Numerical investigation of the influence of pattern topology on the mechanical behavior of PEGDA hydrogels

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Abstract

Poly(ethylene glycol) diacrylate (PEGDA) hydrogels can potentially be used as scaffold material for tissue engineered heart valves (TEHVs) due to their good biocompatibility and biomechanical tunability. The photolithographic patterning technique is an effective approach to pattern PEGDA hydrogels to mimic the mechanical behavior of native biological tissues that are intrinsically anisotropic. The material properties of patterned PEGDA hydrogels largely depend on the pattern topology. **Currently, designing patterned PEGDA hydrogels that meet targeted material properties is still a trial-and-error process. Depending on experiments alone to explore the influence of pattern topology is expensive and time-consuming.** In this paper, we adopt a newly proposed computational framework for fibrous biomaterials to numerically investigate the influences of pattern topology, including pattern ratio, orientation and waviness, on the mechanical behavior of patterned PEGDA hydrogels. The material parameters for the base hydrogel and the pattern stripes are directly calibrated from published experimental data. Several experimental observations reported in literature are captured in the simulations, including the nonlinear relationship between pattern ratio and material linear modulus, and the decrease of material anisotropy when pattern ratio increases. We further numerically demonstrate

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that a three-region (toe-heel-linear) stress-strain relationship typically exhibited by biological tissues can be obtained by tuning the pattern geometry and the material properties of the base hydrogel and the pattern stripes. The numerical strategy and simulation results presented in this paper can provide guidance to optimize the pattern design of PEGDA hydrogels toward the targeted material properties, therefore advance the development of TEHVs.

Keywords: PEGDA hydrogel, Pattern topology, Finite Element Method

1. Introduction

Valvular heart disease involves the malfunction of one or more of the four heart valves. The symptoms range from moderate palpitation to lethal heart failure. According to a recent study, an estimate of 4.2 to 5.6 million adults
5 in the U.S. have valvular diseases [1]. Currently, the preferred method to treat heart valve patients is to repair the diseased valves. However, when the valve disease is severe, just repairing is not sufficient and therefore replacement is required [2]. Each year nearly 300,000 valve replacement surgeries are performed worldwide, and this number is predicted to triple in the next 30 years due to
10 the increase of the aging population [2, 3].

Nowadays there are two basic types of artificial valves that are clinically available for replacement, mechanical valves and bioprosthetic valves. Mechanical valves are made of strong durable materials, and in most cases can last throughout the remainder of the patient's lifetime. However, clot-risks are high
15 for patients with this type of valves. Consequently, lifelong treatment with blood thinner is required to keep clots from forming. On the other hand, bioprosthetic valves are created from animal valves, and do not require long-term use of medication. But their relatively short lifespan (from 10 to 20 years) increases the likelihood of extra surgeries. Biocompatibility is another issue related with bio-
20 prosthetic valves, since human bodies tend to reject foreign materials. Moreover, a major disadvantage of both types is that valve tissue growth and remodeling are not allowed after implantation. This is a significant drawback especially for

young patients, whose heart valves must grow over time [4]. With the recent advance in *tissue engineering*, tissue engineered heart valves (TEHVs) provide a promising potential to generate suitable heart valve replacements [5, 6, 7, 8, 9].

TEHVs usually require biodegradable scaffolds to encapsulate cells for tissue growth and remodeling, and simultaneously must provide sufficient mechanical functions after implantation, which is crucial for the survival of the patient [4]. The scaffolds for the most successful TEHVs to date are non-woven fiber mesh or salt-leached foam scaffolds generated from polyglycolic acid and polyhydroxyalkanoates [10, 11, 12]. However, these materials have certain limitations, including: (1) high initial stiffness compared with native tissues; (2) long time required for the polymer to undergo degradation [13]; (3) lack of intrinsic biological functionalization [6]. Because of the biochemical and biomechanical tunability, poly(ethylene glycol) diacrylate (PEGDA) hydrogels have received more and more attention to be used as scaffold materials for TEHVs [14, 15, 16, 6, 7, 8]. PEGDA hydrogel is a soft bio-inspired material that possesses many important features, among which are biocompatibility, resistance to protein absorption, and tunable biochemical and biomechanical properties [17, 16]. Among its various biomedical applications [18, 19, 8], PEGDA hydrogel is being investigated as a scaffold material to fabricate TEHVs.

From the mechanical perspective, hydrogels are isotropic materials, possessing elastic moduli from around 1 kPa [20] to above 100 kPa [21, 22, 9]. In order to mimic the mechanical behavior of native heart valve tissues that are intrinsically anisotropic [23], advanced patterning and fabrication technologies are required. For example, using photolithographic patterning technology, PEGDA hydrogels can be three dimensionally patterned to exhibit desired anisotropic mechanical behavior [15, 14, 16, 9]. In this process, the microscale characteristics, such as crosslinking density and polymer chain length, can also be tailored. More recently, confocal images generated from native tissues are utilized to guide two-photon laser scanning lithography to pattern hydrogel scaffolds, and the original tissue images and generated hydrogel polymer networks are matched with good fidelity [24].

At the microscopic level, hydrogels are networks of crosslinked polymers and
55 solvent molecules [17, 15, 14, 18] that can be modeled as fiber networks. The mi-
croscopic parameters, such as crosslinking density, fiber density and fiber chain
length [25, 26, 27], largely influence the material macro-mechanical behavior.
At the macroscopic level, it would be desirable to have a patterned PEGDA
hydrogel that is a nonlinear, anisotropic material, and can sustain large defor-
60 mations. At the same time, the mechanical properties of patterned PEGDA
hydrogels should have the capability to be tuned by pattern topology.

In literature, native biological tissues are widely reported to exhibit a stress-
strain curve that can be divided into three regions, a toe region followed by a
heel region and a linear region [28, 20, 29, 4]. The toe region shows a relatively
65 low elastic modulus due to the fact that most collagen fibers are in the crimp
state and do not contribute to the material stiffness at small tensile strains.
As the strain increases, the collagen fibers start to be straightened, leading to
the upward bend of the stress-strain curve in the heel region. The linear region
exhibits a significantly larger linear modulus, since most collagen fibers are fully
70 straightened and contribute to the total material stiffness. In order to mimic
the mechanical behavior of native biological tissues, it is important to properly
design the pattern topology so that hydrogels can exhibit a similar three-region
stress-strain relationship.

Although many efforts have been devoted to study the material property
75 of hydrogels in recent years, the structure-function relationship of patterned
PEGDA hydrogel has not yet been fully understood. Consequently, designing
patterned PEGDA hydrogels that meet specific functional requirements is still
a trial-and-error process. Depending on experiments alone to investigate the
influence of pattern topology is time-consuming and expensive. On the other
80 hand, numerical methods, especially the finite element method (FEM), com-
bined with experiments, can provide better descriptions of PEGDA hydrogels
with complex geometric patterns, and eventually facilitate the material design
process.

In this paper, we adopt a newly proposed computational framework [30] to

85 numerically investigate the influence of pattern topology, including pattern ratio, orientation, and waviness, on the mechanical behavior of patterned PEGDA hydrogels. The material parameters used in the FE simulations are calibrated from published experimental data [9]. The nonlinear relationship between the pattern ratio and the hydrogel stiffness, which is a physical phenomenon reported by different researchers [16, 9], is successfully recovered. More importantly, the typical toe-heel-linear (three-region) shape of the stress-strain relationship is also captured in the numerical simulation by properly tuning the hydrogel properties. The numerical strategy and simulation results presented in this research can provide helpful guidances toward designing the pattern geometry of PEGDA hydrogels to mimic the targeted behavior of native heart valve tissues.

2. Methods

In this section, the photolithographic patterning technique [16, 9] used to pattern PEGDA hydrogel is briefly reviewed. The numerical strategy adopted by this paper is also introduced. Based on this strategy, the material parameters used in FE simulations are calibrated from the published experimental data. Using the calibrated material parameters, the influence of pattern geometry on the mechanical behavior of patterned PEGDA hydrogels is thoroughly investigated.

105 2.1. Photolithographic patterning

Photolithographic patterning techniques are widely used to pattern PEGDA hydrogels. Here, the patterning process adopted in [16, 9] is briefly described. PEGDA hydrogel with a high molecular weight (MW), in this case 20 kilodalton (kDa), was prepared as the base hydrogel by crosslinking under the collimated UV light. Next, the base hydrogel was soaked in the 3.4 kDa PEGDA solution to allow the low MW PEGDA diffuse into the high MW polymer network. Then, the hydrogel sample covered with a pre-designed photomask was exposed

under the collimated UV light. In the hydrogel under the transparent area of the photomask, 3.4 kDa PEGDA molecules were crosslinked into the 20 kDa
115 base hydrogel due to the exposure under the collimated UV light. Finally, the patterned hydrogel was soaked in the phosphate buffered saline (PBS) to remove the unreacted PEGDA molecules. Fig. 1 shows a sample of patterned PEGDA hydrogel with 20% pattern ratio. We refer to the base hydrogel soaked in the 3.4 kDa PEGDA solution and completely exposed under the UV light without
120 using a photomask as the 100% pattern. For the detailed PEGDA fabrication and patterning process, interested readers are referred to Fig. 1 in [16] or Fig. 2 in [9].

Fig. 1. Patterned PEGDA hydrogel sample (20% pattern ratio), image courtesy of Dr. Xing Zhang, Rice University

2.2. Base hydrogel and pattern stripe

From the photolithographic patterning process, it can be seen that patterned PEGDA hydrogel samples are made of two different components, the base hydrogel and the pattern stripes. The base hydrogel contains 20 kDa PEGDA molecules exclusively. On the other hand, the pattern stripes contain 20 kDa PEGDA molecules and crosslinks formed between 20 kDa and 3.4 kDa molecules. In the finite element (FE) simulation, the base hydrogel is described with the modified neo-Hookean constitutive model

$$\Psi_m(\mathbf{F}) = \frac{1}{4}K(J^2 - 1 - 2\ln J) + \frac{1}{2}G(\bar{I}_1 - 3), \quad (1)$$

where K is the bulk modulus, G is the shear modulus, $J = \det \mathbf{F}$ is the de-
 125 terminant of the deformation gradient tensor $\mathbf{F}$, and $\bar{I}_1 = J^{-\frac{2}{3}} \text{tr}(\mathbf{F}^T \mathbf{F})$ is the
 modified tensor invariant. The volumetric term in Eq. 1 serves as a penalty to
 approximately capture the material incompressibility.

Pattern stripes are modeled as polymer networks embedded in the base
 hydrogel. This modeling choice enables us to capture the nonlinear relationship
 130 between the material stiffness and the pattern ratio of PEGDA hydrogels, which
 is demonstrated in the later part of this work. The fiber network generated by
 a random walk algorithm is used to represent the crosslinks formed between the
 3.4 kDa and 20 kDa PEGDA molecules. The random walk algorithm has several
 control parameters. The fiber volume fraction ratio μ and the fiber diameter d
 135 determine the density of the fiber network. Each fiber chain is piecewise linear
 and composed of multiple fiber segments with the length l_{seg} . The initial angle
 θ_0 of individual fibers controls the network anisotropy. The relative angle θ_r
 between neighbouring segments follow the uniform distribution between $-\theta_{max}$
 and θ_{max} . The magnitude of θ_{max} influences the network tortuosity. For the
 details of the random walk algorithm, interested reader is referred to [30].

Fig. 2. (a) Pattern stripe meshed with four-node quad elements, the fiber network is generated
 by the random walk algorithm representing the crosslinks formed between 3.4 kDa and 20 kDa
 PEGDA molecules; (b) Projection of the DOFs of fiber segment to the DOFs of element nodes

140

Fig. 2a shows a pattern stripe meshed with four-node quad elements. Each
 finite element contains multiple piecewise linear fiber chains, and each fiber
 chain is composed of multiple segments. When a fiber segment crosses element

boundaries, the coordinates of the intersections formed by the segment and the
 145 element boundaries are calculated, and the segment is divided into multiple
 sub-segments. The coordinates of the intersections between fiber segments and
 element boundaries, the crosslinks among different fiber chains, and the two
 endpoints of each fiber segment are collected in $L = \{(x_i, y_i), i = 1, 2, 3, \dots\}$.

Inside an isoparametric element, the natural coordinates ξ and the Cartesian
 coordinates $\mathbf{x}$ have the following relationship:

$$\mathbf{x}(\xi) = N_a(\xi)\mathbf{x}_a^{(e)}, \quad (2)$$

where subscript a is the element nodal number, $\mathbf{x}_a^{(e)}$ is the element nodal co-
 150 ordinates of the e -th element, and N_a is the element interpolation function
 associated with node a . Based on Eq. 2, a mechanism illustrated in Fig. 2b is
 adopted to project the discrete degrees of freedom (DOFs) of fiber segments to
 the DOFs of element nodes. The natural coordinates ξ corresponding to the
 Cartesian coordinates of the fiber network recorded in L can also be obtained
 155 via the Newton-Raphson iterations.

The total strain energy of pattern stripes is expressed as follows

$$\Pi(\mathbf{u}) = \int_{\Omega_0} \Psi_m(\mathbf{F}(\mathbf{u})) d\Omega + \sum_{i=1}^N \Psi_f(\lambda^{(i)}(\mathbf{u})), \quad (3)$$

where $\mathbf{F}$ is the deformation gradient tensor, Ψ_m is the strain energy function
 representing the contribution from the base hydrogel and takes the form of
 Eq. 1, Ψ_f is the fiber strain energy function representing the contribution from
 the i -th segment, N is the total number of fiber segments contained in the fiber
 network, Ω_0 is the reference domain, and $\lambda^{(i)}$ is the stretch ratio of the i -th fiber
 segment. After spatial discretization, the total strain energy becomes

$$\Pi(\mathbf{u}) = \sum_{e=1}^{N_{el}} \Pi^{(e)}(\mathbf{u}) = \sum_{e=1}^{N_{el}} \left(\Pi_m^{(e)}(\mathbf{u}) + \Pi_f^{(e)}(\mathbf{u}) \right), \quad (4)$$

$$\Pi_m^{(e)}(\mathbf{u}) = \int_{\Omega_0^{(e)}} \Psi_m(\mathbf{F}(\mathbf{u})) d\Omega, \quad \Pi_f^{(e)}(\mathbf{u}) = \sum_{i=1}^{N^{(e)}} \Psi_f(\lambda^{(i)}(\mathbf{u})), \quad (5)$$

where N_{el} is the total number of finite elements in the discretized system, $\Pi^{(e)}(\mathbf{u})$ is the strain energy of the e -th element and decoupled into two parts, the contributions from the base hydrogel $\Pi_m^{(e)}(\mathbf{u})$ and the embedded crosslinks formed between the 3.4 kDa and 20 kDa molecules $\Pi_f^{(e)}(\mathbf{u})$, $\Omega_0^{(e)}$ is the spatial domain occupied by the e -th element in the reference configuration, and $N^{(e)}$ is the number of fiber segments contained inside the e -th element.

The fiber segments are modeled as truss elements in the FE simulation with the assumption that fiber stretching is the main deformation mode [29, 27]. Since it is a widely accepted assumption that wavy fibers can only sustain tensile forces, but are not able to resist compression [31, 32, 33], the contribution from a fiber segment is switched off if its stretch ratio $\lambda < 1$, indicating the compressive status. Considering the linear stress-strain relationship of the PEGDA hydrogels reported in the experiment [9], the fiber strain energy function Ψ_f takes a polynomial form

$$\Psi_f(\lambda) = \begin{cases} \frac{1}{2}k_f L_0^2 \frac{(\lambda - 1)^2}{\lambda^{z_f}} & \lambda \geq 1 \\ 0 & 0 < \lambda < 1, \end{cases} \quad (6)$$

where k_f is the axial elastic modulus of fiber segment, z_f is a dimensionless parameter controlling the fiber nonlinearity, L_0 is the segment original length, and λ is the stretch ratio. It can be verified that $\Psi_f(\lambda)|_{\lambda=1} = 0$ and $\partial\Psi_f(\lambda)/\partial\lambda|_{\lambda=1} = 0$, indicating the continuity of the fiber strain energy and its first derivative at $\lambda = 1$, and a stress-free state when no deformation occurs. Furthermore, the dimensionless parameter z_f has to satisfy the constraint $z_f \leq 1$ to guarantee the convexity of the fiber strain energy function (Eq. 6). The detailed convexity analysis is shown in the Appendix.

For an arbitrary fiber segment contained in an element, $\mathbf{X}^{(1)}$ and $\mathbf{X}^{(2)}$ are used to represent the Cartesian coordinates of its two endpoints in the reference configuration. $\mathbf{x}^{(1)}$ and $\mathbf{x}^{(2)}$ are used to represent the corresponding coordinates in the deformed configuration. Based on Eq. 2, the stretch ratio of the fiber

segment is expressed as follows:

$$\lambda = \frac{l}{L_0} = \frac{\|\mathbf{x}^{(2)} - \mathbf{x}^{(1)}\|_2}{\|\mathbf{X}^{(2)} - \mathbf{X}^{(1)}\|_2} = \frac{\|N_a(\boldsymbol{\xi}^{(2)})\mathbf{x}_a - N_a(\boldsymbol{\xi}^{(1)})\mathbf{x}_a\|_2}{\|N_A(\boldsymbol{\xi}^{(2)})\mathbf{X}_A - N_A(\boldsymbol{\xi}^{(1)})\mathbf{X}_A\|_2}. \quad (7)$$

170

Following a variational approach [30], the component forms of the element residual $\mathbf{R}_f^{(e)}$ and the stiffness matrix $\mathbf{K}_f^{(e)}$ contributed by a fiber segment under tension ($\lambda \geq 1.0$) are derived:

$$R_{f,iA}^{(e)} = k_f \frac{\lambda - 1}{\lambda^{z_f+1}} \left(1 - \frac{z_f}{2} + \frac{z_f}{2}\lambda^{-1}\right) (x_i^{(2)} - x_i^{(1)}) (N_A(\boldsymbol{\xi}^{(2)}) - N_A(\boldsymbol{\xi}^{(1)})) \quad (8)$$

and

$$\begin{aligned} K_{f,AiBj}^{(e)} = & k_f \frac{\lambda - 1}{\lambda^{z_f+1}} \left(1 - \frac{z_f}{2} + \frac{z_f}{2}\lambda^{-1}\right) \\ & (N_A(\boldsymbol{\xi}^{(2)}) - N_A(\boldsymbol{\xi}^{(1)})) (N_B(\boldsymbol{\xi}^{(2)}) - N_B(\boldsymbol{\xi}^{(1)})) \delta_{ij} \\ & + \frac{k_f}{L_0^2 \lambda^{z_f+2}} \left[\frac{z_f(z_f+2)}{2} \lambda^{-2} + (1 - z_f^2) \lambda^{-1} + \frac{z_f(z_f-2)}{2} \right] \\ & \left[(x_i^{(2)} - x_i^{(1)}) (N_A(\boldsymbol{\xi}^{(2)}) - N_A(\boldsymbol{\xi}^{(1)})) \right] \\ & \left[(x_j^{(2)} - x_j^{(1)}) (N_B(\boldsymbol{\xi}^{(2)}) - N_B(\boldsymbol{\xi}^{(1)})) \right], \end{aligned} \quad (9)$$

where A, B are the element nodal numbers, i, j are the Cartesian directions, $x_i^{(1)}$ and $x_i^{(2)}$, $\boldsymbol{\xi}^{(1)}$ and $\boldsymbol{\xi}^{(2)}$ are the Cartesian and natural coordinates of the two endpoints of the i -th segment, and N_A is the element interpolation function of node A .

The total element residual $\mathbf{R}^{(e)}$ and the stiffness matrix $\mathbf{K}^{(e)}$ are the sum of the contributions from the base hydrogel and the fiber network representing the crosslinks contained in the corresponding element

$$\mathbf{R}^{(e)} = \mathbf{R}_m^{(e)} + \sum_i \mathbf{R}_{f_i}^{(e)}, \quad \mathbf{K}^{(e)} = \mathbf{K}_m^{(e)} + \sum_i \mathbf{K}_{f_i}^{(e)}. \quad (10)$$

Comparing the modeling strategies for the base hydrogel and the pattern, the strain energy of a finite element can be generalized as follows:

$$\Pi^{(e)}(\mathbf{u}) = \begin{cases} \Pi_m^{(e)}(\mathbf{u}) & N^{(e)} = 0 \\ \Pi_m^{(e)}(\mathbf{u}) + \Pi_f^{(e)}(\mathbf{u}) & N^{(e)} > 0. \end{cases} \quad (11)$$

175 Recall that $N^{(e)}$ represents the number of fiber segments contained in the e -th element. When $N^{(e)} = 0$, the element is only composed of the base hydrogel represented by $\Pi_m^{(e)}(\mathbf{u})$. When $N^{(e)} > 0$, the element is composed of the base hydrogel $\Pi_m^{(e)}(\mathbf{u})$ reinforced by the crosslink network, the contribution of which is represented by $\Pi_f^{(e)}(\mathbf{u})$.

180 The published experimental data from the uniaxial tension tests of the base hydrogel and 100% pattern [9] are used to calibrate the material parameters needed for the FE simulations. Fig. 3 shows the boundary conditions used in the 2D plane stress FE simulation that mimic the experimental setup.

Fig. 3. Clamped-clamped boundary conditions are applied at the two ends of the hydrogel samples to mimic the setup of the uniaxial tension test

Recall that the base hydrogel is described by the modified neo-Hookean model (Eq. 1) with Poisson's ratio $\nu = 0.49$ to approximately capture the material incompressibility. The only material parameter to determine is Young's modulus E , which is calibrated by solving the following optimization problem

$$E = \arg \min_E \sum_{i=1}^m \|P_{11}(E, \lambda^{(i)}) - P_{11}^*(\lambda^{(i)})\|_2, \quad (12)$$

185 where P_{11} is the nominal stress (force divided by the sample cross section area in the initial configuration) in the loading direction obtained from the FE simulation, P_{11}^* is the counterpart obtained from the experiment, λ is the sample stretch ratio (strain), and m is the number of available experimental data pairs. Unless otherwise specified, in the following stress refers to the nominal stress and strain refers to the sample stretch ratio.

The 100% pattern sample is modeled as a fiber network (representing the crosslinks formed between 20 kDa and 3.4 kDa molecules) embedded in the

base hydrogel. According to Eq. 4, the total strain energy of the 100% pattern sample is decoupled into the contributions from the base hydrogel and the crosslinks formed between 3.4 kDa and 20 kDa molecules. Therefore, the Young's modulus E of the base component in the pattern stripe takes the same value as calibrated according to Eq. 12. Fig. 3b shows a 100% pattern sample with an embedded fiber network synthetically generated via the 2D random walk algorithm. Since the parameters of the crosslinks are not available, part of the parameter values are chosen from the range reported in the literature. For example, the fiber diameter $d = 0.45$ nm [20]. The crosslink network in the pattern sample is isotropic, therefore all the fiber chains are evenly distributed in different orientations without a preferred direction, $\theta_0 \sim \text{unif}(0^\circ, 360^\circ)$. The relative angle between neighbouring segments follows a uniform distribution, $\theta_r \sim \text{unif}(-90^\circ, 90^\circ)$. The fiber volume fraction ratio (crosslink density) μ , the segment length l_{seg} , the fiber axial stiffness matrix k_f , and the fiber nonlinear parameter z_f are treated as an ensemble and calibrated by solving the following optimization problem

$$(\mu, l_{seg}, k_f, z_f) = \arg \min_{(\mu, l_{seg}, k_f, z_f)} \sum_{i=1}^m \|P_{11}(\mu, l_{seg}, k_f, z_f, \lambda^{(i)}) - P_{11}^*(\lambda^{(i)})\|_2, \quad (13)$$

190 where P_{11} and P_{11}^* are the nominal stresses obtained from the FE simulation and the experiment of the 100% pattern sample, respectively.

2.3. Pattern topology

The mechanical behavior of patterned PEGDA hydrogel largely depends on the pattern topology. The FE simulation result of the hydrogel sample with
 195 20% pattern ratio is firstly compared with the experimental data reported in [9]. Then, a thorough numerical investigation is performed to examine the influence of different pattern geometries, including pattern ratio, orientation and waviness, on the material mechanical behavior. Through this process, the nonlinear relationship between the pattern ratio and the material linear stiff-
 200 ness, which is an important phenomenon reported in the literature, is recovered. Furthermore, the three-region (toe-heel-linear) stress-strain relationship that is

typically exhibited by biological tissues is also captured by tuning the hydrogel property.

2.3.1. Pattern ratio

During the photolithographic patterning process, the pattern ratio of hydrogel samples can be tuned according to the pre-designed photomask. In the experiment [9], PEGDA hydrogel samples with parallel pattern stripes were synthesized. The width of each pattern stripe was $100\ \mu\text{m}$, and the width of the base hydrogel between two neighbouring pattern stripes was $400\ \mu\text{m}$. Therefore, the pattern ratio of the sample was 20%. In the uniaxial tension test, the hydrogel samples with 20% pattern ratio were stretched along the parallel and perpendicular directions with respect to the stripe orientation, respectively. Fig. 4 shows the boundary conditions used in the FE simulation mimicking the setup of the experiments. The crosslink network contained in the pattern stripes are generated via the random walk algorithm. The material parameters calibrated from the base hydrogel and the 100% pattern according to Eqs. 12 and 13 directly used in the FE simulation, the numerical results obtained from which are compared with the experimental data.

Fig. 4. Boundary conditions used in the FE simulations to mimic the setup of the uniaxial tension tests of hydrogel samples with 20% pattern ratio

Through similar approaches, hydrogel samples with other pattern ratios (40%, 60% and 80%) are generated via the random walk algorithm, and their mechanical behavior is numerically investigated to quantify the influence of pattern ratio.

2.3.2. Pattern orientation

In the uniaxial tension tests of the hydrogel sample with 20% pattern ratio, the loading direction is either parallel (0°) or perpendicular (90°) to the pattern orientation. In the FE simulations, the mechanical behavior of hydrogel samples with 20% pattern ratio that form different angles with respect to the loading direction is numerically investigated to quantify the influence of pattern orientation. Fig. 5 shows the hydrogel samples in which the pattern stripes form different angles with respect to the uniaxial tension loading. The crosslink network in the pattern stripes is generated via the random walk algorithm, and the material parameters for the base hydrogel and the pattern stripes take the values calibrated according to Eqs. 12 and 13.

Fig. 5. Pattern stripes form different angle θ with respect to the tensile loading

2.3.3. Pattern waviness

In the experiments conducted in [9], all the pattern stripes were straight and parallel to each other. With the help of the numerical strategy, the influence of more complex pattern geometries can be explored in FE simulations. For example, wavy patterns can be adopted to tune the material mechanical behavior. A rectangular PEGDA hydrogel sample patterned with a single sinusoidal-shape stripe is numerically constructed. In the 2D plane stress FE simulation, clamped-clamped boundary conditions are used. The hydrogel sample has the dimensions of 25×5 mm and undergoes displacement controlled uniaxial ten-

sion. The influence of the pattern waviness, characterized by the ratio between the amplitude A and the half wavelength L of the sinusoidal pattern stripe, is quantified numerically. We investigate the influence of A/L following two approaches. First, the half wavelength L is fixed and the amplitude A varies. This approach generates the same number of half waves N in the sample (Fig. 6). Then, the amplitude A is fixed and the half wavelength L varies, generating different number of half waves N in the sample (Fig. 7).

Fig. 6. Hydrogel sample with a sinusoidal stripe under uniaxial tension, the half wavelength L is fixed and the amplitude A varies

Fig. 7. Hydrogel sample with a sinusoidal stripe under uniaxial tension, the amplitude A is fixed and the half wavelength L varies

One of the design objectives is to ensure patterned PEGDA hydrogels possess a stress-strain relationship similar to the one typically exhibited by native biological tissues, as shown in Fig. 8. The shape of the stress-strain curve of the patterned PEGDA hydrogel shown in Figs. 6 and 7 mainly depends on two

factors, the relative stiffness between the pattern stripe and the base hydrogel,
 255 and the pattern stripe waviness. Recall that the material parameters of the
 pattern stripe (μ, l_{seg}, k_f, z_f) are calibrated as an ensemble, where μ represents
 the crosslink density. Generally speaking, the pattern stiffness increases as the
 crosslink density μ increases. In the FE simulation, by means of tuning the
 value of μ while keeping the other parameters constant, the influence of the
 260 relative stiffness between the pattern stripe and the base hydrogel on the shape
 of the stress-strain curve of patterned PEGDA hydrogels is investigated.

Fig. 8. A typical stress-strain curve of biological tissues can be divided into three regions, a toe region followed by a heel region and a linear region

3. Results

The generation of different geometric patterns in the hydrogel samples is
 based on the random walk algorithm and automated in MATLAB[®]. According
 265 to the component forms of the element residual and stiffness matrix shown in
 Eqs. 8 and 9, a user element subroutine is implemented in an open source finite
 element analysis package FEAP [34].

In the random walk algorithm used for generating the fiber network, the
 initial angle θ_0 of each fiber chain and the relative angle θ_r between two neigh-
 270 boring fiber segments are random variables and follow two uniform distributions,
 respectively. The starting point of each fiber chain is also random. As a result,
 even with the same values of the algorithmic parameters, the generated fiber

network varies between identical simulations. To consider the influence of the stochastic nature of the network on the numerical results, the Monte Carlo (MC) method is combined with FE simulations to capture the material average behavior. In this research, we focus on the material stress-strain relationship and the following MC approach is adopted:

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for  $i = 1 : N$  do
    Random walk algorithm;
    Finite Element simulation;
    Mean stress  $\bar{\sigma} = \sum_{j=1}^i \sigma(j)/i$ ;
end for
std =  $\sqrt{\frac{\sum_{i=1}^N (\sigma - \bar{\sigma})^2}{N}}$ 

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where N is the number of MC simulations, $\bar{\sigma}$ and std are the mean stress and the standard deviation corresponding to a specific strain level.

In the following FE simulations, hydrogel samples undergoing uniaxial tension are modeled as 2D plane stress problems with clamped-clamped boundary conditions to mimic the experiment setup. All the samples have the dimensions of 25×5 mm, the same as used in the experiment, unless otherwise specified. Four-node quadrilateral elements are used. $N = 500$ MC simulations are performed to obtain the mean and standard deviation of the sample stress-strain relationship. One of the advantages of using the element formulation adopted (Eq. 11) is that for hydrogels with complex geometric patterns, structured FE meshes can still be used. This is due to the fact that the contribution from the crosslink network is directly considered at the element level. For example, Fig. 9 shows a hydrogel sample with a sinusoidal pattern stripe meshed by four-node quadrilateral elements. The crosslink network in the pattern stripe is generated via the random walk algorithm. The i th element only contains the base hydrogel, therefore $N^{(i)} = 0$ (recall that $N^{(i)}$ represents the number of segments contained in that element) and the element is described by the modified neo-Hookean model. On the other hand, the j th element contains part of the pattern stripe, therefore $N^{(j)} > 0$ and the element is described by the

modified neo-Hookean model enhanced by the contribution from that part of the crosslink network.

Fig. 9. Hydrogel sample with a sinusoidal pattern stripe meshed by four-node quadrilateral elements

Table 1 shows the material parameters of the base hydrogel and the 100% pattern sample calibrated from the experimental data, according to Eqs. 12 and 13. Recall that the base hydrogel is described by the neo-Hookean model, containing two material parameters Young’s modulus E and Poisson’s ratio ν . The pattern is composed of the base hydrogel enhanced by the crosslinks formed between 20 kDa and 3.4 kDa molecules. The Young’s modulus E and Poisson’s ratio ν used in the pattern represent the mechanical properties of its base component and take the same values as the base hydrogel. The material parameters μ , l_{seg} , k_f , z_f used in the pattern represent the mechanical properties of its embedded fiber network. Fig. 10 shows the comparison of the stress-strain curves obtained from the FE simulations and the experimental data of the base hydrogel and 100% pattern samples under uniaxial tension, where $N = 500$ MC simulations are used to obtain the mean and the standard deviation of the stress-strain relationship.

Comparing Figs. 10a and 10b it can be seen that the pattern sample is much stiffer than the base hydrogel due to the crosslink network between 3.4 kDa and 20 kDa in the former. Note that the material parameters of the crosslink network (μ , l_{seg} , k_f , z_f) are calibrated here as an ensemble, and the optimization problem (Eq. 13) typically has multiple local minima. Consequently, the value

Table 1. Material parameters of the base hydrogel and the 100% pattern sample calibrated from uniaxial tension tests

Parameters	E (kPa)	ν	μ	l_{seg} (μm)	k_f (Nmm^{-1})	z_f
Base hydrogel	5.5	0.49	–	–	–	–
100% pattern	5.5	0.49	0.01	75	0.04	0.4

Fig. 10. Calibration: comparison of stress-strain relationships obtained from simulations (FEM) and experiments (Exp 1,2,3), experimental data courtesy of Dr. Xing Zhang and Dr. Jane Grande-Allen, Rice University [9]

of individual parameters in the ensemble (μ, l_{seg}, k_f, z_f) may not be uniquely
 325 determined. That is to say, there may exist multiple combinations of crosslink
 network parameters that fit the experimental data shown in Fig. 10b equally
 well, and the group listed in Table 1 is only one of them. However, since these
 material parameters all have straightforward physical or geometric meaning,
 they can ideally be measured from experiments. The current parameter fitting
 330 process is only a compromising approach due to lack of detailed information
 about the microscopic structure of the fabricated material. In the following
 FE simulations, the material parameters listed in Table 1 are used for the base
 hydrogel and the pattern stripes of PEGDA hydrogels with different pattern
 geometries.

335 In the experiment carried out in [9], the hydrogel samples with 20% pattern
 ratio were stretched along the direction that was either parallel or perpendicular
 to the pattern stripes. Since pattern stripes are much stiffer than the base
 hydrogel, patterned PEGDA hydrogels are anisotropic, with higher stiffness
 along the pattern orientation. Fig. 11 shows the comparison of the stress-strain
 340 curves obtained from the FE simulations and the experiments of the hydrogel
 samples with 20% pattern ratio under parallel or perpendicular tensile loading,
 where $N = 500$ MC simulations are used. The numerical results match the
 experimental data well in both cases.

Fig. 11. Validation: comparison of stress-strain relationships obtained from simulations (FEM) and experiments (Exp 1,2,3) of hydrogel samples with 20% pattern ratio, experimental data courtesy of Dr. Xing Zhang and Dr. Jane Grande-Allen, Rice University [9]

The influence of the pattern ratio on the mechanical behavior of PEGDA
 345 hydrogel is further numerically investigated. The sample dimensions and the
 boundary conditions are kept the same as the counterparts used in the case for
 the 20% pattern ratio. The relationship between the pattern ratio and linear
 modulus (the slope of the linear portion of the stress-strain curve) of patterned
 PEGDA hydrogels is shown in Fig. 12. For the 0% (base hydrogel), 20% and
 350 100% pattern ratios, the FE results match the experimental data well in both the
 parallel and perpendicular loading cases. Scrutinizing the experimental data,
 it can be seen that the linear modulus (100 kPa) of the 100% pattern sample

is 20 times larger than the value (4.5 kPa) of the base hydrogel (0%). When the pattern ratio is increased from 0% to 20%, the material linear modulus is increased from 4.5 kPa to 40 kPa, which is 40% of the 100% pattern sample (Fig. 12a). When the pattern ratio is increased to 80%, the linear modulus predicted by the FE simulation is almost as large as the value of the 100% pattern sample, indicating a saturation state for the crosslink network formed between 3.4 kDa and 20 kDa PEGDA molecules.

Fig. 12. Relationship between pattern ratio and linear moduli of patterned hydrogels

Fig. 13 shows the influence of the pattern ratio on the material anisotropy of patterned PEGDA hydrogels predicted by FE simulations. When the pattern ratio is 20%, the ratio between the material linear moduli for the parallel loading and the perpendicular loading is around 3.8. When the pattern ratio is increased to 80%, the ratio decreases to around 1.5, indicating that the material anisotropy becomes less obvious.

The nonlinear relationship between the pattern ratio and the material linear modulus, the saturation phenomenon of the crosslinking, and the decrease of the material anisotropy with the increase of the pattern ratio, were all reported in the literature, see [16, 9]. The above phenomena are all successfully captured in the FE simulations, demonstrating the capabilities of the adopted numerical strategy.

In the uniaxial tension test of the hydrogel sample with 20% pattern ratio,

Fig. 13. (a) Relationship between pattern ratio and material anisotropy of patterned hydrogels; (b) Ratio between material linear moduli in the parallel and perpendicular directions

the pattern stripes are either parallel or perpendicular with respect to the loading direction. The influence of other pattern orientations (θ) are also examined numerically. Fig. 14 shows the relationship between the pattern orientation and the linear modulus of the hydrogel sample with 20% pattern ratio. The linear moduli predicted by the FE simulations decrease as the angle θ formed between the pattern stripes and the tensile loading direction increases, indicating that the adopted numerical strategy is capable to capture the material anisotropy.

Fig. 14. Relationship between pattern orientation θ and linear modulus of the hydrogel sample with 20% pattern ratio

Recall that for the hydrogel patterned with a sinusoidal stripe, which is generated via the random walk algorithm, the pattern waviness is characterized by the ratio between the amplitude A and the half wavelength L of the sinusoidal pattern stripe (Fig. 6). In the FE simulation, the material parameters used for the base hydrogel and the sinusoidal pattern stripe are from the values listed in Table 1. Since the standard deviation is negligible compared with the mean in the MC simulations, only the mean stress-strain relationships are reported from now on. From the simulation results shown in Fig. 15, it can be seen that the sample linear modulus decreases when the pattern stripe becomes wavier (A/L increases), although the sample with a wavier stripe has a larger pattern ratio.

Fig. 15. Prediction: relationship between the pattern waviness (A/L) and the linear modulus of the hydrogel sample patterned with one sinusoidal stripe

As shown in Fig. 15, although the hydrogel sample is patterned with a sinusoidal stripe, the stress-strain curve obtained from the FE simulation is still linear. In order to acquire the three-region (toe-heel-linear) stress-strain relationship typically exhibited by biological tissues, the relative stiffness of the sinusoidal pattern stripe with respect to the base hydrogel is tuned by increasing the crosslink density μ in the simulation. Fig. 16 shows the relationship between the crosslink density μ and the linear modulus of the pattern stripe normalized with respect to the base hydrogel. The linear relationship between μ and the pattern stripe stiffness is a manifestation of the underlying affine fiber kinematics

[35, 36, 37, 38] of the adopted numerical strategy.

Fig. 16. Prediction: relationship between the crosslink density μ and the linear modulus of pattern stripe normalized with respect to the base hydrogel

400 In the following FE simulations, the value of the crosslink density μ in the pattern stripe is increased from 0.01 to 0.10, and the rest of the material parameters shown in Table 1 are kept the same. Fig. 17a shows the influence of μ on the stress-strain relationship of the hydrogel sample patterned with a sinusoidal-shape stripe (Fig. 6), where $A/L = 0.4$. It can be seen that the slope and the length of the toe region on the stress-strain curves are not influenced
405 by the crosslink density μ . This is because when the sample stretch is small ($\lambda < 1.1$), most crosslink fibers are still in the crimp state and do not contribute to the material stiffness. Therefore, the material global behavior is not influenced by the amount of fibers contained in the material (the crosslink density) at this stage. However, the slope of the linear region on the stress-strain
410 curve increases as the value of μ increases. This is because when the stretch is large enough ($\lambda > 1.3$), most crosslink fibers are straightened, and the material stiffness is directly proportional to the crosslink density μ . Fig. 17b shows the stress-strain curves normalized with respect to the stress when the sample stretch ratio $\lambda = 2.0$. It can be seen that the three-region (toe-heel-linear) characteristic of the stress-strain curve becomes more obvious when the crosslink
415 density μ (or the relative stiffness of the pattern stripe with respect to the base

hydrogel) increases.

Fig. 17. Prediction: influence of the crosslink density μ on the stress-strain relationship of the hydrogel sample patterned with a sinusoidal stripe, $A/L = 0.4$

Fig. 18 shows how the change of the pattern waviness relates to different regions on the nonlinear stress-strain curve. When the stretch ratio λ is small (from 1.0 to 1.2), the stress-strain curve is in the toe-region due to the large waviness of the pattern stripe. The heel region approximately begins at $\lambda = 1.2$, marked by point (b), and ends at $\lambda = 1.4$, marked by point (c) on the curve. After the exit of the heel region, the stripe waviness decreases significantly compared with the initial stage, contributing to a larger material modulus in the linear region. Fundamentally, the increase of the material stiffness is triggered by two mechanisms, the straightening of individual fibers at the microscale, and the straightening of the pattern stripe at the macroscale.

The shape of the three-region stress-strain curve can be further tuned by adjusting the number of half waves N of the sinusoidal pattern stripe (Fig. 7). Fig. 19 shows the influence of the number of half waves N (A is fixed and L varies) on the length of the toe region and the slope of the linear region obtained from the FE simulation. In Fig. 19b, the toe-region length is normalized to the total sample stretch ratio $\lambda = 2.0$. It can be seen that when N increases, the length of the toe region increases, and the slope of the linear region after the upward heel decreases.

Fig. 18. Straightening of pattern stripe at the macroscale and individual fibers at the microscale

Fig. 19. Prediction: influence of the number of half waves N of the sinusoidal pattern stripe on the stress-strain relationship

The material parameters of the base hydrogel and the pattern stripe(s) used in the above FE simulations are directly calibrated from the experiment and listed in Table 1. From the experimental data shown in Fig. 10, we can see that the pattern stripe behaves linearly under uniaxial tension. Therefore, a wavy (sinusoidal) shape of stripe is necessary to ensure that the patterned hydrogel sample possesses a toe-heel-linear stress-strain relationship, as shown in Figs. 17 and 18. However, by properly tuning the mechanical properties of the fiber component in the pattern stripe, the FE simulation predicts that hydrogel samples

with straight stripes can also exhibit a toe-heel-linear stress-strain relationship.

Fig. 20 shows the comparison between the FE simulations and the experimental data of porcine aortic valve tissues [39] in the circumferential and radial directions. Two different pattern geometries are used in the FE simulations, respectively. In the first pattern geometry, the sample with the dimensions of 15×5 mm, the same as used in the experiment [39], contains 20% straight pattern stripes. The material parameters for the stripe are $\mu = 0.01$, $k_f = 0.4 \text{ Nmm}^{-1}$ and $z_f = -16$ (satisfying the convexity constraint $z_f \leq 1.0$). In the second pattern geometry, the sample contains a sinusoidal stripe with the waviness represented by $A/L = 0.2$ in the circumferential direction and $A/L = 0.3$ in the radial direction, respectively. The material parameters for the stripe are $\mu = 0.1$, $k_f = 0.4 \text{ Nmm}^{-1}$ and $z_f = -5$. The two different pattern geometries with different sets of material parameter values can both attain the toe-heel-linear stress-strain relationship and fit the experimental data equally well. In the pattern design process, which pattern geometry to be adopted depends on the targeted material behavior, such as the stress-strain relationship, and the material properties of the base and the pattern hydrogels.

Fig. 20. Comparison between the FE simulations and the experimental data of porcine aortic valve tissues, experimental data are obtained from [39]

4. Discussions

Among many potential biomedical applications, patterned PEGDA hydrogels can be used as scaffold materials to fabricate tissue engineered heart valves (TEHVs). The material properties of patterned PEGDA hydrogels largely depend on the pattern topology. In this paper, we adopt a newly proposed computational framework [30] combined with published experimental data [9] to numerically quantify the influence of various pattern topology, including pattern ratio, orientation and waviness, on the mechanical behavior of PEGDA hydrogels patterned by the photolithographic technology. Several important phenomena that were reported in literature are successfully captured in the FE simulations.

In the adopted numerical strategy, the base hydrogel is modeled as an isotropic material by the modified neo-Hookean constitutive law. The pattern stripes are modeled as fiber networks (representing the crosslinks formed between the 3.4 kDa and 20 kDa PEGDA molecules) embedded in the base hydrogel. The two different material components (the base hydrogel and the pattern stripes) contained in the patterned PEGDA hydrogel are unified through an element formulation strategy based on Eq. 11, in which the contribution from the crosslinks (fiber network) is captured at the element level. This element formulation strategy makes the spatial discretization of the FE simulation convenient, for instance, see Fig. 9, since structured FE meshes can still be used even in complex pattern geometries.

The material parameters for the base hydrogel and the pattern are calibrated from the published experimental data. The base hydrogel is described by the modified neo-Hookean model with two material parameters, Young's modulus E and Poisson's ratio ν . The parameter calibration process for the base hydrogel is relatively straightforward, since the material is assumed to be nearly incompressible ($\nu = 0.49$), leaving E the only parameter to be determined. The parameters of the fiber network contained in the pattern include the fiber volume fraction ratio (crosslinking density) μ , the fiber segment length l_{seg} , the axial

elastic modulus k_f and the dimensionless parameter z_f of individual fibers. The material parameter calibration process for the pattern is more complex in the sense that there may exist multiple groups of parameter values of (μ, l_{seg}, k_f, z_f) fitting the experimental data equally well. Fitting these parameters as an ensemble is only a compromising approach adopted in this paper, due to lack of the quantitative information about the microscopic structure of the pattern. However, recently many efforts have been devoted to study the mechanical behavior of a single fiber [40, 41, 42]. Also, quantitative geometric information regarding microscopic fiber arrangement can be acquired via advanced image processing technologies such as confocal reflection imaging [20, 43] or scanning electron microscopy (SEM) [25]. All these advances make it possible to uniquely determine the material parameters of the crosslink network from experiments.

The nonlinear relationship between the pattern ratio and the linear modulus of patterned PEGDA hydrogels was reported by different researchers in the literature [16, 9]. This phenomenon is successfully captured by the numerical strategy adopted in this paper. At the same time, the decrease of the material anisotropy when the pattern ratio increases, which was observed in the experiment [16], is also captured in the FE simulation. Currently, only the numerical results of hydrogel samples with 0%, 20% and 100% pattern ratios are compared with the experimental data (Fig. 12). To further verify the nonlinear relationship between the pattern ratio and the material linear modulus obtained from the numerical investigation, more experiments (hydrogel samples with other pattern ratio) are still needed.

In the context of TEHVs, patterned PEGDA hydrogels are designed to mimic the mechanical behavior of native heart valve tissues, so that the valve can provide sufficient mechanical functions after initial implantation, which is crucial for the survival of the patient [4]. The numerical results presented in this paper predict that patterned hydrogels can be designed via two approaches to possess a toe-heel-linear stress-strain relationship exhibited by many native biological tissues. When pattern stripes behave according to a linear stress-strain relationship (Fig. 10b), as reported in the experiment by [9], wavy patterns

are necessary to ensure the hydrogel sample possess a three-region stress-strain curve. An alternative approach is to increase the nonlinearity of the fiber component contained in pattern stripes. Following this approach, the FE simulation demonstrates that hydrogel samples with straight patterns can also exhibit a nonlinear stress-strain relationship (Fig. 20). In general, there may exist different combinations of material properties and pattern geometries to get a similar stress-strain relationship. In the actual pattern design process, which pattern geometry to be adopted largely depends on the targeted stress-strain relationship and the material properties of the base and the pattern. Furthermore, during the fabrication and patterning process of hydrogels, there are many constraints on the base and the pattern properties. For example, only certain range of crosslinking density μ or fiber stiffness k_f could be reached. In order to design a patterned hydrogel satisfying certain targeted mechanical properties, the constraints on the material properties also need to be considered, making the whole design process become a constrained optimization problem.

The relative stiffness between the pattern stripe and the base hydrogel influences the stress-strain relationship of patterned hydrogels. The FE simulation suggests that the toe-heel-linear behavior occurs when the stripe is much stiffer than the base hydrogel. This observation is supported by the comparison of material properties of extracellular matrix (ECM) and collagen fibers reported in literature. The mechanical properties of several tissue-specific ECM measured by atomic force microscopy are summarized in [44]. For example, the elasticity of ECM in smooth muscle is from 5 to 9 kPa [45], and the elasticity of ECM in skeletal muscle varies from 8 to 17 kPa [46, 47]. The elastic moduli of single collagen fibers reported in the literature are rather scattered. For example, the elastic moduli for single fibers extracted from different rat tails are reported to be between 100 MPa and 360 MPa [48]. The (tangent) elastic moduli of extruded bovine achilles tendon collagen fibers are reported to be 359.6 ± 28.4 MPa [49]. In [50], the elastic moduli of wet and dry rat tail tendon fibers are reported to be 570 MPa and 2690 MPa. The high ratio between the single fiber and ECM stiffness ($> 10^4$) in native biological tissues confirms the

FE prediction that the pattern strip needs to be much stiffer than the base
555 hydrogel to generate the toe-heel-linear behavior.

In this paper, the base hydrogel is made of 20 kDa PEGDA molecules, and
the crosslinks in the pattern stripes are formed between the 3.4 kDa and 20
kDa PEGDA molecules. Although the quantitative results obtained from the
numerical simulations only apply to hydrogels fabricated from above PEGDA
560 molecule weights, the numerical strategy presented in this paper can be used to
examine the material properties of hydrogels made of other molecule weights.

In the photolithographic patterning process described in [9], the collimated
UV light is evenly distributed and the crosslinking density remains constant in
the thickness direction of the hydrogel sample. For hydrogels patterned with 2D
565 geometries discussed in this research, the anisotropy caused by material hetero-
geneousness mainly occurs in the plane formed between the sample length and
width directions. Because the material properties do not vary in the thickness
direction, 2D formulations should be sufficient to capture the most dominant
features of the mechanical behavior of patterned hydrogels under discussion.
570 However, 2D formulations may not be enough to represent a strongly micro-
heterogeneous material. For instance, hydrogels are patterned into a layered-
structure with each layer having a different 2D pattern geometry or properties,
see [8]. In this case, the material properties do vary in the thickness direction
and a 3D computational framework needs to be used to consider the anisotropy
575 caused by material heterogeneousness in all the three directions.

Though not shown in this paper, the adopted computational strategy is also
implemented in 3D. An extra spatial angle is introduced in the random walk
algorithm to generate the three-dimensional fiber network structure. A similar
projection scheme shown in Fig. 2b is used to describe the deformation of a fiber
580 segment by the nodes of a 3D element. The component forms of the element
residual and the stiffness matrix contributed by a fiber segment in 3D take the
same forms as shown in Eqs. 8 and 9.

PEGDA hydrogels are designed as scaffolds to encapsulate cells for tissue
growth and remodeling. In literature, there are many experimental studies

585 exploring the cellular behaviors, such as adhesion, spreading and proliferation,
 when cells are encapsulated inside hydrogel or other ECM environment [16, 6,
 51, 52]. For example, research shows that ECM elasticity plays an important
 role in cell differentiation processes [44]. In order to include cellular behaviors
 in the simulation, besides the proper spatial discretization strategy, this future
 590 development heavily relies on the availability of accurate mathematical model(s)
 describing the cell-cell and cell-matrix interactions, which still requires more
 theoretical and experimental studies.

Appendix A

A necessary and sufficient condition for Eq. 6 to be convex is that

$$\frac{\partial^2 \Psi_f(\lambda)}{\partial \lambda^2} \geq 0, \quad \forall \lambda \geq 1. \quad (14)$$

The second derivative of $\Psi_f(\lambda)$ can be written as,

$$\frac{\partial^2 \Psi_f(\lambda)}{\partial \lambda^2} = \frac{k_f L_0^2}{2\lambda^{z_f+2}} [(z_f^2 - 3z_f + 2)\lambda^2 + (2z_f - 2z_f^2)\lambda + (z_f^2 + z_f)]. \quad (15)$$

For the above function to be non-negative, the coefficient of the λ^2 term has to
 be positive

$$z_f^2 - 3z_f + 2 > 0 \quad \Rightarrow \quad z_f > 2 \text{ or } z_f < 1. \quad (16)$$

Under the condition shown in Eq. 16, when the discriminant of the quadratic
 part of Eq. 15 is non-positive, that is,

$$(2z_f - 2z_f^2)^2 - 4(z_f^2 - 3z_f + 2)(z_f^2 + z_f) = 8z_f^2 - 8z_f \leq 0 \quad \Rightarrow \quad 0 \leq z_f \leq 1,$$

the condition shown in Eq. 14 always holds. Therefore, when $0 \leq z_f < 1$, the
 595 fiber strain energy function shown in Eq. 6 is always convex.

Recall that fibers cannot resist compression, thus, Eq. 14 only needs to hold
 when $\lambda \geq 1.0$. The constraint on z_f can be relaxed. The two roots of Eq. 15
 are

$$\lambda_{1,2} = \frac{-(2z_f - 2z_f^2) \pm \sqrt{8z_f^2 - 8z_f}}{2(z_f^2 - 3z_f + 2)}, \quad 8z_f^2 - 8z_f > 0.$$

Under the condition shown in Eq. 16, when $\lambda \geq \lambda_1$ or $\lambda \leq \lambda_2$, the condition shown in Eq. 14 holds. On the other hand, since $\lambda \geq 1.0$, as long as $\lambda_1 \leq 1.0$, the condition shown in Eq. 14 will hold for $\forall \lambda \in [1.0, +\infty)$. That is,

$$\lambda_1 = \frac{-(2z_f - 2z_f^2) + \sqrt{8z_f^2 - 8z_f}}{2(z_f^2 - 3z_f + 2)} \leq 1.0 \quad \Rightarrow \quad z_f < 0. \quad (17)$$

Finally, when $z_f = 1$,

$$\frac{\partial^2 \Psi_f(\lambda)}{\partial \lambda^2} = \frac{k_f L_0^2}{\lambda^3} > 0. \quad (18)$$

In summary, in order to guarantee the convexity of the fiber strain energy function shown in Eq. 6, the material parameter z_f has to satisfy the constraint $z_f \leq 1$.

Acknowledgements

600 The authors thank Prof. Jane Grande-Allen and Dr. Xing Zhang for providing the experimental data used in this work and for useful discussions and suggestions. This work was partially funded by NSF, Grant No. 1463432.

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PATTERN GEOMETRIES

EXPERIMENTS

hydrogel experiments (provide calibrated parameters)

COMPUTATIONAL MODELING

pattern at microscale

projection scheme

NUMERICAL RESULTS AND PREDICTIONS