Predicting and understanding collagen remodeling in human native heart valves during early development

Citation for published version (APA):

Ristori, T., Bouten, C. V. C., Baaijens, F. P. T., & Loerakker, S. (2018). Predicting and understanding collagen remodeling in human native heart valves during early development. *Acta Biomaterialia*, 80, 203-216.
<https://doi.org/10.1016/j.actbio.2018.08.040>

DOI:

[10.1016/j.actbio.2018.08.040](https://doi.org/10.1016/j.actbio.2018.08.040)

Document status and date:

Published: 15/10/2018

Document Version:

Author's version before peer-review

Please check the document version of this publication:

- A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
- The final author version and the galley proof are versions of the publication after peer review.
- The final published version features the final layout of the paper including the volume, issue and page numbers.

[Link to publication](#)

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal.

If the publication is distributed under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license above, please follow below link for the End User Agreement:

www.tue.nl/taverne

Take down policy

If you believe that this document breaches copyright please contact us at:

openaccess@tue.nl

providing details and we will investigate your claim.

Predicting and understanding collagen remodeling in human native heart valves during early development

T. Ristori^{a,b}, C.V.C. Bouten^{a,b}, F.P.T. Baaijens^{a,b}, S. Loerakker^{a,b,*}

^a*Department of Biomedical Engineering, Eindhoven University of Technology, PO Box 513, 5600 MB Eindhoven, The Netherlands*

^b*Institute for Complex Molecular Systems, Eindhoven University of Technology, PO Box 513, 5600 MB Eindhoven, The Netherlands*

Abstract

The hemodynamic functionality of heart valves strongly depends on the distribution of collagen fibers, which are their main load-bearing constituents. It is known that collagen networks remodel in response to mechanical stimuli. Yet, the complex interplay between external load and collagen remodeling is poorly understood. In this study, we adopted a computational approach to simulate collagen remodeling occurring in native fetal and pediatric heart valves. The computational model accounted for several biological phenomena: cellular (re)orientation in response to both mechanical stimuli and topographical cues provided by collagen fibers; collagen deposition and traction forces along the main cellular direction; collagen degradation decreasing with stretch; and cell-mediated collagen prestretch. Importantly, the computational results were well in agreement with previous experimental data for all simulated heart valves. Simulations performed by varying some of the computational parameters suggest that cellular (re)orientation in response to mechanical stimuli is a fundamental mechanism for the emergence of the circumferential collagen alignment usually observed in native heart valves. On the other hand, the tendency of cells to coalign with collagen fibers is essential to maintain and reinforce that circumferential alignment during development.

Keywords: Computational modeling, Collagen remodeling, Native heart valve, Contact guidance, Mechanical stimuli

1. Introduction

The functionality of semilunar heart valves strongly depends on the orientation of collagen fibers, the main load-bearing constituents of these tissues. Collagen fibers in native semilunar heart valve leaflets are mainly circumferentially aligned [1, 2, 3]. This distribution causes the circumferential direction of

*Corresponding author

Email address: S.Loerakker@tue.nl (S. Loerakker)

heart valve leaflets to be stiffer than the radial one [1, 3]. In fact, due to this mechanical anisotropy, leaflets can restrict circumferential deformations while simultaneously enlarging in the radial direction, thereby favoring a proper heart valve closure [4, 5]. Identifying the cues and processes determining the alignment of collagen fibers is therefore desired. This is particularly relevant for the field of heart valve tissue engineering, that aims at the development of functional living heart valve replacements with the potential to grow and remodel with patients. Although tissue-engineered heart valves with a proper functionality for up to one year can be developed with current techniques [6, 7, 8, 9], further studies are needed to ensure that these tissues are provided with the correct stimuli for a physiological collagen remodeling, as this is necessary for a correct long-term functionality.

The evolution of collagen orientation in heart valves over a long-term period can be analyzed by investigating native heart valves. Concretely, it has been observed that the collagen fiber anisotropy increases with age in native aortic valves, in line with the magnitude of blood pressure [2, 3]. The increase in alignment is smaller in pulmonary valves, which correlates with the lower blood pressure [3]. Yet, the complex interplay between external load and collagen remodeling is poorly understood.

In this context, due to their versatility and predictive potential, computational models can be employed to simulate collagen remodeling and test hypotheses. Early computational models have confirmed the importance of mechanical stimuli for the collagen remodeling occurring in heart valves [10, 11]. However, the assumptions at the basis of these early computational models were mainly phenomenological. More recently, we have proposed a computational approach, based on biophysical concepts, to study the short-term heart valve tissue remodeling [12]. Motivated by previous experimental studies, the computational model that was adopted took into account several biological phenomena: the reorientation of cells in response to mechanical stimuli [13, 14, 15]; the collagen deposition [16] and traction forces [17] exerted by cells in their main direction; the cell-mediated collagen prestretch [18]; and the degradation of collagen decreasing with stretch [19]. With those main computational features, the short-term tissue remodeling occurring in tissue-engineered heart valves was simulated in that study. However, this previous computational approach did not account for the effects that collagen fibers have on the (re)orientation of cells.

Cells reorient not only in response to mechanical, but also topographical stimuli, such as the ones provided by collagen fibers. Both in two- and three-dimensional environments, it has been observed that cells tend to align with collagen fibers [20, 21], a phenomenon that has often been referred to as contact guidance. In a previous study, we have proposed a computational model to predict the (re)orientation of cells in response to both mechanical stimuli and contact guidance [22]. This was achieved by extending the computational model that was previously proposed by Obbink-Huizer et al. [23] for the prediction of cellular (re)orientation in response to mechanical stimuli. In the present study, we adopt a similar approach to extend the computational framework presented in Loerakker et al. [12], with the aim of accounting for the effects that con-

tact guidance has on the (re)orientation of cells in heart valves. The extended computational method is used to simulate and understand the early collagen fiber remodeling in native heart valves. In particular, native fetal and pediatric heart valves were simulated, and the computational results were compared with experimental data from the literature [3]. Finally, the relative importance of the (re)orientation of cells in response to mechanical stimuli and contact guidance was tested by varying the parameters associated with these two biological phenomena.

2. Methods

In this study, a computational framework for the simulation of short-term tissue remodeling [12] was extended to understand long-term collagen remodeling of native heart valves. This adaptation was realized by including the effects of topographical stimuli on cellular (re)orientation, similar to Ristori et al. [22]. In addition, changes with respect to the valve geometry, loading profile, material properties of the collagen fiber network, and cellular density and contractility were necessary to account for the differences between aortic and pulmonary valves, and the changes of these parameters with age. These changes were motivated by previous experimental studies [2, 3]. In what follows, we briefly describe the previous computational approach and highlight the changes proposed to simulate tissue remodeling in native heart valves. For a complete discussion and motivation of the several features of the modeling framework, we refer the reader to previous studies [24, 23, 12, 25, 22].

2.1. Modeling of tissue remodeling

Similar to previous studies [24, 12], the collagenous tissue of heart valves was modeled as a mixture of cells, collagen fibers, and other isotropic matrix constituents, where the total Cauchy stress $\boldsymbol{\sigma}$ equals

$$\boldsymbol{\sigma} = \boldsymbol{\sigma}_{sf} + \boldsymbol{\sigma}_{cf} + \boldsymbol{\sigma}_{mc}, \quad (1)$$

with $\boldsymbol{\sigma}_{sf}$ the active cellular stress exerted via stress fibers, $\boldsymbol{\sigma}_{cf}$ represents the collagen fiber stress, and $\boldsymbol{\sigma}_{mc}$ considers the remaining isotropic matrix components. Collagen and stress fibers were assumed to be distributed only within the plane of the tissue. The fiber distributions were approximated by considering a finite number of directions $N \in \mathbb{N}$, with a resolution of 6° . In particular, given two orthogonal vectors $\vec{v}_1$ and $\vec{v}_2$, the i -th direction in the original configuration was characterized by the unit vector

$$\vec{e}_{f0}^i = \cos(\gamma^i) \vec{v}_1 + \sin(\gamma^i) \vec{v}_2, \quad (2)$$

with γ^i the angle between this vector and $\vec{v}_1$. $\vec{v}_1$ was chosen to correspond to the circumferential directions in heart valves. $\vec{v}_2$ was then determined as the unit vector orthogonal to both $\vec{v}_1$ and the vector $\vec{n}$, describing the normal of the surface of the tissue to model.

2.1.1. Stress fiber stress and remodeling

90 The total stress fiber stress was modeled as

$$\boldsymbol{\sigma}_{sf} = \frac{1}{N} \sum_{i=1}^N \varphi_{sf}^i \sigma_{sf}^i \vec{e}_f^i \vec{e}_f^i, \quad (3)$$

where $\vec{e}_f^i$ represents the unit vector in the current configuration, while φ_{sf}^i and σ_{sf}^i are, respectively, the stress fiber volume fraction and exerted stress in the direction i . This latter term was assumed to depend on the strain ε^i and strain rate $\dot{\varepsilon}^i$ experienced by stress fibers in the i -th direction, such that

$$\sigma_{sf}^i = \sigma_{max} f_{\varepsilon}(\varepsilon^i) f_{\dot{\varepsilon}}(\dot{\varepsilon}^i), \quad (4)$$

95 where σ_{max} quantifies the maximum cell traction. In agreement with the Green-Lagrange strain definition, the strain was calculated from the global stretch as $\varepsilon^i = 0.5 \left((\lambda_f^i)^2 - 1 \right)$, where λ_f^i is the global stretch along direction i . With $\mathbf{F}$ indicating the deformation gradient tensor, λ_f^i was computed as $\lambda_f^i = \sqrt{\vec{e}_{f0}^i \cdot \mathbf{F}^T \cdot \mathbf{F} \cdot \vec{e}_{f0}^i}$. The functions $f_{\varepsilon}(\varepsilon^i)$ and $f_{\dot{\varepsilon}}(\dot{\varepsilon}^i)$ were derived from
100 previous studies [26, 27, 23] and consider the effects of strain and strain rate on the stress fiber stress. In particular,

$$f_{\dot{\varepsilon}}(\dot{\varepsilon}^i) = \frac{1}{1 + 2/\sqrt{5}} \left(1 + \frac{k_v \dot{\varepsilon}^i + 2}{\sqrt{(k_v \dot{\varepsilon}^i + 2)^2 + 1}} \right), \quad (5)$$

where k_v characterizes the decrease of σ_{sf}^i due to stress fiber shortening. The function $f_{\varepsilon}(\varepsilon^i)$ is represented as a summation of active and passive components:

$$f_{\varepsilon}(\varepsilon^i) = f_{\varepsilon,a}(\varepsilon^i) + f_{\varepsilon,p}(\varepsilon^i), \quad (6)$$

where

$$f_{\varepsilon,a}(\varepsilon^i) = \exp \left(- (\varepsilon^i / \varepsilon_0)^2 \right), \quad (7)$$

and

$$f_{\varepsilon,p}(\varepsilon^i) = \begin{cases} 0, & \text{if } \varepsilon^i < 0, \\ (\varepsilon^i / \varepsilon_1)^2, & \text{if } \varepsilon^i \geq 0. \end{cases} \quad (8)$$

Here, ε_0 describes the decrease of σ_{sf}^i for non-zero values of ε^i , while ε_1 char-
105 acterizes the rate of increase of σ_{sf}^i due to stress fiber extension.

In the previous computational approach [12], the remodeling of stress fibers solely depended on the strain and strain rate experienced by stress fibers. In Ristori et al. [22] we extended the evolution law for stress fiber remodeling by taking the phenomenon of contact guidance into account. In the present
110 study we adopt a similar approach, by describing the evolution of the stress fiber volume fraction φ_{sf}^i in direction i as

$$\frac{d\varphi_{sf}^i}{dt} = \left(k_0^f + k_1^f \sigma_{max} f_{\varepsilon,a}(\varepsilon^i) f_{\dot{\varepsilon}}(\dot{\varepsilon}^i) + f_{cg}(\varphi_{cf}^i) \right) \varphi_m - k_d \varphi_{sf}^i. \quad (9)$$

Here, k_0^f and k_d quantify, respectively, the basal stress fiber formation and dissociation. The effects that mechanical stimuli have on stress fiber remodeling, which are modeled with the functions $f_{\varepsilon,a}$ and $f_{\dot{\varepsilon}}$ introduced with Eqs. (5) and (7), scale with the maximum cell traction σ_{max} and the parameter k_1^f . The term φ_m represents the monomeric actin volume fraction, which is related to the stress fiber volume fractions φ_{sf}^i and the total actin volume fraction ϕ_a via a conservation law for actin monomers:

$$\phi_a = \varphi_m + \frac{1}{N} \sum_{i=1}^N \varphi_{sf}^i. \quad (10)$$

Finally, $f_{cg}(\varphi_{cf})$ is a monotonically increasing function that describes the effects that the volume fraction of collagen fibers along direction i has on cellular (re)orientation. This term was introduced in Ristori et al. [22] to model contact guidance. In that study, a three-parameter sigmoid function was proposed for f_{cg} . To simplify our modeling framework and reduce the number of parameters, in this work we modeled contact guidance as linearly increasing with φ_{cf}^i , with a proportionality constant g_{cg} :

$$f_{cg}(\varphi_{cf}^i) = g_{cg} \varphi_{cf}^i. \quad (11)$$

2.1.2. Collagen fiber stress and remodeling

The total collagen fiber stress σ_{cf} was defined as

$$\sigma_{cf} = \sum_{i=1}^N \varphi_{cf}^i \sigma_{cf}^i \vec{e}_f^i \vec{e}_f^i, \quad (12)$$

where σ_{cf}^i is the collagen fiber stress and φ_{cf}^i is the collagen volume fraction in direction i . This latter quantity is related to the total collagen volume fraction ϕ_{cf} by the conservation law

$$\phi_{cf} = \sum_{i=1}^N \varphi_{cf}^i. \quad (13)$$

The magnitude of collagen fiber stress was assumed to depend on the elastic stretch experienced by collagen fibers, according to the exponential law introduced by Driessen et al. [28]:

$$\sigma_{cf}^i = \begin{cases} \frac{k_1 k_2}{k_3} (\exp(k_3((\lambda_e^i)^2 - 1)) - 1), & \text{if } \lambda_e^i < 1, \\ k_1 (\lambda_e^i)^2 (\exp(k_2((\lambda_e^i)^2 - 1)) - 1), & \text{if } \lambda_e^i \geq 1. \end{cases} \quad (14)$$

In this equation, k_1 and k_2 are material parameters that describe the increase of collagen fiber stress in response to extension. The stress in response to compression, dependent on $k_3 \gg 0$, was introduced in Loerakker et al. [24] to improve numerical convergence without affecting the quality of the results. Note that the magnitude of collagen fiber stress is not directly dependent on the global

stretch λ_f^i , but it depends on the elastic part of the global stretch, indicated
 140 with λ_e^i , which is obtained from

$$\lambda_f^i = \lambda_e^i \lambda_g^i. \quad (15)$$

This partition of the global stretch λ_f^i into an elastic part λ_e^i and a growth part
 λ_g^i was proposed by Loerakker et al. [24] to model the cell-mediated collagen
 fiber prestretch. The magnitude of λ_g^i was identified by assuming that cells
 contract collagen fibers to a preferred degree, providing mechanical equilibrium
 145 between collagen and stress fiber stress, such that

$$\sigma_{cf,p}^i = \sigma_{sf}^i, \quad (16)$$

where $\sigma_{cf,p}^i$ is the preferred collagen fiber stress. From this preferred stress, a
 corresponding elastic stretch $\lambda_{e,p}^i$ can be calculated via Eq. (14). In turn, given
 the global stretch λ_f^i , the value of $\lambda_{e,p}^i$ leads to a preferred magnitude of λ_g^i via

$$\lambda_{g,p}^i = \lambda_f^i / \lambda_{e,p}^i. \quad (17)$$

Only collagen crimp was considered, such that $\lambda_{g,p}^i \leq 1$. Finally, it was assumed
 150 that cells contract collagen fibers such that λ_g^i tends to the preferred $\lambda_{g,p}^i$ with
 a rate characterized by a time constant τ_λ :

$$\frac{d\lambda_g^i}{dt} = \frac{1}{\tau_\lambda} (\lambda_{g,p}^i - \lambda_g^i). \quad (18)$$

Similar to stress fibers, also the remodeling of collagen fibers was described with
 a first-order ordinary differential equation:

$$\frac{d\varphi_{cf}^i}{dt} = \frac{\varphi_{sf}^i}{\phi_a - \varphi_m} \sum_{j=1}^N \left[f_{deg}(\varepsilon_e^i, \varphi_{cf}^j) \right] - f_{deg}(\varepsilon_e^i, \varphi_{cf}^i), \quad (19)$$

where the first term in the right-hand side models the deposition of collagen
 155 performed by cells in the stress fiber directions, motivated by experimental
 studies [16]. The second term describes the collagen fiber degradation, which
 decreases with increasing elastic strain according to a monotonic decreasing
 sigmoid function [19, 29]:

$$f_{deg}(\varepsilon_e^i, \varphi_{cf}^i) = \left(D_{min} + \frac{D_{max} - D_{min}}{1 + 10^{2.5(\varepsilon_e^i/\varepsilon_{trans} - 1)}} \right) \frac{\varphi_{cf}^i}{\tau_{cf}}. \quad (20)$$

Here, D_{min} and D_{max} are the minimum and maximum collagen fiber degra-
 160 dation fraction, respectively. Similar to the strain ε^i , the elastic strain ε_e^i is
 defined as $\varepsilon_e^i = 0.5((\lambda_e^i)^2 - 1)$, while τ_{cf} is a time constant characterizing the
 rate of collagen remodeling. Finally, the term ε_{trans} is the transition strain,
 which corresponds to the inflection point that the function $f_{deg}(\varepsilon_e^i, \varphi_{cf}^i)$ has
 with respect to ε_e^i .

Table 1: Parameters varying across the valves. Only the percentage of the default values of σ_{max} and g_{cg} are reported because these default values are identified with the results of the simulations of fetal and pediatric heart valves, respectively.

Valve	F 19w	A 2y	P 2y	A 5y	P 5y	References
Radius (mm)	1.5	5.5	6.5	7.0	9.0	[3]
Thickness (mm)	0.21	0.68	0.52	0.68	0.60	[3]
Pressure (kPa)	1.6	5.6	1.1	7.1	1.1	[3]
Heart rate (bpm)	155	113	113	98	98	[3, 30, 31, 32]
G (kPa)	11.1	11.4	5.0	53.4	12.8	[3]
k_1 (kPa)	8.9	0.1	0.55	0.1	0.48	[3]
k_2 (-)	0.92	8.93	4.82	8.77	3.52	[3]
ε_{trans} (-)	0.2	0.32	0.27	0.32	0.25	-
ϕ_a (-)	0.050	0.033	0.033	0.023	0.023	[2]
σ_{max} (kPa)	100%	42%	22%	22%	22%	[2]
g_{cg} (s ⁻¹)	0	100%	100%	100%	100%	[2]

Observe that the degradation law introduced in Eq. (20) is slightly different than the one adopted in our previous computational approach [12]. In that case, the transition strain ε_{trans} was identified by hypothesizing that the collagen degradation is at the minimum level when $\sigma_{cf}^i \geq \sigma_{max}$. This assumption was not maintained in the present work. One of the goals of the current study was to simulate tissue remodeling in developing native heart valves. For such tissues, Oomen et al. [3] observed large variations for the material parameters relative to the collagen fibers k_1 and k_2 , across the heart valves. These two parameters are strictly related to σ_{cf}^i , as shown in Eq. (14). Thus, their large variations would entail large fluctuations across valves also of ε_{trans} , if the previous assumption was considered, which was judged unphysiological. In the present study, therefore, the transitions strains were determined by assuming that they correspond to the level of stretch at which there is the transition between the first and second curve of the bilinear fit of the stress-stretch curve of collagen fibers (Eq. (14)). The degradation law in Eq. (20) was slightly modified from the one adopted in previous studies, to take this change of hypothesis into account. The values of the transition strain for the different native heart valves simulated in this study are reported in Table 1.

2.1.3. Stress of isotropic tissue components

The contribution to the stress by the isotropic tissue components, such as proteoglycans and cellular passive constituents, was captured with a compressible Neo-Hookean material law:

$$\boldsymbol{\sigma}_{mc} = \phi_{mc} \left(\kappa \frac{\ln(J)}{J} \mathbf{I} + \frac{G}{J} (\mathbf{B} - J^{2/3} \mathbf{I}) \right), \quad (21)$$

where ϕ_{mc} is the volume fraction of these isotropic components, while κ and G are the compression and shear moduli, respectively. Given the shear modulus G ,

the compression modulus was computed as $\kappa = \frac{2G(1+\nu)}{3(1-2\nu)}$, where ν is the Poisson's ratio. Finally, $J = \det(\mathbf{F})$ and $\mathbf{B} = \mathbf{F} \cdot \mathbf{F}^T$.

2.2. Numerical implementation

2.2.1. Valve geometry

The valve geometry was defined as in Loerakker et al. [12], with changes of radius and thickness to take experimentally observed geometries into account [3]. The valve geometry was derived from three spheres and a circle having the same radius of the valve to model. The spheres touch in a single point, corresponding to the center of the circle, while the sphere centers lie on the circle and, thus, on a single plane. Three planes are then constructed, corresponding to the ones identified by the intersections between pairs of spheres. The parts of the spheres which are inside one of the other spheres, or outside the cylinder identified by the circle, were removed. The valve surface is then extracted by unifying the surfaces of the planes and spheres that are below the plane identified by the circle. The surface of each leaflet of the valve corresponds to the surface extracted from one sphere and two planes. Each leaflet can then be obtained by extruding this surface, taking the thickness of the valve to model into account. In contrast to our previous study where valve radius and thickness were assigned considering typical values for tissue-engineered heart valves, here we incorporated the values measured by Oomen et al. [3] for native heart valves (Table 1).

2.2.2. Boundary conditions

Due to symmetry, only one half of one leaflet was modeled. The half leaflet was discretized into 79 quadratic brick elements with full integration (C3D20 elements). The nodes at the outer edge of the valve were fixed, assuming the deformations of the aortic wall to be negligible. Due to symmetry, the normal displacements of the nodes at the symmetry edge were assumed to be zero. Pressure was then applied on the arterial side of the valve, by assuming the pressure difference over the valve to be zero during systolic phase, maximal during diastolic phase, and to vary linearly in between. The magnitude of the hemodynamic pressure and heart rate were chosen based on previous studies [3, 30, 31, 32], and are reported in Table 1.

Directly applying all load cycles would lead to excessive computational costs to simulate years of tissue remodeling. Therefore, as in our previous computational approach [12], approximations were considered. First of all, for the major part of the simulation, the maximum pressure was applied on the valve to follow the evolution of the strains in the configuration corresponding to the diastolic phase. Only a finite number of unloading cycles were considered to update the strains present in the systolic phase. The influence that the dynamic nature of the loading cycles have on the tissue remodeling were then considered using the following numerical approximations.

2.2.3. Numerical approximations

Stress fiber remodeling, as modeled in 2.1.1, depends on both the strain and strain rate experienced by stress fibers. Considering the dynamic nature of

the loading conditions of heart valves is therefore essential to predict a correct stress fiber distribution. This was achieved via the computational approach that we previously introduced in Ristori et al. [25]. In particular, motivated by an analytical approximation of the solution of Eq. (9), we assumed that each stress fiber volume fraction φ_{sf}^i tends to a preferred value

$$\varphi_{sf,p}^i = \frac{\bar{a}_i}{\sum_{j=1}^N \bar{a}_j / N + k_d} \phi_a, \quad (22)$$

where

$$\bar{a}_i = \frac{1}{T} \int_0^T \left[k_0^f + k_1^f \sigma_{max} f_{\varepsilon,a}(\varepsilon^i(t)) f_{\dot{\varepsilon}}(\dot{\varepsilon}^i(t)) + f_{cg}(\varphi_{cg}^i) \right] dt, \quad (23)$$

with T the period of the heart beat. The integrand in Eq. (23) was determined by assuming that $\varepsilon^i(t=0)$ and $\varepsilon^i(t=T)$ are both equal to the value of ε^i computed during the systolic phase, $\varepsilon^i(t=T/2)$ is equal to the value of ε^i computed during the diastolic phase, and by assuming that the values in between can be found by linear interpolation. Finally, it was assumed that the stress fiber volume fraction φ_{sf}^i tends towards the preferred value $\varphi_{sf,p}^i$ with a rate characterized by a time constant τ_{sf} :

$$\frac{d\varphi_{sf}^i}{dt} = \frac{1}{\tau_{sf}} (\varphi_{sf,p}^i - \varphi_{sf}^i). \quad (24)$$

For more details, we refer the reader to previous studies [12, 25].

For the collagen fiber prestretch, a similar approach was adopted: the preferred value of growth stretch $\lambda_{g,p}^i$, defined in Eq. (17), was calculated as the average between the preferred growth stretch calculated for the strains present at the systolic phase and at the diastolic phase.

Finally, the effects of elastic strains on collagen remodeling, which are present in Eq. (20), were taken into account by assuming that collagen degradation depends on the average between the elastic strains present during the systolic and diastolic phases.

2.2.4. Initial conditions

To initiate the simulations, initial conditions must be set for the collagen fiber volume fractions φ_{cf}^i , the stress fiber volume fractions φ_{sf}^i and the growth stretches λ_g^i . Such as in Obbink-Huizer et al. [23], stress fibers were assumed to be initially depolymerized in each heart valve, such that $\varphi_{sf}^i = 0$ for all $i = 1, \dots, N$ and $\varphi_m = \phi_a$. Moreover, the collagen fiber prestretch was assumed to be initially absent ($\lambda_g^i = 1$).

Due to the inclusion of the effects of contact guidance, the final collagen fiber distribution significantly depended on the initial one. Similar to previous studies [24, 12], the initial distribution for the simulation of the fetal heart valve was chosen isotropic, such that $\varphi_{cf}^i = \phi_{cf}/N$ for all $i = 1, \dots, N$. For the simulation of the remaining heart valves, the initial collagen distribution was assumed to

be equal to the final distribution predicted for the younger heart valve of the same type.

2.2.5. Material parameters

The majority of the material parameters were taken from Loerakker et al. [12]. Some changes were made based on previous experimental studies on native heart valves [2, 3]. First, the shear modulus G and the material parameters associated with the collagen fiber stress (k_1 and k_2) were taken from the measurements of Oomen et al. [3]. The values of g_{cg} , ϕ_a , and σ_{max} were chosen specifically for each valve, with each change motivated by the study of Aikawa et al. [2]. In that study, on the one hand, it was observed that the cell density and contractility decrease with age; therefore, the values of ϕ_a and σ_{max} were scaled accordingly (Table 1). On the other hand, it was seen that the collagen fiber thickness increases from early fetal valves (younger than 19 weeks of age) to late fetal valves (between 20 and 39 weeks of age), and then stabilizes during the pediatric phase. Therefore, by hypothesizing that the effects of contact guidance depend on the collagen fiber thickness, we assumed this phenomenon to be negligible for early fetal valves and, conversely, significant for pediatric valves. This assumption was motivated also by previous computational and experimental studies demonstrating that cells cultured on grooved substrates undergoing cyclic strain respond to mechanical stimuli in case of relatively thin topographical patterns, while they respond to contact guidance for relatively large patterns [33]. Given these observations, for fetal valves we chose $\phi_a = 0.05$ and $g_{cg} = 0$, while σ_{max} was fitted by comparing the experimental and predicted collagen fiber distributions. Once approximating the value of σ_{max} for fetal valves, for the 2-year-old aortic valve we scaled ϕ_a and σ_{max} (according to Aikawa et al. [2], Table 1), and we fitted the value of g_{cg} with the experimental data. For the remaining heart valves, the value of g_{cg} that was determined with the simulation of 2-year-old aortic valve was kept unchanged, while again ϕ_a and σ_{max} were scaled according to Aikawa et al. [2] (see Table 1). The values of the parameters that do not change across the heart valves are reported in Table 2.

2.3. Comparison between simulations and previous experimental data

Via confocal microscopy, Oomen et al. [3] obtained the collagen distribution present in the belly region of native heart valves. In our study, these data were visually and quantitatively compared with the collagen distribution that the computational model predicted in the element located at the center of the belly region. The quantitative comparison was done using a goodness-of-fit approach. In particular, a coefficient of determination was defined as:

$$R = 1 - \frac{\sum_{k=1}^{360} (\varphi_{exp}^k - \varphi_{cf}^k)^2}{\sum_{k=1}^{360} (\varphi_{exp}^k - \bar{\varphi}_{exp})^2}, \quad (25)$$

where φ_{exp}^k and φ_{cf}^k are the percentage of collagen fibers from the experimental data and computational simulations, respectively, which are aligned between

Table 2: Parameter set for computational simulations, from Loerakker et al. [12]

Model component	Parameter	Value
SFs	ε_0	0.12 (-)
	ε_1	0.17 (-)
	k_v	50 s
	k_0^f	$1.5 \cdot 10^{-6} \text{s}^{-1}$
	k_1^f	$7.0 \cdot 10^{-7} \text{s}^{-1} \text{Pa}^{-1}$
	k_d	$1.0 \cdot 10^{-3} \text{s}^{-1}$
CFs	ϕ_{cf}	0.5 (-)
	k_3	100 (-)
	D_{min}	0.1 (-)
	D_{max}	1.0 (-)
Remodeling rates	τ_{sf}	5 min
	τ_λ	1 h
	τ_{cf}	12 h
Isotropic component	ϕ_{mc}	$1 - \phi_{cf} - \phi_a$
	ν	0.3 (-)

an angle $(0.5(k-1)-90)^\circ$ and an angle $(0.5k-90)^\circ$ with respect to the circumferential direction. The value of φ_{cf}^k was determined by scaling and interpolating the collagen volume fractions φ_{cf}^i derived from the computational simulations. The term $\bar{\varphi}_{exp}$ indicates the average of the experimental data: $\bar{\varphi}_{exp} = \sum_{k=1}^{360} \varphi_{exp}^k / 360$. In this case, the coefficient of determination R defined in equation (25) quantifies the agreement of the predictions of the computational model with the experimental data, compared with assuming an isotropic configuration for the collagen fibers in the heart valves. The closer the computational model predicts the experimental data, the closer R is to +1. The value of R is zero when the prediction error resulting from the computational model is the same as the one resulting from an isotropic collagen fiber configuration. Finally, R has negative values when an isotropic collagen fiber configuration more closely matches the experimental data compared to the predictions of the computational model.

3. Results

3.1. Collagen remodeling of fetal heart valves

First, the computational approach was applied to predict the collagen architecture in native fetal heart valves. As contact guidance is expected to have a negligible role, g_{cg} was set to zero and these simulations could be used to

Figure 1: Cell traction is necessary for the emergence of a circumferential collagen fiber alignment in fetal heart valves. A: Comparison between the collagen fiber distribution in the belly region of fetal heart valves quantified in a previous study [3] and the one predicted by the computational model with different values of maximum cell traction σ_{max} (reported in the legend). The direction v_1 corresponds to the circumferential direction in the heart valve leaflet. If $\sigma_{max} = 0$, the simulation predicted an isotropic collagen fiber distribution. Increasing values of maximum cell traction correspond to increasing circumferential alignment of collagen fibers. B: Histogram of the performance of the computational model with different values of maximum cell traction σ_{max} , quantified with the coefficient of determination defined in Eq. (25). The maximum performance is obtained for $\sigma_{max} = 50$ kPa, with $R = 0.75$. C: Radial strain distribution in the final loaded configuration of the fetal heart valve, as predicted by the computational model. The opening of the heart valve in the loaded configuration increases for increasing values of σ_{max} .

325 estimate the value of maximum cell traction σ_{max} for native fetal heart valves and understand its influence on collagen remodeling.

The computational results show that cell traction is necessary to obtain the circumferential collagen orientation observed in Oomen et al. [3] (Fig. 1). When cell traction was set to zero, the resulting collagen fiber architecture was approximately isotropic (Fig. 1A, results with $\sigma_{max} = 0$ kPa). Conversely, non-zero values of σ_{max} resulted in an anisotropic collagen fiber organization that was mainly circumferentially aligned, with this alignment increasing with σ_{max} (Fig. 1A).

335 The quantitative comparison of the experimental and computational results demonstrated that the best predictions, in the sense of goodness-of-fit, were obtained with $\sigma_{max} = 50$ kPa (Fig. 1B). We observe that the simulations with $\sigma_{max} = 50$ kPa also predicted a fairly good closure of the fetal heart valve, and that this closure decreased for higher values of σ_{max} (Fig. 1C). Considering

Figure 2: Contact guidance is a necessary mechanism to maintain and reinforce the circumferential collagen fiber alignment during development towards 2-year-old aortic valves. A: Comparison between the collagen fiber distribution in the belly region of 2-year-old aortic valves observed in a previous study [3] and the one predicted by the computational model with different values of g_{cg} (reported in the legend), proportional to the effects of contact guidance. If $g_{cg} = 0$ s^{-1} , the simulation predicted an almost isotropic collagen fiber distribution. Increasing values of g_{cg} correspond to increasing circumferential alignment of collagen fibers. B: Histogram of the performance of the computational model with different values of g_{cg} . The agreement between experimental and computational results increases for increasing values of g_{cg} . A plateau is reached at approximately $g_{cg} = 500$ s^{-1} . C: Radial strain distribution in the final loaded configuration of the 2-year-old aortic valve, as predicted by the computational model. The computational model predicted good valve closure for every value of g_{cg} .

these results, the maximum cell traction for fetal heart valves was estimated to be $\sigma_{max} = 50$ kPa.

3.2. Collagen remodeling of 2-year-old aortic valves

Simulations of 2-year-old aortic valves were then performed considering previous experimental studies [2, 3] for the choice of the heart valve geometry, collagen fiber material properties, total actin volume fraction, and maximum cell traction (Table 4.1). The circumferential distribution predicted for fetal heart valves with $\sigma_{max} = 50$ kPa was chosen as initial condition for the remodeling of these 2-year-old valves. Despite that, if contact guidance was not considered, the simulations predicted an isotropic collagen distribution at 2 years of age (Fig. 2A, $g_{cg} = 0$ s^{-1}). This is in disagreement with Oomen et al. [3], who reported collagen fibers to be aligned circumferentially (Fig. 2A). Conversely, with the inclusion of the effects of contact guidance, a circumferential collagen orientation was obtained in agreement with experimental data. The degree of

alignment predicted by the simulations increased with g_{cg} (Fig. 2A), together with the agreement with the experimental results (Fig. 2B), until a plateau was reached for values of g_{cg} around 500 s^{-1} .

These results suggest that the phenomenon of contact guidance is necessary to maintain and reinforce the circumferential collagen alignment that was present at the fetal age. In particular, contact guidance can induce a positive feedback on the collagen and cell distribution: on the one hand, circumferential collagen fibers induce cells to orient circumferentially; on the other hand, these aligned cells deposit more collagen in this circumferential direction, thereby increasing the effects of contact guidance.

Interestingly, varying g_{cg} did not have significant effects on the closure of 2-year-old aortic valves, which were predicted to efficiently close in every case (Fig. 2C). It can be observed, anyway, that increasing values of g_{cg} led to a slight increase of radial strains, which favor valve closure. Given that a reasonable agreement between experimental and computational results was obtained for $g_{cg} = 500 \text{ s}^{-1}$ and that a further increase of g_{cg} did not entail a significant improvement (Fig. 2C), we chose this value as a reference for the effects of contact guidance.

3.3. Collagen remodeling of other pediatric heart valves

The computational model with contact guidance was further applied to simulating three other native heart valves: a 2-year-old pulmonary valve, and 5-year-old aortic and pulmonary valves. The parameter g_{cg} identified with the simulations of 2-year-old aortic valve was unchanged, while the maximum cell traction σ_{max} was scaled in agreement with previous experimental studies (Table 1). The results of the computational simulations with these parameters are shown in Fig. 3, together with the results for the fetal and 2-year-old aortic valves obtained with the chosen parameters.

The computational simulations predicted collagen distributions qualitatively in agreement with the experimental results for all heart valves. In particular, all heart valves present a circumferential alignment with different degrees of anisotropy. In case of aortic valves, this anisotropy increases with age, going from fetal (Fig. 3A) to 5-year-old aortic valves (Fig. 3C). In contrast, in case of pulmonary valves, the alignment only increases from fetal to 2-year-old valves (Fig. 3D), and it remains stable until 5-year-old valves (Fig. 3E).

Strikingly, the computational predictions were reasonably in agreement with the experiments also from a quantitative point of view, as represented by the values of R reported in each graph (Fig. 3). This is especially significant because these results were obtained with minimal changes of parameters, that were necessary to consider the differences between aortic and pulmonary valves, and the changes of these parameters with age, as observed in experimental studies [2, 3].

3.4. The importance of cell traction and contact guidance

Further simulations were performed to evaluate the relative importance of cell traction and cellular (re)orientation in response to both mechanical and

Figure 3: The computational model can successfully predict the collagen fiber distribution in fetal and pediatric (2- and 5-year-old) aortic and pulmonary heart valves. In each panel, the comparison between experimental and computational results is shown with a graph, for fetal (A) and pediatric (B-E) heart valves. Furthermore, in these panels, the values of the coefficient of determination R are indicated (all above 0.75), and spider-plots representing the collagen fiber distribution predicted by the computational simulations are shown. In particular, for these spider-plots, the length of the lines is proportional to the amount of collagen fibers along that particular direction, as predicted by the computational model, where the horizontal and vertical directions correspond, respectively, to the circumferential and radial directions in the heart valve leaflet. Below each panel, the final loaded configuration as predicted by the computational simulations is reported.

topographical stimuli for the final collagen distribution. To this aim, we first simulated 5-year-old heart valves by changing the parameters associated with these phenomena. In particular, the simulations without contact guidance were performed by assuming $g_{cg} = 0 \text{ s}^{-1}$. The simulations without cell traction were performed by setting $\sigma_{max} = 0 \text{ kPa}$. Simulations with cell traction, but without a reorientation response of cells due to mechanical stimuli, were obtained by: setting σ_{max} to its original value (11 kPa in case of 5-year-old valves); increasing the value of the basal actin stress fiber production from the original k_0^f to $k_0^f + k_1^f \sigma_{max}$; and, later, set the parameter associated to cellular reorientation in response to strain to $k_1^f = 0 \text{ s}^{-1} \text{ Pa}^{-1}$. The simulations were performed with several variations of the parameters, as shown in the table in Fig. 4.

From the results of these simulations, it can be seen that contact guidance is again fundamental to maintain the circumferential alignment of collagen fibers. In particular, the simulation of 5-year-old heart valves performed with $g_{cg} = 0 \text{ s}^{-1}$ predicted an isotropic (or slightly radial) organization of collagen fibers for both the aortic and pulmonary valve (Fig. 4A and 4B), in strong disagreement with experimental data (Fig. 4C). Considering contact guidance without cell traction was also not sufficient to correctly predict collagen remodeling. In fact, simulations with $g_{cg} = 500 \text{ s}^{-1}$ and $\sigma_{max} = 0 \text{ kPa}$ also had computational results in disagreement with experiments. This was particularly evident for 5-year-old pulmonary valves which, in that case, presented an excessive circumferential alignment (Fig. 4B). On the other hand, the (re)orientation of cells in response to mechanical stimuli does not seem significantly important in this case, since a good level of agreement between computational and experimental data was achieved by setting to zero the parameter k_1^f associated with the reorientation potential of cells in response to mechanical stimuli, and by increasing the basal stress fiber formation accordingly (Fig. 4, parameter set V).

Given the results for 5-year-old heart valves, we simulated 2-year-old heart valves with changes of parameters to investigate whether a physiological collagen remodeling can be obtained in these heart valves without the reorientation of cells in response to mechanical stimuli. Again, the computational simulations showed that contact guidance is fundamental to obtain the circumferential collagen alignment (Fig. 5). Most importantly, these computational results show that considering cellular realignment in response to mechanical stimuli is fundamental to predict the high degree of alignment observed in 2-year-old heart valves. In fact, a decrease of the agreement between computational and experimental results was observed when the reorientation of cells in response to mechanical stimuli was not taken into account.

4. Discussion

The distribution of collagen fibers strongly influences the biomechanics and functionality of heart valves. Therefore, predicting and understanding the remodeling of collagen fibers is desired. The aim of this study was to understand the collagen remodeling in human native heart valves during early development.

Figure 4: Simulation of 5-year-old heart valves with variation of computational parameters. A-B: Comparison, for aortic (A) and pulmonary (B) valves, between the experimental data and the collagen fiber distribution predicted by the computational simulation with varying values of the parameters g_{cg} , σ_{max} , k_0^f , and k_1^f , as reported in the table on the right. In the table, the overbars indicate the reference parameters, adopted to obtain the results in Fig. 3. C: Performance of the computational model. A good agreement between experimental and computational results is obtained only when σ_{max} and g_{cg} are not equal to zero.

440 To this aim, we adopted a computational approach to simulate tissue remodeling
in fetal (19-weeks-old) and pediatric (2- and 5-year-old) heart valves.

4.1. Agreement between computational results and experimental data

Importantly, the collagen distributions predicted by the computational simulations exhibited a good level of qualitative and quantitative agreement with
445 the experimental data reported in a previous study [3], for all heart valves that
were simulated (Fig. 3). This is especially notable if we consider that these
predictions were obtained by varying the computational parameters only when
required to consider the variations between aortic and pulmonary valves, and
between valves of different age groups [2, 3].

450 The quantitative agreement between experiments and simulations could further
improve by considering that the scaling of the maximum cell traction for the
simulation of the distinct heart valves was based on the study of Aikawa et al.
[2], while the experimental collagen distributions were taken from Oomen et al.

Figure 5: Simulation of 2-year-old heart valves with variation of computational parameters. A-B: Comparison, for aortic (A) and pulmonary (B) valves, between the experimental data and the collagen fiber distribution predicted by the computational simulation with varying values of the parameters g_{cg} , σ_{max} , k_0^f , and k_1^f , as reported in the tables on the right. C: Performance of the computational model. Choosing $k_1^f = 0 \text{ s}^{-1} \text{ Pa}^{-1}$ leads to computational results less closely matching the experimental data.

[3]. Considered this discrepancy and the biological variation between individuals, the agreement between computational and experimental data is remarkably good.

4.2. Cell traction and (re)orientation in response to mechanical stimuli are fundamental for initiation of circumferential alignment

Given the good level of agreement between computational and experimental data, we can derive information about the importance of the different mechanisms driving collagen remodeling during development. For example, from the simulation of fetal heart valves with different values of maximum cell traction (Fig. 1), we can understand that cell traction is fundamental for the circumferential collagen fiber orientation to arise. In fact, the computational simulations of fetal heart valves predicted a circumferential collagen fiber alignment only if a non-zero value of cell traction was chosen; an approximately isotropic distribution was predicted otherwise. We observe that the maximum cell traction is also correlated with the reorientation of cells in response to mechanical stimuli (Eq.

(9)). Consequently, when σ_{max} is set to zero, this reorientation response is neglected. It thus appears that both cell traction and reorientation in response to mechanical stimuli are necessary for the collagen organization to arise in the very early development.

The importance of these two phenomena for a physiological collagen remodeling was also confirmed at a later stage of development, when simulating 2- and 5-year-old valves. In fact, excessive alignment was predicted for 5-year-old pulmonary valves with no cell traction (Fig. 4B), while a lower level of anisotropy was predicted for 2-year-old heart valves if the cellular reorientation response due to the experienced strain and strain rate was not considered (Fig. 5). Overall, these results suggest that cell traction and reorientation in response to mechanical stimuli are fundamental mechanisms driving the physiological collagen remodeling in native heart valves, especially in the relatively early stage of development.

4.3. Contact guidance is essential to maintain and reinforce collagen alignment

The computational simulations further indicate that the tendency of cells to coalign with collagen fibers is fundamental for the maintenance and reinforcement of the circumferential collagen alignment obtained during the early stage of development. Computational simulations of 2- and 5-year-old heart valves performed without considering such phenomenon had results strongly in disagreement with experimental data (Figs. 2, 4 and 5).

In pediatric native heart valves, the importance of contact guidance for the remodeling of collagen is particularly evident because, as reported by Aikawa et al. [2], the maximum cell traction of cells in these valves is low compared to the fetal stage and, consequently, also their reorientation potential in response to mechanical stimuli is diminished (Eq. (9)).

4.4. Main limitation

One of the main limitations of this study is the fact that the change of the material parameters performed when passing from fetal to 2-year-old valves, and further to 5-year-old valves, was assumed to occur instantaneously, together with the change of heart valve radius, thickness, and blood pressure. This assumption was necessary because no extensive information on the evolution over time of such parameters is present in the literature. With further information, computational models predicting the heart valve growth over time in response to the in vivo biomechanical stimuli experienced by heart valves could be developed, with an approach similar to computational models for the growth of other native tissues [34]. Such models could then be implemented in the computational framework proposed in this study, together with evolution laws for the material parameters.

4.5. Future applications

Although improvements are possible, the computational framework proposed in this study presents a large number of potential applications. For example,

a similar approach could be adopted to investigate the tissue remodeling of adolescent and adult native heart valves [3], the long-term remodeling of tissue-engineered heart valves, the evolution of native pulmonary heart valves undergoing the Ross procedure [35] or other cardiovascular tissues, such as arteries. Furthermore, while in this study we mainly focused on analyzing the importance that cellular (re)orientation in response to contact guidance and mechanical stimuli has on collagen remodeling, future studies could also address other mechanisms that are assumed to determine the collagen fiber distribution.

5. Conclusion

In this study, we adopted a computational approach to understand collagen remodeling in human native heart valves during early development. Importantly, the computational simulations were able to successfully predict the collagen fiber distribution in native fetal (19-week-old) and pediatric (2- and 5-year-old) aortic and pulmonary valves with little adjustment of parameters. Additional simulations performed by varying computational parameters suggested that the reorientation of cells in response to mechanical cues is fundamental for the circumferential alignment of collagen fibers to arise during heart valve development, while the tendency of cells to coalign with collagen fibers is necessary to maintain and enhance such alignment.

6. Disclosures

The authors have declared that no conflicts of interests exist.

7. Acknowledgements

This research has received funding from the People Programme (Marie Curie Actions) of the European Union’s Seventh Framework Programme FP7-People-2012-ITN “TECAS” under grant agreement No 317512, and from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 654513. The authors would like to acknowledge D. van Geemen and P.J.A. Oomen for using their previously acquired data base on native human heart valve properties, which was obtained in collaboration with the Heart Valve Bank, Erasmus Medical Center Rotterdam (A.J. van den Bogaardt , A.J.J.C. Bogers) and Leiden University Medical Center (M.J. Goumans).

References

- [1] K. L. Billiar, M. S. Sacks, Biaxial mechanical properties of the natural and glutaraldehyde treated aortic valve cusp—Part I: Experimental results, *Journal of Biomechanical Engineering* 122 (1) (2000) 23–30. [arXiv:Sack2000a](#), doi:10.1115/1.429624.

URL <http://biomechanical.asmedigitalcollection.asme.org/article.aspx?articleid=1399079>

- 550 [2] E. Aikawa, P. Whittaker, M. Farber, K. Mendelson, R. F. Padera, M. Aikawa, F. J. Schoen, Human semilunar cardiac valve remodeling by activated cells from fetus to adult: implications for postnatal adaptation, pathology, and tissue engineering, *Circulation* 113 (10) (2006) 1344–1352. doi:10.1161/CIRCULATIONAHA.105.591768.

555 URL <http://circ.ahajournals.org/cgi/doi/10.1161/CIRCULATIONAHA.105.591768>

- [3] P. Oomen, S. Loerakker, D. van Geemen, J. Neggers, M.-J. Goumans, A. van den Bogaardt, A. Bogers, C. Bouten, F. Baaijens, Age-dependent changes of stress and strain in the human heart valve and their relation with collagen remodeling, *Acta Biomaterialia* 29 (2016) 161–169. doi:10.1016/j.actbio.2015.10.044.

560 URL <http://linkinghub.elsevier.com/retrieve/pii/S1742706115301732>

- [4] M. S. Sacks, W. David Merryman, D. E. Schmidt, On the biomechanics of heart valve function., *Journal of biomechanics* 42 (12) (2009) 1804–24. doi:10.1016/j.jbiomech.2009.05.015.

565 URL <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2746960&tool=pmcentrez&rendertype=abstract>

- [5] S. Loerakker, G. Argento, C. W. J. Oomens, F. P. T. Baaijens, Effects of valve geometry and tissue anisotropy on the radial stretch and coaptation area of tissue-engineered heart valves., *Journal of biomechanics* 46 (11) (2013) 1792–800. doi:10.1016/j.jbiomech.2013.05.015.

570 URL <http://www.ncbi.nlm.nih.gov/pubmed/23786664>

- [6] Z. Syedain, J. Reimer, M. Lahti, J. Berry, S. Johnson, R. T. Tranquillo, Tissue engineering of acellular vascular grafts capable of somatic growth in young lambs, *Nature Communications* 7 (2016) 12951. doi:10.1038/ncomms12951.

575 URL <http://www.nature.com/doifinder/10.1038/ncomms12951>

- [7] L. A. Bockeria, O. Svanidze, A. Kim, K. Shatalov, V. Makarenko, M. Cox, T. Carrel, Total cavopulmonary connection with a new bioabsorbable vascular graft: First clinical experience, *Journal of Thoracic and Cardiovascular Surgery* 153 (6) (2017) 1542–1550. doi:10.1016/j.jtcvs.2016.11.071.

580 URL <http://dx.doi.org/10.1016/j.jtcvs.2016.11.071>

- 585 [8] J. Kluin, H. Talacua, A. I. Smits, M. Y. Emmert, M. C. Bruggmans, E. S. Fioretta, P. E. Dijkman, S. H. Söntjens, R. Duijvelshoff, S. Dekker, M. W. Janssen-van den Broek, V. Lintas, A. Vink, S. P. Hoerstrup, H. M. Janssen, P. Y. Dankers, F. P. Baaijens, C. V. Bouten, In situ heart valve tissue

- engineering using a bioresorbable elastomeric implant – From material
 590 design to 12 months follow-up in sheep, *Biomaterials* 125 (2017) 101–117.
 doi:10.1016/j.biomaterials.2017.02.007.
- [9] P. W. Serruys, Y. Miyazaki, A. Katsikis, M. Abdelghani, M. B. Leon,
 R. Virmani, T. Carrel, M. Cox, Y. Onuma, O. I. Soliman, Restorative valve
 595 therapy by endogenous tissue restoration: Tomorrow’s world? Reflection
 on the EuroPCR 2017 session on endogenous tissue restoration, *EuroInt-
 ervention* 13 (AA) (2017) AA68–AA77. doi:10.4244/EIJ-D-17-00509.
- [10] N. J. B. Driessen, C. V. C. Bouten, F. P. T. Baaijens, Improved prediction
 of the collagen fiber architecture in the aortic heart valve, *Journal of Biome-
 600chanical Engineering* 127 (2) (2005) 329–336. doi:10.1115/1.1865187.
 URL [http://biomechanical.asmedigitalcollection.asme.org/
 article.aspx?articleid=1414019](http://biomechanical.asmedigitalcollection.asme.org/article.aspx?articleid=1414019)
- [11] N. J. B. Driessen, M. A. J. Cox, C. V. C. Bouten, F. P. T. Baaijens,
 Remodelling of the angular collagen fiber distribution in cardiovascular
 605 tissues., *Biomechanics and modeling in mechanobiology* 7 (2) (2008)
 93–103. doi:10.1007/s10237-007-0078-x.
 URL [http://www.pubmedcentral.nih.gov/articlerender.fcgi?
 artid=2792349&tool=pmcentrez&rendertype=abstract](http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2792349&tool=pmcentrez&rendertype=abstract)
- [12] S. Loerakker, T. Ristori, F. P. Baaijens, A computational analysis of cell-
 mediated compaction and collagen remodeling in tissue-engineered heart
 610 valves, *Journal of the Mechanical Behavior of Biomedical Materials* 53
 (2016) 173–187. doi:10.1016/j.jmbbm.2015.10.001.
- [13] U. Faust, N. Hampe, W. Rubner, N. Kirchgeßner, S. Safran, B. Hoff-
 mann, R. Merkel, Cyclic stress at mHz frequencies aligns fibro-
 blasts in direction of zero strain, *PLoS ONE* 6 (12) (2011) e28963.
 615 doi:10.1371/journal.pone.0028963.
 URL [http://www.pubmedcentral.nih.gov/articlerender.fcgi?
 artid=3241701&tool=pmcentrez&rendertype=abstract](http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3241701&tool=pmcentrez&rendertype=abstract)
- [14] J. Foolen, V. S. Deshpande, F. M. W. Kanters, F. P. T. Baaijens, The
 influence of matrix integrity on stress-fiber remodeling in 3D, *Biomaterials*
 620 33 (30) (2012) 7508–7518. doi:10.1016/j.biomaterials.2012.06.103.
 URL <http://www.ncbi.nlm.nih.gov/pubmed/22818650>
- [15] A. Tondon, H. J. Hsu, R. Kaunas, Dependence of cyclic stretch-induced
 stress fiber reorientation on stretch waveform, *Journal of Biomechanics*
 45 (5) (2012) 728–735. doi:10.1016/j.jbiomech.2011.11.012.
 625 URL <http://dx.doi.org/10.1016/j.jbiomech.2011.11.012>
- [16] J. H.-C. Wang, F. Jia, T. W. Gilbert, S. L.-Y. Woo, Cell orientation deter-
 mines the alignment of cell-produced collagenous matrix, *J Biomech* 36 (1)
 (2003) 97–102. doi:10.1016/S0021-9290(02)00233-6.

- URL <http://www.ncbi.nlm.nih.gov/pubmed/12485643>{%}5Cn<http://www.sciencedirect.com/science/article/pii/S0021929002002336>
- 630 [17] M. Ghibaudo, A. Saez, L. Trichet, A. Xayaphoummine, J. Browaeys, P. Silberzan, A. Buguin, B. Ladoux, Traction forces and rigidity sensing regulate cell functions, *Soft Matter* 4 (9) (2008) 1836–1843. doi:10.1039/b804103b. URL <http://xlink.rsc.org/?DOI=b804103b>
- 635 [18] A. S. Meshel, Q. Wei, R. S. Adelstein, M. P. Sheetz, Basic mechanism of three-dimensional collagen fibre transport by fibroblasts., *Nature cell biology* 7 (2) (2005) 157–164. doi:10.1038/ncb1216.
- [19] K. E.-K. Wyatt, J. W. Bourne, P. A. Torzilli, Deformation-dependent enzyme mechanokinetic cleavage of type I collagen, *Journal of Biomechanical Engineering* 131 (5) (2009) 051004. doi:10.1115/1.3078177.
- 640 URL <http://biomechanical.asmedigitalcollection.asme.org/article.aspx?articleid=1475682>
- [20] S. Guido, R. T. Tranquillo, A methodology for the systematic and quantitative study of cell contact guidance in oriented collagen gels. Correlation of fibroblast orientation and gel birefringence, *Journal of cell science* 105 (Pt 2) (1993) 317–331.
- 645 [21] J. Friedrichs, A. Taubenberger, C. M. Franz, D. J. Muller, Cellular remodelling of individual collagen fibrils visualized by time-lapse AFM, *Journal of Molecular Biology* 372 (3) (2007) 594–607. doi:10.1016/j.jmb.2007.06.078.
- 650 [22] T. Ristori, T. Notermans, J. Foolen, N. Kurniawan, C. Bouten, F. Baaijens, S. Loerakker, Modeling the combined effects of collagen and cyclic strain on cellular orientation in collagenous tissues (2017).
- [23] C. Obbink-Huizer, C. W. J. Oomens, S. Loerakker, J. Foolen, C. V. C. Bouten, F. P. T. Baaijens, Computational model predicts cell orientation in response to a range of mechanical stimuli., *Biomechanics and modeling in mechanobiology* 13 (1) (2014) 227–36. doi:10.1007/s10237-013-0501-4.
- 655 URL <http://www.ncbi.nlm.nih.gov/pubmed/23708875>
- [24] S. Loerakker, C. Obbink-Huizer, F. P. T. Baaijens, A physically motivated constitutive model for cell-mediated compaction and collagen remodeling in soft tissues., *Biomechanics and modeling in mechanobiology* 13 (5) (2014) 985–1001. doi:10.1007/s10237-013-0549-1.
- 660 URL <http://www.ncbi.nlm.nih.gov/pubmed/24370853>
- [25] T. Ristori, C. W. J. Oomens, F. P. T. Baaijens, S. Loerakker, Efficient computational simulation of actin stress fiber remodeling, *Computer methods in biomechanics and biomedical engineering* 19 (12) (2016) 1347–1358. doi:10.1080/10255842.2016.1140748.
- 665

- [26] V. S. Deshpande, R. M. McMeeking, A. G. Evans, A bio-chemo-mechanical model for cell contractility, *Proceedings of the National Academy of Sciences of the USA* 103 (45) (2006) 14015–14020.
- [27] F. J. Vernerey, M. Farsad, A constrained mixture approach to mechanosensing and force generation in contractile cells, *Journal of the Mechanical Behavior of Biomedical Materials* 4 (8) (2011) 1683–1699. doi:10.1016/j.jmbbm.2011.05.022.
URL <http://www.ncbi.nlm.nih.gov/pubmed/22098869>
- [28] N. J. B. Driessen, A. Mol, C. V. C. Bouten, F. P. T. Baaijens, Modeling the mechanics of tissue-engineered human heart valve leaflets., *Journal of biomechanics* 40 (2) (2007) 325–34. doi:10.1016/j.jbiomech.2006.01.009.
URL <http://www.ncbi.nlm.nih.gov/pubmed/16529755>
- [29] T. Heck, W. Wilson, J. Foolen, A. Cilingir, K. Ito, C. van Donkelaar, A tissue adaptation model based on strain-dependent collagen degradation and contact-guided cell traction, *Journal of Biomechanics* 48 (5) (2015) 823–831. doi:10.1016/j.jbiomech.2014.12.023.
- [30] S. Fleming, M. Thompson, R. Stevens, C. Heneghan, A. Plüddemann, I. MacOnochie, L. Tarassenko, D. Mant, Normal ranges of heart rate and respiratory rate in children from birth to 18 years of age: A systematic review of observational studies, *The Lancet* 377 (9770) (2011) 1011–1018. doi:10.1016/S0140-6736(10)62226-X.
URL [http://dx.doi.org/10.1016/S0140-6736\(10\)62226-X](http://dx.doi.org/10.1016/S0140-6736(10)62226-X)
- [31] P. C. Struijk, V. J. Mathews, T. Loupas, P. A. Stewart, E. B. Clark, E. A. Steegers, J. W. Wladimiroff, Blood pressure estimation in the human fetal descending aorta, *Ultrasound in Obstetrics and Gynecology* 32 (5) (2008) 673–681. doi:10.1002/uog.6137.
- [32] U. A. Stock, J. P. Vacanti, Cardiovascular physiology during fetal development and implications for tissue engineering., *Tissue Eng* 7 (1) (2001) 1–7. doi:10.1089/107632701300003241.
URL <http://www.ncbi.nlm.nih.gov/pubmed/11224919>
- [33] L. Prodanov, J. te Riet, E. Lamers, M. Domanski, R. Luttge, J. J. van Loon, J. A. Jansen, X. F. Walboomers, The interaction between nanoscale surface features and mechanical loading and its effect on osteoblast-like cells behavior, *Biomaterials* 31 (30) (2010) 7758–7765. doi:10.1016/j.biomaterials.2010.06.050.
URL <http://linkinghub.elsevier.com/retrieve/pii/S0142961210008306>
- [34] E. Kuhl, Growing matter: a review of growth in living systems., *Journal of the mechanical behavior of biomedical materials* 29 (2014) 529–43. doi:

10.1016/j.jmbbm.2013.10.009.

URL <http://www.ncbi.nlm.nih.gov/pubmed/24239171>

- ⁷¹⁰ [35] D. Ross, Homograft replacement of the aortic valve, *Lancet* 2 (7254) (1961) 487. doi:10.1016/S0140-6736(62)90345-8.