ABSTRACT
Heart valves are inhomogeneous microstructure with nonlinear anisotropic properties and constantly experience different stress states during cardiac cycles. However, how tissue-level mechanical forces can translate into altered cellular stress states remains unclear, and associated biomechanical regulation in the tissue has not been fully understood. In the current study, we use an image-based finite element method to investigate factors contributing the stress distributions at both tissue- and cell-levels inside the healthy heart valve tissues. Effects of tissue microstructure, inhomogeneity, and anisotropic material property at different diastole states are discussed to provide a better understanding of structure-mechanics-property interactions, which alters tissue-to-cell stress transfer mechanisms in heart valve tissue. To the best of the authors’ knowledge, this is the first study reporting on the evolution of stress fields at both the tissue- and cellular-levels in valvular tissue, and thus contributes toward refining our collective understanding of valvular tissue micromechanics while providing a computational tool enabling further study of valvular cell-tissue interactions.

INTRODUCTION
To date, there are at least 250,000 people suffering heart valve diseases in the United States [1, 2], and the population is rapidly expanding. The disease is caused by the disordered tissue homeostatic mechanisms that pathologically vary the collagen fiber microstructure, material properties, or specific chemical concentrations and property. As a result, the mechanical stresses and strains are considered as key factors for the structure-mechanics-property relations tightly coupled in the biomechanical regulation of the heart valve tissue homeostasis. At the tissue level, the heart valves are inhomogeneous microstructure with nonlinear anisotropic properties and constantly experience different stress states during cardiac cycles. Studies have shown that from a computational biomechanics perspective, the tissue has previously been modeled as a homogeneous medium, thereby underestimating tissue-cell mechanical interactions due to the simplified representation of the highly heterogeneous collagen fiber network [3-6]. At the cellular-level, isolated heart valve interstitial cells (VICs) are observed to be modulated by mechanical force environment [7-17]. The altered VICs’ phenotype and morphology triggered by mechanical forces induce biomechanical regulation, such as contraction, collagen synthesis, and extracellular matrix (ECM) remodeling [7, 13-17]. Therefore, how tissue corresponds with functions of VICs, and how VICs and ECM work together to maintain, repair, and regenerate healthy heart valve tissue should be addressed as a whole continuum.

We have previously established an image-based finite element analysis (FEA) enabling the investigations of the overall stress distributions of heart valve tissues [18, 19]. The imaged-based FEA approach is capable of simulating physiologic biaxial stretching of valvular tissue and cells from photomicrographs of histological tissue sections. The study incorporated collagen fiber heterogeneous architectures and VIC’s random distributions and compares localized stresses on VICs within two different constitutive models. The result indicated that the collagen fiber architecture, cell distributions and anisotropic materials properties of the tissue are key factors of how stresses are transferred from the ECM to cells. It is observed the anisotropic materials property of the tissue mitigates the overall mechanical stresses inside heart valve tissues, comparing to the models incorporating the isotropic materials property [18, 19].

In the current study, we utilized the developed imaged-based FEA further to study the stress evolutions at and cell levels during diastole of pulmonary valve (PV). To better visualize the stress transmission and reception between cells and ECM in the heart valve tissue during diastole, piecewise linear (to represent nonlinear behavior) anisotropic tissue-level...
finite element models are incorporated to conduct virtual biaxial experiments. The stress evolution around a single cell is studied to illustrate critical effects of the collagen fiber directional dependency, the location of the cell, and the overall tissue structure-property relations.

MATERIAL AND METHODS

The Image-Based Finite Element Analysis

In the process of the FEA modeling, the selected histological photomicrograph (1,000 x 1,000 pixels) captures collagen fibers microstructure and various cell morphologies (Fig. 1a-b). The four-node quadrilateral plane stress element is applied, and the boundary conditions are varied from 10% to 35% of equal-biaxial stretching by the increment of 5% (Fig. 1c). Furthermore, the nine regions shown in Fig. 1d are defined to better visualize the stress distribution in tissue samples. In order to understand and quantify the stress distribution when tissue is stretched up to 35%, the stress distribution around a single cell nucleus is studied. By varying the amount of stretching (Fig. 1c), different linear anisotropic material properties are applied to describe the nonlinear anisotropic material property of the heart valve tissue during diastole [18, 20], as described in the next section.

The stress distribution around a cell nucleus and the resulting cellular deformation caused by the external stretching on the matrix could provide better understanding for the mechanism of stress transfer from matrix to the cell. A representative VIC nucleus is selected from the region 2 of the image (Fig. 1d). Since it is difficult to obtain the location data of the cell in the original photomicrograph (Fig. 1e), the boundary of the VIC nucleus is selected based on the distinct $\sigma_1$ contour of the 35% stretching model. To evaluate the strain and stress distributions of the said VIC nucleus, twenty-one locations around the cell boundary are defined (Fig. 1f). Due to the rigid body motion of the cell, it is noted that the contour of the VIC nucleus after stretching is slightly different from the outline of the original cell nucleus in the photomicrograph. During 0 to 35% of equibiaxial stretching at the tissue level, the stress distribution and the cellular deformation is clearly presented.

The Stiffness Matrix of Tissue Models

It is observed that the PV tissue exhibits nonlinear anisotropic material behavior [20]. Since the stress-strain relation of PV tissues was determined in our previous study via biaxial testing [20], the three piecewise linearized elastic orthotropic material properties under different strain stretching are used to obtain orthotropic stiffness matrices for the anisotropic material properties of finite element models (Table 1).

Due to the thinness of this specimen (4 µm), the finite element model in the current study is considered to be a two-dimensional plane stress with equibiaxial boundary conditions. For the plane stress material ($\sigma_{12} = 0, \tau_{23} = \tau_{13} = 0$), the constitutive equation can be reduced to

$$
\begin{bmatrix}
\varepsilon_1 \\
\varepsilon_2 \\
\gamma_{12}
\end{bmatrix} =
\begin{bmatrix}
S_{11} & S_{12} & 0 \\
S_{21} & S_{22} & 0 \\
0 & 0 & S_{66}
\end{bmatrix}
\begin{bmatrix}
\sigma_1 \\
\sigma_2 \\
\tau_{12}
\end{bmatrix},
$$

where $S_{ij}$ is the compliance matrix ($i, j = 1, \ldots, 6$), $\varepsilon$ and $\gamma$ are strains, and $\sigma$ and $\tau$ are stresses. The two-dimensional compliance matrix for the orthotropic material can be determined via moduli of elasticity $E$, the Poisson’s ratio $\nu$, and the shear moduli $G$ [21, 22]:

$$
[S] =
\begin{bmatrix}
S_{11} & S_{12} & 0 \\
S_{21} & S_{22} & 0 \\
0 & 0 & S_{66}
\end{bmatrix} =
\begin{bmatrix}
\frac{1}{E_1} & -\frac{\nu_{12}}{E_2} & 0 \\
-\frac{\nu_{12}}{E_1} & \frac{1}{E_2} & 0 \\
0 & 0 & \frac{1}{G_{12}}
\end{bmatrix},
$$

and the stiffness matrix $[C]$ consequently can be obtained as follows:

<table>
<thead>
<tr>
<th>$x$</th>
<th>$y$</th>
<th>$z$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-18% of strain</td>
<td>18-28% of strain</td>
<td>28-35% of strain</td>
</tr>
<tr>
<td>C-dir</td>
<td>R-dir</td>
<td>C-dir</td>
</tr>
<tr>
<td>C-dir</td>
<td>R-dir</td>
<td>C-dir</td>
</tr>
<tr>
<td>C-dir</td>
<td>R-dir</td>
<td>C-dir</td>
</tr>
<tr>
<td>C-dir</td>
<td>R-dir</td>
<td>C-dir</td>
</tr>
<tr>
<td>$E$ (kPa)</td>
<td>$\pm 0.79$</td>
<td>$\pm 6.01$</td>
</tr>
<tr>
<td>$\nu_{12}$</td>
<td>$\pm 0.79$</td>
<td>$\pm 6.01$</td>
</tr>
<tr>
<td>$G_{12}$</td>
<td>$\pm 0.79$</td>
<td>$\pm 6.01$</td>
</tr>
</tbody>
</table>

Table 1. Moduli of elasticity $E$ (kPa) at circumferential ($C$) and radial ($R$) directions of pulmonary valve tissue at different equibiaxial strain regions.
The Poisson’s ratio $\nu_{12}$ is 0.45 for orthotropic collagen fibers, and $\nu_{21}$ is derived from the relation $\nu_{21} = \nu_{12}E_2/E_1$, while cells and voids are considered as isotropic materials ($E_{\text{cell}} = 0.9$ kPa and $\nu_{\text{cell}} = 0.45$; $E_{\text{void}} = 0.01$ kPa and $\nu_{\text{void}} = 0.45$) [7]; the shear moduli are obtained from the relation $G_{ij} = \sqrt{E_iE_j / \left[ 2(1 + \nu_{ij}) \right]}$, where $i,j=1,2$. As a result, three stiffness matrices for the porcine PV tissue are calculated based on data in Table 1 and are shown below:

$$
[C] = [S]^T = \begin{bmatrix}
\frac{E_1}{1-\nu_{12}\nu_{21}} & \frac{E_1\nu_{21}}{1-\nu_{12}\nu_{21}} & 0 \\
\frac{E_1\nu_{12}}{1-\nu_{12}\nu_{21}} & \frac{E_2}{1-\nu_{12}\nu_{21}} & 0 \\
0 & 0 & G_{12}
\end{bmatrix}
$$

RESULTS AND DISCUSSION

The FEA results illustrate the distinct features of stress distribution in heart valve samples under equibiaxial stretching. Figures 2-3 present stress fields from the PV model within nine regions under 30% stretching, where an overlapped coordinate from 160 to 910 pixels is denoted. The overall circumferential stresses ($\sigma_1$) are apparently higher than radial stresses ($\sigma_2$) and shear stresses ($\tau_{12}$) (not shown) in all models. Stress concentrations are observed around perimeters of cell nuclei. To understand the changes in peak stresses around cells in the PV tissue model during equibiaxial stretching, three locations are selected. They are denoted by an arrow (A), a box (B), and a circle (C) in regions 4, 5, and 7, respectively (Fig. 2-3). The results illustrates that the tissue-level overall circumferential stresses ($\sigma_1$) are apparently higher than radial stresses ($\sigma_2$). It is concluded that localized peak stresses around cell nuclei are directional-dependent, suggesting the preferred collagen fiber direction has greater effects on how forces are transited to cells. Such higher stresses may be mediated by VICs for biomechanical regulations.

Figure 4 illustrates a nonlinear stress evolution for the selected VIC. It is observed that $\sigma_1$ rapidly increases and reaches the maximum at locations 4-9 on the cell boundary.
The maximum values of $\sigma_2$ are found at locations 1-3 and 21 on the cell boundary (Fig. 4). It is suggested that higher cellular activities occur along the boundary parallel to the preferred collagen fiber direction. This clearly indicates that the increasing effective load in the circumferential direction due to the collagen fiber direction is a very critical factor for the peak stresses in cells.

Figure 5 illustrates a nonlinear strain evolution for the selected VIC. It is observed that $\varepsilon_2$ exhibit much more nonlinear response than $\varepsilon_1$ does. It could be attributed by collagen fiber directions: compliant materials properties are generally observed along the radial direction of heart valve tissue. Therefore, VICs experience larger deformation along the radial direction, even though they are subjected by larger stress stimulations along the circumferential direction (Fig. 4).

Stress transfer from the matrix to the cells is highly dependent on matrix mechanical and biochemical properties, collagen fiber microstructure, and VIC distribution and morphology. The potential cellular activity around the boundary of the cell in the mechanical environment is considered to be corresponding with the stress transfer [23, 24]. It is considered that VICs have a great influence on the mechanics of the heart valve by the cell-matrix interaction [8-12, 25-30]. Observations of VICs clarify that biomechanical properties of heart valves highly correspond with cellular functions, that is modulated by the mechanical environment [23], such as tissue synthesizing and remodeling mediated by VICs [24]. It has been reported that VICs are able to change their phenotypes and functions to mediate tissue growth, repair and remodeling while sensing mechanical stimuli [7, 13-17]. Moreover, phenotypes of VICs are important to the mechanics of the heart valve and biomechanical regulation of valve function from cellular level to tissue level. Mechanical forces (stress) or deformations (strain) subjected on the ECM influence the regulation of cell phenotypes [31, 32]. Increases of the aggregation of the cytoskeleton, the degree of cell spreading, and the contractile force on the ECM via cellular deformation may contribute changes of cell phenotypes.

CONCLUSION

The current study not only demonstrates the stress transmission and reception between cells and the matrix in heart valve tissue, but it also provides an insight into the relationship between the heart valve mechanics, microstructure and material properties. In addition, the developed virtual experiments provide a necessary complement to the visualization of the overall stress distributions in heart valves. This approach, by incorporating the collagen fiber architecture, cell distributions, and the anisotropic material properties of the ECM, highlights the stress evolutions around VICs during diastole. Through the investigation of matrix-to-cell mechanical interactions in heart valves, it contributes to a better understanding of cellular mechanotransduction, cell migration, matrix synthesis, and tissue remodeling in heart valves.

REFERENCES


