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Modeling the elastic anisotropy of woven hierarchical tissues: experimental comparison on biological materials and design of a new class of scaffolds

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Abstract. This paper models the elastic properties of 2-D woven hierarchical tissues, assuming an orthotropic material of warp and fill yarns at level 0. Considering matrix transformation and stiffness averaging, stiffness matrices of warp and fill yarns of the tissue at level i are employed to calculate those of the tissue at level i+1. We compare our theory with another approach from the literature on tendons and experiments on leaves performed by ourselves. The result shows the possibility of designing a new class of hierarchical 2-D scaffolds with desired elastic anisotropy, better matching the anisotropy of the biological tissues and thus maximizing the regeneration.

Introduction

Many soft biological tissues exhibit anisotropic, inhomogeneous and nonlinear mechanical behaviors [1,2,3], because of the random orientation and mechanical properties of the collagen molecule, e.g. the heart valve tissue [4]. Besides, the structure of a tissue may be described at different several hierarchical levels, with dimensions ranging from the nano-scale to the macro-scale; for instance, in describing a tendon, there are five distinctive levels from collagen molecule to the tendon itself [5,6]. Accordingly, numerous contributions are today devoted to create hierarchical scaffolds in order to match the mechanical and structural properties of natural tissues at all the hierarchical levels, a key requirement to maximize the tissue regeneration.

On one hand, Moutos et al. [7] developed a three-dimensional woven composite scaffold with the proper anisotropy for cartilage tissue engineering, and experimental results show that the mechanical properties are comparable to those of the native articular cartilage. Several papers have reported the anisotropic characteristics of woven fabric suggesting a similar mechanical behavior between woven fabric and soft tissues. In this regard, woven fabric seems to be suitable for designing biological tissues.

On the other hand, the structural hierarchy is difficult to be produced. Traversa et al. [8] developed a hierarchical scaffold basing on traditional methods and Ahn et al. [9] developed a hierarchical structure by combining solid free-form fabrication (SFF) with electro-spinning process; both structures improved the cell proliferation and differentiation. However, traditional methods are not so easy to control the fabrication process.

In this paper, hierarchical woven fabrics are studied at all the hierarchical levels to match the elastic anisotropy of natural tissues. Based on matrix transformation and stiffness averaging, the anisotropy of the scaffolds is controlled by changing the orientation angles of fill and warp yarns and/or the volumetric fraction of fibers at different hierarchical levels. Prediction results of tendons using an approach from the literature and experimental results performed by ourselves on leaves are compared with the hierarchical theoretical predictions, showing a relevant agreement.

Hierarchical Theory

The structure is modeled as a continuum at each level [10] and yarns are assumed to be orthotropic; matrix transformation [11] is used to deal with the warp and fill yarns' orientation and stiffness averaging by volumetric fraction of warp and fill yarns [12] is employed to calculate the stiffness matrix of the hierarchical woven tissue.

First, three sets of coordinate systems (Local coordinate systems: $1^{(i)}_W - 2^{(i)}_W$ for warp yarns and $1^{(i)}_F - 2^{(i)}_F$ for fill yarns; Global coordinate system: $x^{(i)} - y^{(i)}$) are introduced (Fig. 1). Then, the elastic matrices for the hierarchical structure are mathematically expressed as:

$$\begin{cases} [Q]^{(i-1)}_{F,F} = \left[T(\beta^{(i)}_{F,F})\right]^{-1}\left[Q^*\right]^{(i-1)}_F\left[T(\beta^{(i)}_{F,F})\right] \\ [Q]^{(i-1)}_{F,W} = \left[T(\alpha^{(i)}_{F,W})\right]^{-1}\left[Q^*\right]^{(i-1)}_W\left[T(\alpha^{(i)}_{F,W})\right] \end{cases} ; \begin{cases} [Q]^{(i-1)}_{W,F} = \left[T(\beta^{(i)}_{W,F})\right]^{-1}\left[Q^*\right]^{(i-1)}_F\left[T(\beta^{(i)}_{W,F})\right] \\ [Q]^{(i-1)}_{W,W} = \left[T(\alpha^{(i)}_{W,W})\right]^{-1}\left[Q^*\right]^{(i-1)}_W\left[T(\alpha^{(i)}_{W,W})\right] \end{cases} \tag{1}$$

$$\begin{cases} [Q]^{(i)}_F = v^{(i)}_{F,F}[Q]^{(i-1)}_{F,F} + v^{(i)}_{F,W}[Q]^{(i-1)}_{F,W} = v^{(i)}_{F,F}\left(\left[T(\beta^{(i)}_{F,F})\right]^{-1}\left[Q^*\right]^{(i-1)}_F\left[T(\beta^{(i)}_{F,F})\right]\right) + v^{(i)}_{F,W}\left(\left[T(\alpha^{(i)}_{F,W})\right]^{-1}\left[Q^*\right]^{(i-1)}_W\left[T(\alpha^{(i)}_{F,W})\right]\right) \\ [Q]^{(i)}_W = v^{(i)}_{W,F}[Q]^{(i-1)}_{W,F} + v^{(i)}_{W,W}[Q]^{(i-1)}_{W,W} = v^{(i)}_{W,F}\left(\left[T(\beta^{(i)}_{W,F})\right]^{-1}\left[Q^*\right]^{(i-1)}_F\left[T(\beta^{(i)}_{W,F})\right]\right) + v^{(i)}_{W,W}\left(\left[T(\alpha^{(i)}_{W,W})\right]^{-1}\left[Q^*\right]^{(i-1)}_W\left[T(\alpha^{(i)}_{W,W})\right]\right) \end{cases} \tag{2}$$

where, $\left[Q^*\right]^{(i-1)}_F, \left[Q^*\right]^{(i-1)}_W$ are the stiffness matrices of fill and warp yarns at level (i−1) in the local systems at level i; $\left[T(\beta^{(i)}_{F,F})\right]$, $[Q]^{(i-1)}_{F,F}$ and $v^{(i)}_{F,F}$ ($\left[T(\beta^{(i)}_{F,W})\right]$, $[Q]^{(i-1)}_{F,W}$ and $v^{(i)}_{F,W}$) are transformation matrix, after-transformation stiffness matrix and volumetric fraction of fill (warp) yarns at level (i-1), composing the fill yarns at level i; $\left[T(\beta^{(i)}_{W,F})\right], [Q]^{(i-1)}_{W,F}$ and $v^{(i)}_{W,F}$ ($\left[T(\beta^{(i)}_{W,W})\right]$, $[Q]^{(i-1)}_{W,W}$ and $v^{(i)}_{W,W}$) are transformation matrix, after-transformation stiffness matrix and volumetric fraction of fill (warp) yarns at level (i-1), composing the warp yarns at level i; $[Q]^{(i)}_F$, $[Q]^{(i)}_W$ are stiffness matrices of fill and warp yarns at level i in the global coordinate system.

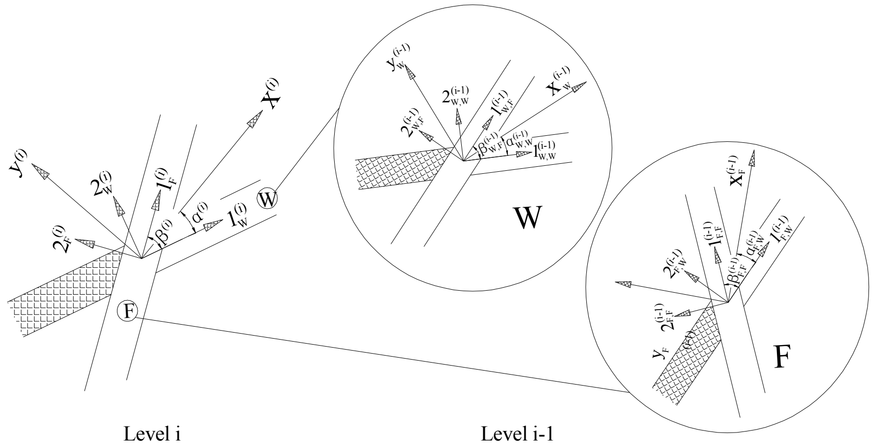

Fig. 1 Schematic of hierarchical woven tissue

In particular, this process can be used in the presence of just one type of fiber, e.g. by removing the warp yarns. Then, adding a matrix term, Eq. (1) and Eq. (2) can be rearranged as:

$$[Q]^{(i)} = \prod_{j}^{i} v^{(j)}_f \times \left[T(\sum_{j=1}^{i}\beta^{(j)})\right]^{-1}\left[Q^*\right]^{(0)}_f\left[T(\sum_{j=1}^{i}\beta^{(j)})\right] + (1 - \prod_{j=1}^{i} v^{(j)}_f)[Q]_M \tag{3}$$

where, $v^{(j)}_f$ and $\beta^{(j)}$ are the volumetric fraction and orientation angle of the fiber at the j[th] level, respectively; $\left[Q^*\right]^{(0)}_f$, $[Q]_M$ are the elastic matrices of the fiber at level 0 in the local coordinate system and the matrix.

Influence of the constituents on the overall elasticity of tendons

Tendons are typical hierarchical tissue and have five levels that are collagen molecule, collagen fibril, collagen fiber, fascicle and tendon. Here, tendon is treated as a hierarchical woven tissue, which only has fill yarns, composed by two phases, i.e. collagen and matrix; proteoglycan and water are treated as the matrix. The hierarchical model of tendons is shown in Fig. 2.

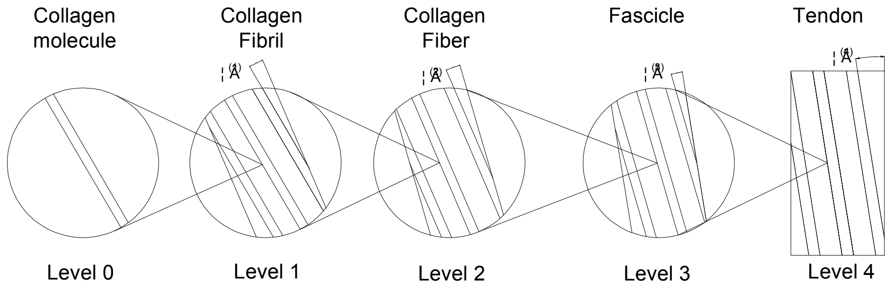

Fig. 2 Hierarchical model of tendons

Mow et al. [13] gave the weight percentage of the constituents in tendons, i.e. 23% for collagen, 7% for proteoglycan and 70% for water. Thus, the volumetric fractions can be derived from the densities: 1.2 g/cm^2 for collagen [14], 1.4 g/cm^2 for proteoglycan [15] and 1.0 g/cm^2 for water. Thus, the volumetric fractions 21% and 79% are obtained for collagen and matrix. Here, the elastic constants of tendons are: E_1=750 MPa; E_2=12 MPa; μ_{12}=2.98; G_{12}=5 MPa [16,17,18]; whereas for the matrix: E=1 MPa; μ=0.25; G_{12}=0.4 MPa [19]. Treating the elastic constants of tendon and matrix as input parameters and considering the conditions of $\beta^{(i)}=0$ and $v_f^{(i)}=0.677$ deduced from $v_f=21\%$, the elastic constants of collagen molecule are obtained by employing Eq. (3): $E_1^C=3536$ MPa; $E_2^C=53.2$ MPa; $\mu_{12}^C=3.16$ and $G_{12}^C=22.3$ MPa. With the material constants of collagen molecule and matrix, we investigate the influence of the collagen orientation and volumetric fraction of fiber.

Influence of collagen orientation. The previous description about the collagen orientation of tendons is parallel. Fechete et al. [20] reported that the angular distribution of collagen fibrils around the symmetric axis of the tendon was described by a Gaussian function with a standard deviation of 12^o±1^{o}and with the center of the distribution at 4^o±1^o. Accordingly, employing the result of collagen molecule, the hierarchical prediction of Young's modulus is investigated here and compared with a different approach from the literature [12], and the result is plotted in Fig. 3. It shows that the result determined by the different theory is in close agreement, even if slightly lower than that determined by our hierarchical theory.

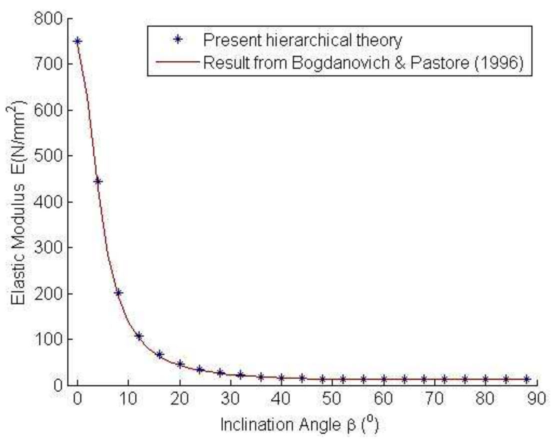

Fig. 3 Comparison of elastic modulus of tendons *vs* collagen orientation angle between hierarchical theory and literature

Influence of the total volume of collagen. The volumetric fraction of collagen molecule is another important parameter influencing the material constants. Varying in the range 10%–30%, with incremental 4%, the influence on each hierarchical level is reported in Fig. 4. The results quantify how the elastic properties are improved as the total volume of collagen increases.

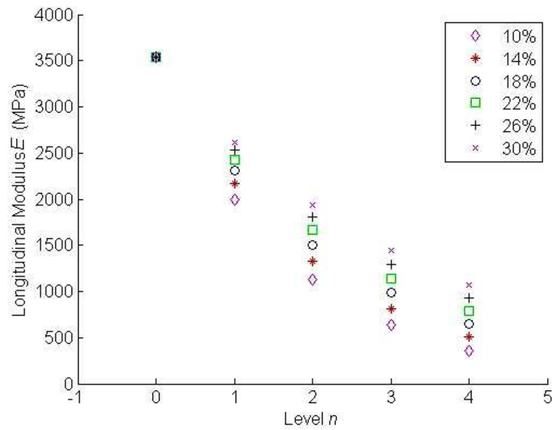

Fig. 4 Longitudinal elastic modulus of tendon constituents *vs* related hierarchical levels for different volumetric fractions

Experiments on the Aechmea aquilegia leaf

Experimental procedure. In order to investigate the relationship between material constants and fiber orientation, we carried out *ad hoc* tensile tests employing a MTS micro-tensile machine. A leaf

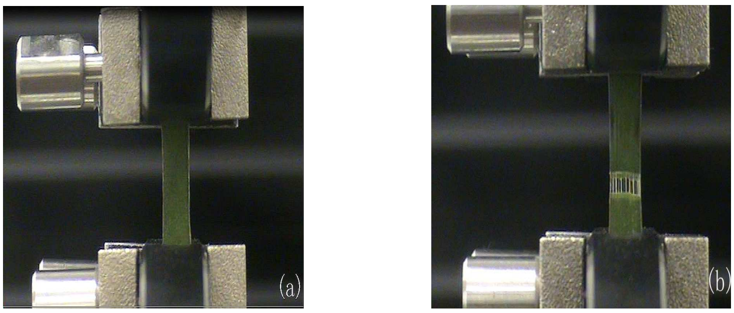

Fig. 5 Experimental process: (a) loading before failure; (b) failure with yield of emerging fibers.

of the Aechmea aquilegia was cut into 30 specimens with dimension 30mm×3mm×0.4mm; fiber inclination angles vary from 0^{o} to 90^{o} with 10^{o} incremental. The whole process was displacement controlled with loading speed 1mm/min (Fig. 5). After that, specimens were examined using SEM (Fig. 6).

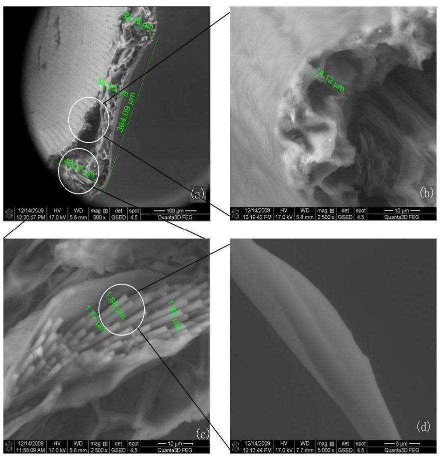

Fig. 6 The structural hierarchy of the aechmea aquilegia leaf

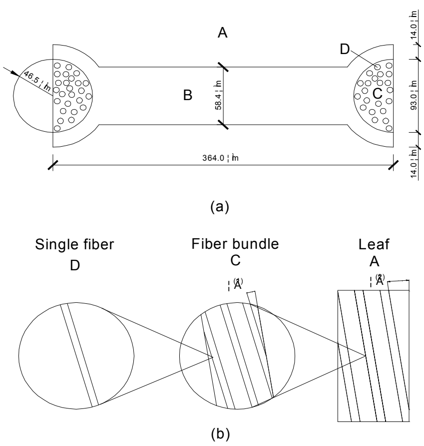

Fig. 7 Hierarchical model of the aechmea aquilegia leaf

Experimental results and prediction of the hierarchical theory. The results of peak stress (or strength), peak strain and Young's modulus are listed in Table 1. It shows that, generally, they decrease as the fiber orientation angle increases.

Table 1 Experimental results on Aechmea aquilegia leaf

Angle (°)	0	10	20	30	40	50	60	70	80	90
Peak stress [MPa]	11.3±0.1	8.9±0.1	6.8±1.5	4.8±0.7	3.2±0.9	3.7±0.3	2.0±0.5	2.6±0.4	2.8±0.3	2.1±0.6
Peak strain [mm/mm]	0.17±0.00	0.21±0.00	0.19±0.03	0.18±0.02	0.16±0.05	0.17±0.05	0.12±0.04	0.15±0.06	0.20±0.01	0.12±0.03
Young's modulus [MPa]	127.0±3.5	87.2±7.2	62.1±4.4	47.8±4.4	29.3±2.2	31.2±3.7	18.5±1.9	21.3±3.0	16.4±1.9	18.7±0.3

Due to the schematic of the crack mouth (Fig. 5(b)) and the direct SEM experimental observations (Fig. 6), a hierarchical model composed by three levels, in which A, B, C and D parts in Fig. 7 are corresponding to Fig. 6(a)-(d), is built. The four fitting elastic parameters of the leaf are obtained by the experimental data of the Young's modulus in Table 1; we accordingly calculate: E_1=121.8MPa; E_2=19.3MPa; μ_{12}=0.26; G_{12}=10.9MPa. The matrix is assumed to be isotropic with E=19.3 MPa; μ=0.25; G_{12}=7.7 MPa. The volumetric fraction $v_f^{(1)}=0.9$ is estimated and $v_f^{(2)}$ is calculated from SEM observations, as ~26.5%. Finally, treating the elastic constants of the leaf and matrix as input parameters and considering the conditions of $v_f^{(1)}=0.9$; $v_f^{(2)}=0.265$ and $\beta^{(1)}=\beta^{(2)}=0$, the material constants of single fiber are derived using Eq. (3): E_1=449 MPa; E_2=16 MPa; μ_{12}=0.30 and G_{12}=21 MPa. With the given elastic constants of the matrix and single fiber, the comparison between experimental data and present hierarchical theory is plotted in Fig. 8.

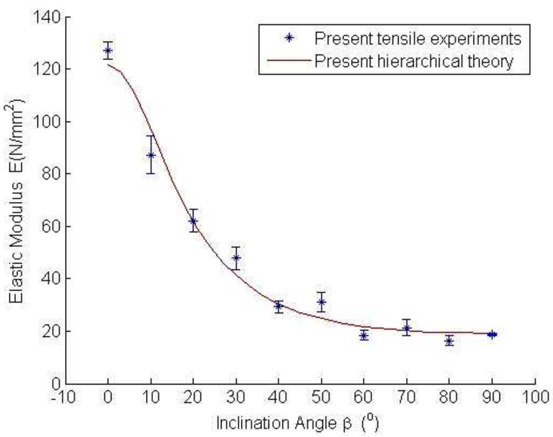

Fig. 8 Comparison between theoretical prediction and experimental data

Conclusion

The method stated in this paper shows the possibility of designing hierarchical tissues with desired elastic properties. According to the analysis on tendon and aechmea aquilegia leaf, the mechanical performance of bio-inspired tissues can be optimized by altering the orientation angle and volumetric fraction of fibers. Thus, starting from the elastic properties of natural tissues, this theory allows us to select an appropriate combination of orientation angles and volumetric ratios of fill and warp yarns in order to obtain the elastic constants close to those of the natural tissues at each hierarchical level, thus maximizing the tissue regeneration.

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