predicted collagen distributions for these conditions are very similar to the stress fiber distributions reported in Fig. 8b. In contrast, the main directions of the collagen networks at aortic pressure conditions were perpendicular to the main directions of the stress fiber networks at 50% and 10% contractility (right column in Fig. 9b). This can be explained by the presence of large circumferential strains when the aortic diastolic pressure was applied to the valve, which protected the collagen fibers in this direction from degradation. Apparently, although collagen production occurred mainly in the radial direction due to the alignment of the stress fibers, the external load was dominant in the collagen remodeling process under these conditions.
4. Discussion

The goal of the present study was to simulate tissue remodeling in TEHVs, in order to predict the risk of valvular insufficiency as a result of leaflet retraction in valves implanted in the pulmonary and aortic position. Our computational framework was based on a recently developed constitutive model for cell-mediated compaction and collagen remodeling (Loerakker et al., 2014), which we applied to a heart valve geometry to predict the remodeling process of valves subjected to dynamic pulmonary and aortic pressure conditions, and no transvalvular pressure at all representing a worst-case scenario. In addition, we varied the intrinsic cell contractility in the model for all loading conditions to investigate its effect on the ultimate remodeling process.

The time scale of tissue remodeling is typically much larger than the time scale over which the loading conditions on the valve vary in time. For example, in the present study, we needed to simulate 14 days of remodeling to reach a steady state configuration for all valves, whereas the loads on the valve vary over a time period of less than a second. To simulate collagen remodeling, it is not necessary to include these dynamic variations in the load in a computational model, as good predictions can be obtained by assuming that this process only depends on the average or maximal load experienced by the tissue (Driessen et al., 2008; Kuhl and Holzapfel, 2007; Martufi and Gasser, 2012; Valentín et al., 2013). Similar assumptions can be made for the collagen prestretch that is induced by cell traction. However, the stress fiber remodeling process itself clearly depends on the variation in strains and strain rates during each heart beat (Tondon and Hsu, 2012; Wei et al., 2008), and they should therefore be included in the modeling framework to obtain accurate predictions for these loading conditions.

Including all these temporal variations of the pressure over a remodeling period of 14 days would not be possible due to excessive computational costs. Therefore, to be able to use our computational framework in combination with dynamic loading conditions, we derived an analytical approximation for predicting the final outcome of the stress fiber remodeling process based on the variation in strains and strain rates during each heart beat (Ristori et al., 2015). Using this approximation, these variations were indirectly included in the model via Eq. (14), and only a limited amount of complete load cycles was required to update the strains in the minimally loaded configuration (once every 8.4 h). For the remainder of the time period, it was sufficient to only apply the maximum pressure load to the valve. As demonstrated by Ristori et al. (2015), this approach results in a tremendous decrease in computational costs, particularly in case of complex deformations, while still giving accurate predictions of the final stress fiber organization. So in summary, to simulate the remodeling process in response to dynamic mechanical stimuli, we hypothesized that collagen prestretch and remodeling could be predicted based on the maximally and minimally loaded configuration, whereas the temporal variations in strain and strain rate required for predicting the stress fiber remodeling process were included in the computational framework mainly indirectly via the analytical approximation.

Next to predicting the effects of different pressure conditions, we used the model to investigate the effects of cell contractility on the remodeling process. To discriminate between different cell phenotypes, we varied the value of $\sigma_{\text{max}}$ in the stress fiber model, which represents the (maximal) intrinsic contractility of the cells. Since the original value corresponds to the very contractile myofibroblast phenotype, and most likely represents an upper limit to the degree of cell contractility of cells inside a heart valve, we only investigated the effects of decreasing $\sigma_{\text{max}}$ in the current study. Changing this parameter to study the effect of different cell types has been used in other studies as well (Ronan et al., 2013, 2014). It should, however, be noted that differences in cell behavior due to differences in cell phenotype or differences between a healthy and diseased state of the cell, may also be characterized by changing other parameters. Since the purpose of the current study was to investigate the effect of cell contractility itself, we chose $\sigma_{\text{max}}$ as our main parameter of interest. The effect of this parameter on the stress fiber remodeling process is rather large, as it influences both the contractility as well as the formation of the stress fibers. Changing other parameter values could, therefore, lead to different or more subtle effects, and could be the subject of future studies.

Before investigating the effects of the pulmonary and aortic loading conditions, we used our computational framework to predict remodeling of TEHVs not subjected to any pressure at all. The main reason for having a reference simulation without any external load applied to the valve, was to assess the effect of the external mechanical stimuli on the remodeling process by comparing the predictions with and without pressure application. Secondly, remodeling without any load on the valve represents a worst-case scenario of what could happen to the valve once it becomes insufficient, as the transvalvular pressure in the diastolic phase will drop tremendously when valve closure is incomplete. And finally, these simulations allowed us to compare our predictions with experimental observations as a verification of the model. The results of these simulations showed that the amount of tissue compaction can become very severe without any pressure applied to the valve, resulting in almost complete straightening of the leaflets in combination with a triangular opening of the valve. These findings were corroborated by previous experimental observations. Furthermore, due to the relatively unconstrained compaction in the radial direction, the stress fibers and collagen fibers in these simulations both modeled towards circumferentially aligned networks, where the degree of alignment increased with the contractility of the cells.

The simulations with dynamic pulmonary pressure conditions showed that there is a high risk of valvular insufficiency for these loading conditions. Due to the low pressure amplitude, valvular insufficiency was only absent when the contractility of the cells was reduced by 90%. Furthermore, it should be noted that, in case of incomplete valve closure, the current simulations only provide a conservative prediction of the degree of insufficiency, because the maximum pressure difference over the valve remained constant throughout the simulations. In reality, the diastolic pressure difference over
the valve will drop when valves become insufficient, which could induce a complete triangular opening of the valve as predicted in the simulations without any pressure application. Therefore, these results strongly suggest that utilizing or attracting contractile cells for TEHVs implanted in the pulmonary position can have a detrimental effect on the functionality of the valves, and may give an explanation for the often observed development of valvular insufficiency in preclinical studies when TEHVs were fabricated using vascular-derived cells and implanted in the pulmonary position (Flanagan et al., 2009; Hoerstrup et al., 2000; Schmidt et al., 2010). In addition, the changes in tissue architecture due to remodeling at pulmonary loading conditions appeared to be very sensitive to the contractility of the cells. With maximum contractility, the stress fibers and collagen fibers aligned primarily in the circumferential direction, which is indeed corresponding with the collagen organization in native heart valves (Martin and Sun, 2012; Sacks et al., 2009). However, a decrease in the contractility of the cells resulted in the development of radially aligned stress fiber and collagen networks, which is not mimicking the native situation and may have an adverse effect on the functionality of the valve. So, interestingly, our predictions suggest that both the development of valvular insufficiency and the evolving tissue architecture are very sensitive to the degree of cell contractility at pulmonary loading conditions, where the optimal results for both factors unfortunately require opposite degrees of cell contractility.

For the aortic loading conditions, fortunately, no presence of valvular insufficiency was predicted for all the investigated degrees of cell contractility, which was due to the high pressure amplitude that successfully prevented the development of leaflet retraction. In addition, in all cases a circumferential alignment of the collagen network was predicted, corresponding to the native tissue architecture. Hence, functional adaptation of TEHVs towards a native tissue architecture without the development of valvular insufficiency may be more natural for valves implanted in the aortic position compared to the pulmonary position. Since there were only small differences in the final outcome of the remodeling process between the different simulations, our predictions suggest that the remodeling process at these loading conditions is dominated by the mechanical stimuli induced by the aortic pressure conditions, and cell contractility and alignment are less relevant. This is confirmed by the fact that the stress fibers in the model oriented towards the radial direction at 50% and 10% contractility, whereas the collagen fibers were primarily oriented in the circumferential direction. Collagen production was assumed to depend on the stress fiber organization, and collagen degradation depended on the elastic strain. Therefore, the only explanation for the fact that these two fiber networks aligned into perpendicular directions can be that the external loading conditions dominated the remodeling process. This result can be explained by the fact that most of the collagen fibers in the circumferential direction were protected from degradation due to the presence of large circumferential stretches. Apparently, only a limited degree of stress fiber alignment was already sufficient for obtaining and preserving a high collagen concentration in this direction.

Of course, the computational framework that we used to predict tissue remodeling and the development of valvular insufficiency has some limitations that need to be addressed. First of all, although the analytical approximation was crucial for enabling these simulations, there is a small inaccuracy in the prediction of the stress fiber organization in the tissue compared to using direct numerical integration of the differential equations. However, this error was still small compared to the difference between the model predictions and experimental observations (Ristori et al., 2015), and is therefore not considered to be significant. In addition, we had to increase the Young’s modulus in the present study to prevent numerical instabilities. A comparison of simulations at low pressure conditions that allowed for using the original value (data not shown) indicates that this increase in Young’s modulus slightly reduces the radial compaction and thereby leads to an underestimation of the circumferential alignment of the collagen fibers. Nevertheless, the general conclusions based on valve closure and main direction of collagen alignment still hold. Furthermore, it is unknown which of the two values is more realistic, and the answer to this question may depend to a large extent on the adopted tissue engineering strategy.

The stress fiber remodeling algorithm has the disadvantage that it depends on the global strains. Although this is not a limitation for studying the final outcome and consequences of short-term tissue remodeling, which was the goal of the present study, it does limit our understanding of the stress fiber remodeling process as the general cell behavior is only phenomenologically captured by the model. Moreover, it is unlikely that cells will continue to respond to global strains in the long term, as the reference configuration of the tissue may evolve over time due to tissue growth. So to study long-term growth of TEHVs, another approach should be adopted to predict stress fiber remodeling. Related to this, uncoupling the amounts of collagen production and degradation in the model should also be introduced when studying tissue growth, to allow for a net change in collagen content and volume fraction. Finally, a potentially important limitation of the current model is the absence of contact guidance, which means that the organization of the cells and stress fibers is dictated by the topology of its surroundings such as the scaffold (de Jonge et al., 2014; Niklason et al., 2010). Including this phenomenon would have probably prevented the radial alignment of the stress fibers in the model when the valves were exposed to the aortic pressure conditions, and it would allow for predicting the effects of the scaffold design on the overall remodeling process. Ultimately, overcoming these limitations will allow for studying long-term tissue growth and adaptation of TEHVs, and contribute to defining a rational scaffold design that will guide the growth and remodeling process towards functional tissue regeneration.

5. Conclusions and recommendations

One of the most critical problems in heart valve tissue engineering is the progressive development of valvular insufficiency due to leaflet retraction. Solving this problem first of all requires a detailed understanding of the remodeling
processes responsible for leaflet retraction, which can subsequently be employed to improve tissue engineering strategies (e.g., improvements in scaffold design). In the present study, we adopted a computational approach to investigate (1) whether there may be differences in tissue remodeling and therefore the development of valvular insufficiency between valves implanted in the pulmonary and aortic position, and (2) how important the intrinsic cell contractility is for the final outcome of the remodeling process. Our predictions suggest that there may indeed be major differences in the outcome of tissue remodeling at pulmonary and aortic pressure conditions, and that the degree of cell contractility can particularly play an important role in the remodeling process at pulmonary pressure conditions.

For valves implanted in the aortic position, the model predictions indicate that valvular insufficiency is not likely to occur as the blood pressure is high enough to prevent the development of leaflet retraction. In addition to this, the collagen network was always predicted to remodel towards a circumferentially aligned network, which is corresponding to the native situation. In contrast, for valves implanted in the pulmonary position, our model predicted that there is a high risk for the development of valvular insufficiency, unless the cell contractility is very low. Conversely, the development of a circumferential collagen network was only predicted at these pressure conditions when cell contractility was high. Overall, these results suggest that tissue remodeling at aortic pressure conditions is much more stable and favorable compared to tissue remodeling at pulmonary pressure conditions. For TEHVs to be implanted in the aortic position, these results imply that the initial scaffold design may be less important, since the external mechanical loads will probably guide the remodeling process towards functional tissue regeneration. For TEHVs to be implanted in the pulmonary position, however, it may be crucial to (1) utilize or attract cell phenotypes with a low intrinsic contractility as this may be the only solution for preventing the development of valvular insufficiency at these pressure conditions, and (2) use aligned scaffolds that can prevent the radial alignment and induce a circumferential alignment of the collagen fibers via the mechanism of contact guidance.

Acknowledgments

We gratefully acknowledge the funding from the European Union's Seventh Framework Programme (FP7/2007-2013) Grant agreement no. 242008), and the Netherlands Cardio-Vascular Research Initiative (CVON 2012-01): the Dutch Heart Foundation, Dutch Federation of University Medical Centers, the Netherlands Organization for Health Research and Development and the Royal Netherlands Academy of Sciences. In addition, we thank Bart Sanders (Department of Biomedical Engineering, Eindhoven University of Technology) for providing the image of a heart valve fabricated using ovine vascular-derived cells.

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