Geometric characterization and simulation of planar layered elastomeric fibrous biomaterials

James B. Carleton^a, Antonio D'Amore^b, Kristen R. Feaver^a,
Gregory J. Rodin^{a,d}, and Michael S. Sacks^a

^aCenter for Cardiovascular Simulation
Institute for Computational and Engineering Sciences
Department of Biomedical Engineering
University of Texas at Austin, Austin, TX 78712 U.S.A.

^bMcGowan Institute for Regenerative Medicine
University of Pittsburgh, Pittsburgh, PA 15261 U.S.A.

^cDepartment of Aerospace Engineering and Engineering Mechanics
University of Texas at Austin, TX 78712 U.S.A.

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For correspondence:

Michael Sacks, Ph.D.

W. A. "Tex" Moncrief, Jr. Simulation-Based Engineering Science Chair I

Institute for Computational Engineering and Sciences (ICES)

The University of Texas at Austin

201 East 24th Street, ACES 5.438

1 University Station, C0200

Austin TX 78712-0027 U.S.A.

email: msacks@ices.utexas.edu

phone: (512) 232-7773

fax: (512) 471-8694

ABSTRACT

Many important biomaterials are composed of multiple layers of networked fibers. While there is a growing interest in modeling and simulation of the mechanical response of these biomaterials, a theoretical foundation for such simulations has yet to be firmly established. Moreover, correctly identifying and matching key geometric features is a critically important first step for performing reliable mechanical simulations. The present work addresses these issues in two ways. First, using methods of geometric probability we develop theoretical estimates for the mean linear and areal fiber intersection densities for two-dimensional fibrous networks. These densities are expressed in terms of the fiber density and the orientation distribution function, both of which are relatively easy-to-measure properties. Secondly, we develop a random walk algorithm for geometric simulation of two-dimensional fibrous networks which can accurately reproduce the prescribed fiber density and orientation distribution function. Furthermore, the linear and areal fiber intersection densities obtained with the algorithm are in agreement with the theoretical estimates. Both theoretical and computational results are compared with those obtained by post-processing of SEM images of actual scaffolds. These comparisons reveal difficulties inherent to resolving fine details of multilayered fibrous networks. The methods provided herein can provide a rational means to define and generate key geometric features from experimentally measured or prescribed scaffold structural data.

LIST OF SYMBOLS

55	LIST OF SYMBOLS
56	A - domain area
57	f - orientation density function (ODF)
58	g - probability density function (PDF) of n
59	L - total fiber length
60	L_B - box edge length
61	$\hat{L}_B$ - normalized box edge length
62	L_f - single fiber length
63	L_t - test segment length
64	m - number of segments in the domain
65	n - number of segments intersecting test segment
66	n_f - number of fibers
67	n_s - number of segments in each fiber
68	O - random walk origin
69	s - segment length
70	$\hat{s}$ - normalized segment length
71	Δp - probability that the arbitrary segment intersects the test segment
72	p - probability that any segment intersects the test segment
73	$\mathcal{P}$ - intersection condition parallelogram
74	$\mathcal{S}$ - arbitrary segment
75	$\mathcal{T}$ - test segment
76	z - complex number representation of random walk end-to-end vector
77	α - maximum allowed random walk angle
78	θ_k - angle between k 'th segment in random walk and previous segment
79	λ - linear intersection density
80	μ_λ - mean linear intersection density
81	$\hat{\mu}_\lambda$ - normalized mean linear intersection density
82	μ_ρ - mean areal intersection density
83	$\hat{\mu}_\rho$ - normalized mean areal intersection density
84	ξ - fiber density
85	ρ - areal intersection density
86	φ - absolute orientation angle of test segment
87	φ' - orientation angle of arbitrary segment
88	φ_k - orientation angle of k 'th segment in random walk
89	Ω - domain in Euclidean 2-D space

1. INTRODUCTION

Many important biomaterials are composed of multiple layers of networked fibers. Elastomeric fibrous scaffolds used in engineering soft tissues are a prime example [1]. Since soft tissues undergo large deformations [2, 3], the constituent fibers must have elastomeric characteristics and undergo large macroscopic deformations as a result of large rotations and strains. These characteristics allow the scaffolds to duplicate many of the salient characteristics of the soft tissues they are intended to replace [4]. The scaffolds are also expected to have multiple functions, including facilitation of cell attachment and support of de-novo tissue synthesis and stress transfer [1]. To meet these multi-faceted demands, one must develop a fundamental understanding of the underlying physical processes occurring within the scaffolds across multiple scales [1].

Currently, electro-spinning is a common process for elastomeric scaffold fabrication [1, 5]. This process results in very long, often undulated “continuous” fibers that form dense networks with layered structures. Other approaches focus on reconstituted collagen or fibrin gels [6]. Regardless of the methodology used to create the scaffold structures, it is well-established that the fiber geometry significantly influences the macroscopic mechanical response [7, 8]. Accordingly, there has been a considerable amount of effort devoted to characterization of the fibrous geometry, with the focus on measuring an in-plane fiber orientation density function and establishing its relations with mechanical anisotropy [5, 9-13].

Generally, current approaches to simulating fibrous network geometries are based on: (i) generating Voronoi diagrams [14]; (ii) generating assemblies of straight fibers with prescribed orientations [15]; (iii) using method (ii) to generate a network that is used as the initial state of a dynamics simulation in which the fiber positions and orientations evolve, and cross links are formed [16]; (iv) direct post-processing of scaffold images [10]. While all four approaches have been successfully used for predicting the macroscopic mechanical response in terms of fiber stiffness and volume fraction, the generated geometries simply do not consistently resemble the actual ones and involve somewhat subjective, empirical rules. Most importantly, the geometric parameters necessary for modeling and simulation of other aspects of mechanical behavior of fibrous networks are yet to be defined.

Herein, we rely on methods of geometric probability to develop theoretical estimates for the mean linear and areal fiber intersection densities for two-dimensional fibrous networks. These densities are expressed in terms of the fiber density and the orientation distribution function, both of which are relatively easy-to-measure properties. Secondly, we develop a 2D random walk algorithm capable of generating realistic geometric models of fibrous network layers. This algorithm can greatly benefit further studies concerned with micromechanical modeling by generating geometries that resemble actual ones. We further present simulation results aimed at validating the assumptions underlying the theoretical development, testing the algorithm performance, and establishing the minimum specimen size necessary for capturing macroscopic properties. Finally, we show how the theory and simulations can complement the use of experimental observations to more accurately determine important features of the material's microstructural geometry.

2 - METHODS

2.1 – Derivation of key geometric relationships for planar fibrous networks

The materials considered in the present work can be idealized as a set of multiple, connected planar 2D networks of long, curved fibers. This has been shown to be a very good idealization for electro-spun polyester urethane urea (ES PEUU) scaffolds (Fig. 1), as well as many other important biomaterials. Our approach is thus restricted to layered networks, which can be treated as an assembly of 2D monolayers. We introduce basic quantities that characterize 2D fibrous geometries, the linear and areal intersection densities for planar fibrous networks, and develop simple expressions in terms of the fiber density and orientation density function. These quantities allow estimation of a characteristic segment length between neighboring fiber intersections. The derived expressions significantly simplify the task of microstructural characterization, since the intersection densities are difficult to measure directly.

We begin with a simplified model, in which a fibrous layer is represented as a two-dimensional network whose building blocks are short, straight line segments. At first, we establish some results for such networks of disjoint segments, and then we extend those results

to networks of long, curved fibers composed of connected segments. In the process, we adopt minimal assumptions required to establish simple expressions for the linear and areal intersection densities in terms of the linear fiber density and the orientation density function (ODF). The mathematical problem considered in this section can be classified as a generalization of the classical Buffon's needle problem [17]. In this problem, a needle of known length is dropped onto a floor composed of many parallel floor boards of known width, and we seek the probability that the needle lands on a boundary line between the adjacent floorboards. This problem represents the beginning of the field of geometric probability [18].

Figure 1. Scanning electron microscope (SEM) image of the top layer of an electro-spun polyester urethane urea (PEUU) scaffold [10]. The fiber diameter is approximately 1 micron.

Let us consider a domain $\Omega \in \mathbb{R}^2$ whose area is equal to A . The domain contains m straight line segments, each of length s . We suppose that $m \gg 1$ and assign to the segments an orientation density function (ODF) $f(\varphi)$ such that the probability that any given segment has an orientation between φ and $\varphi + \Delta\varphi$ is $\Delta p = f(\varphi)\Delta\varphi$. The ODF is periodic, such that $f(\varphi) = f(\varphi + \pi)$, and therefore the angle φ can be restricted to the interval $[0, \pi)$. The segment centers are uniformly distributed in Ω following a Poisson's process [19]. It is

expedient to assume that Ω is a unit cell of a periodic structure. Accordingly, the segments protruding outside of Ω can reenter it from the opposite side.

Let us consider a randomly-placed test straight line segment $\mathcal{T}$ of length L_t cutting through some of the m segments in Ω and oriented at an angle φ . Then a segment $\mathcal{S}$, oriented at an angle between φ' and $\varphi' + \Delta\varphi'$, intersects $\mathcal{T}$ if and only if the center of $\mathcal{S}$ is located inside the parallelogram $\mathcal{P}$ with edges of length L_t and s , and angle $|\varphi - \varphi'|$ (**Fig. 2**).

Figure 2. An arbitrary segment $\mathcal{S}$ having length s and orientation angle φ' intersects the test segment $\mathcal{T}$ having length L_t and orientation angle φ if its center lies within the parallelogram $\mathcal{P}$ designated by the dashed lines.

Since the area of $\mathcal{P}$ is equal to $sL_t \sin|\varphi - \varphi'|$, the probability that $\mathcal{S}$ is oriented at an angle between φ' and $\varphi' + \Delta\varphi'$, and intersects $\mathcal{T}$ is equal to

$$\Delta p = \frac{sL_t \sin|\varphi - \varphi'|}{A} f(\varphi') \Delta\varphi' . \quad (1)$$

Accordingly, the probability that any segment, regardless of its orientation, intersects $\mathcal{T}$ is equal to

$$p(\varphi) = \frac{sL_t}{A} \int_0^\pi \sin|\varphi - \varphi'| f(\varphi') d\varphi' . \quad (2)$$

Let n denote the number of intersections along $\mathcal{T}$; the corresponding probability density function is denoted by $g(n)$. Since the position and orientation of each segment are

independent of the positions and orientations of all the other segments, the intersections with $\mathcal{T}$ are independent events. This is a classical setting leading to the binomial distribution with the probability density function (PDF)

$$g(n) = \frac{m!}{n!(m-n)!} p^n (1-p)^{m-n} \quad (3)$$

Using Eq. (2), we express the mean of n as

$$\mu_n(\varphi) = mp = \frac{msL_t}{A} \int_0^\pi \sin|\varphi - \varphi'| f(\varphi') d\varphi'. \quad (4)$$

Define the linear intersection density along the direction φ as

$$l(j) := \frac{n(j)}{L_t}. \quad (5)$$

The mean for this random variable is determined from Eq. (4):

$$\mu_\lambda(\varphi) = \frac{L}{A} \int_0^\pi \sin|\varphi - \varphi'| f(\varphi') d\varphi' = \xi \int_0^\pi \sin|\varphi - \varphi'| f(\varphi') d\varphi'. \quad (6)$$

Here $L = ms$ is the total fiber length and

$$\xi := \frac{L}{A} \quad (7)$$

is the linear fiber density. Thus Eq. (6) constitutes a simple expression for the mean linear intersection density along the direction φ in terms of the linear fiber density ξ and ODF $f(\varphi)$.

Unfortunately such an expression cannot be established for the standard deviation and other higher-order moments, as their dependences on m and s prohibit these two parameters from being combined into the single parameter L . Note that ξ is an easy-to-measure macroscopic parameter, and $f(\varphi)$ is relatively straightforward to obtain from microscopic images, which makes Eq. (6) particularly useful.

Following Morton [20], one may regard the segments as the building blocks of rectifiable curves forming the network, and thus extend Eq. (6) to the entire network. This passage is valid only when the process preserves statistical independence of the segments, which, for our purposes, is a prohibitively restrictive condition. This issue will be addressed in Section 3, where we use simulations to examine implications of the statistical dependence. If the network is isotropic then $f(\varphi) = \pi^{-1}$ and Eq. (6) implies

$$\mu_\lambda = \frac{2\xi}{\pi}. \quad (8)$$

Very similar calculations can be carried out for the areal intersection density ρ . Accordingly, we obtain

$$\mu_\rho = \frac{1}{2} \xi^2 \int_0^\pi \int_0^\pi \sin|\varphi - \varphi'| f(\varphi') f(\varphi) d\varphi' d\varphi. \quad (9)$$

By adopting ξ^{-1} as the characteristic length scale, we can normalize both μ_λ and μ_ρ so that the quantities of interest become dimensionless. Then Eq. (6) and Eq. (9) imply

$$\hat{\mu}_\lambda(\varphi) := \mu_\lambda(\varphi) \xi^{-1} = \int_0^\pi \sin|\varphi - \varphi'| f(\varphi') d\varphi' \quad (10)$$

and

$$\hat{\mu}_\rho := \mu_\rho \xi^{-2} = \frac{1}{2} \int_0^\pi \int_0^\pi \sin|\varphi - \varphi'| f(\varphi') f(\varphi) d\varphi' d\varphi. \quad (11)$$

2.2 – An algorithm for generating networks of fibers.

In this section, we describe an algorithm for generating 2D networks of intersecting fibers possessing the desired geometric characteristics. In the proposed algorithm, the network is generated as a collection of random walks constructed so that the network possesses the prescribed fiber density and an ODF. In the algorithm, each fiber is a piecewise linear curve generated as a random walk involving n_s straight segments of the same length s , so that the

length of one fiber is $L_f = sn_s$. The polar angles (orientations) of the segments for a particular fiber are determined via the following algorithm.

1. Choose an origin O and a polar angle φ_0 ; as in the previous section $\varphi_0 \in [0, \pi)$.
2. Construct the first segment such that its origin is at O , and it deviates from the direction φ_0 by an angle θ_1 which is a realization of a random variable uniformly distributed over the interval $-\alpha \leq \theta_1 \leq \alpha$ with $0 < \alpha \leq \pi$; note that the polar angle of the first segment is $\varphi_1 = \varphi_0 + \theta_1$.
3. Continue the process such that the polar angle of the k -th segment is $\varphi_k = \varphi_{k-1} + \theta_k$, where θ_k is a realization of a random variable, uniformly distributed over the interval $[-\alpha, \alpha]$.

Most frequently, random walks are constructed using $\alpha = \pi$. This case, however, is of little interest to us, as we are interested in constructing fibers that do not depart dramatically from straight lines. For this purpose, α should be small.

According to the algorithm, the end-to-end vector of a fiber formed by n_s segments can be prescribed by the complex number

$$z = s \sum_{k=1}^{n_s} \exp(i\varphi_k). \quad (12)$$

The mean of z is

$$\mu_z = \frac{1}{(2\alpha)^{n_s}} \int_{-\alpha}^{\alpha} \int_{-\alpha}^{\alpha} \dots \int_{-\alpha}^{\alpha} z d\theta_1 d\theta_2 \dots d\theta_{n_s} = s \exp(i\varphi_0) \frac{\beta(1-\beta^{n_s})}{1-\beta} \quad \text{with} \quad \beta = \frac{\sin \alpha}{\alpha}. \quad (13)$$

This equation implies that φ_0 is the expected polar angle of the end-to-end vector. Therefore a desired ODF $f(\varphi)$ for a network can be approximated by generating the initial angles φ_0 for

the individual fibers from the ODF $f(\varphi_0)$. This approximation requires a sufficiently large number of fibers.

The algorithm is formulated in terms of dimensionless parameters, using ξ^{-1} as the normalizing length scale, as in Eq. (10) and Eq. (11); the normalized parameters are denoted by the hat symbol (^). Then for Ω chosen to be a square box of size L_B , the normalization implies the following relationship among the dimensionless parameters:

$$\frac{n_f n_s \hat{s}}{\hat{L}_B^2} = 1 . \quad (14)$$

The algorithm can be summarized as follows:

1. Prescribe a square $L_B \times L_B$ box, total fiber length L , and ODF $f(\varphi)$.
2. Compute ξ from Eq. (7) and normalize all length scales by multiplying them by ξ .
3. Select a sufficiently large n_f for an accurate piecewise-constant approximation of $f(\varphi)$, and generate n_f initial angles φ_0 consistent with this approximation.
4. Select a sufficiently small $\hat{s}$ ($\hat{s} \ll \hat{L}_B$), and compute n_s from Eq. (14).
5. Select the random walk bounding angle α .
6. Generate the network as a collection of n_f random walks each comprised of n_s segments.
7. Compute the intersection points.
8. Compute the actual ODF for the generated network.
9. If the actual and prescribed ODFs are insufficiently close, increase n_f and/or decrease $\hat{s}$, until the two ODFs are sufficiently close.
10. Compute the densities $\lambda(\varphi)$ and ρ .

In summary, to generate a network, one prescribes L_B , L , and $f(\varphi)$, uses the parameters n_f and $\hat{s}$ to control the accuracy, while the angle α is used as an adjustable parameter. The algorithm generates a network whose properties coincide with the prescribed ones. Such a network consists of long piecewise linear fibers that appear curved on the box scale (**Fig. 3**),

thanks to the condition $L_p \gg s$. Note that, if desired, one can introduce splines to smoothen piecewise linear random walks; this option is not pursued in this paper.

Figure 3. A representative simulated network of fibers: (a) Fibers inside a periodic box; (b) Close-up view showing the individual segments and their intersections.

To understand the role of the angle α , let us consider two networks generated using identical parameters except for α (**Fig. 4**). Upon comparison of the generated networks, it becomes evident that small α 's result in almost straight fibers (**Fig. 4a**) whereas large α 's result in tortuous fibers (**Fig. 4b**). We believe that quantification of this geometric feature is essential for mechanical modeling of networks. While we do not pursue this issue in this paper, we observe that in principle the proposed algorithm allows one to control tortuosity.

(a)

(b)

Figure 4. The effect of the angle α on network tortuosity: (a) $\alpha = \frac{\pi}{64}$. (b) $\alpha = \frac{\pi}{8}$. The networks were generated using $L=50,000$, $L_B=1000(\hat{L}_B=50)$, $\xi=0.05$, $\hat{s}=0.15$, $n_f=1$, $n_s=16,667$, $f(\theta)=1/\pi$.

2.3 – Representative volume elements

The determination of material properties must involve sufficiently large specimens, which properly represent the overall (macroscopic) material response. In the pertinent solid mechanics literature, such specimens are referred to as the Representative Volume Elements (RVEs), and we retain this terminology here, despite the fact that we are concerned with 2D networks. A simple way of testing if specimens of a particular size can be treated as RVEs is by examining the statistics for a property of interest. For example, if the property of interest is the areal density of intersections ρ , then all RVEs must yield essentially the same value of ρ . The term essentially means that ρ can have slight variations from one specimen to another, but those variations must be small. In particular, one can demand that the coefficient of variation σ_ρ/μ_ρ corresponding to a set of specimens is small.

The notion of large specimens is meaningless unless one specifies a reference microstructural length scale. For 2-D networks, this scale can be related to the mean areal

density and set equal to $\mu_\rho^{-1/2}$. For the purposes of dimensional analysis, the double integral in Eq. (9) can be set equal to unity, so that we obtain

$$L_B \gg \xi^{-1} \Rightarrow L_B \gg \frac{L_B^2}{L} \Rightarrow L \gg L_B. \quad (15)$$

This equation implies that one can think of RVEs as specimens that are densely filled with fibers.

3 - RESULTS

3.1 - Statistical dependence of segments and minimum box size.

In this section, we compare theoretical and simulation results using the mean areal density μ_ρ as the quantity of interest. We are also concerned with identifying the minimum RVE size necessary for treating μ_ρ as a material rather than specimen property. In comparing computed and theoretical values, it is important to keep in mind that the algorithm does not generate statistically independent segments, because the polar angle of each segment within a fiber depends on the polar angles of the preceding segments, whereas the theoretical estimates are based on the assumption that the segments are statistically independent. Nevertheless it is reasonable to expect that large numbers of long fibers tend to generate samples of segments for which the theoretical estimates should be accurate. We thus present simulation results to confirm this supposition.

For the first set of simulations, we selected $n_s = 1$, $\hat{s} = 0.3$, and $f(\varphi) = \pi^{-1}$; for $n_s = 1$ the value of α becomes irrelevant. The box size $\hat{L}_B$ was treated as an independent variable and the value of n_f was computed from Eq. (14). The corresponding theoretical estimate follows from Eq. (11): $\hat{\mu}_\rho = \pi^{-1}$. Although this case is of little interest to modeling of scaffolds, it allows us to generate statistically independent segments and examine how the computational and theoretical results converge to each other as the network size increases.

Computational results for $\hat{\mu}_\rho$ versus $\hat{L}_B$ (**Fig. 5**) were obtained using ten realizations per box size. The error bars represent one standard deviation for those samples. This plot shows that for $\hat{L}_B \geq 100$ the standard deviation becomes very small and $\hat{\mu}_\rho \approx \pi^{-1}$. This suggests that the box size for computing macroscopic properties should be at least $100\xi^{-1}$; a more precise definition of the minimum RVE size must involve a prescribed error tolerance.

Figure 5. The normalized areal intersection density $\hat{\mu}_\rho$ versus the normalized box size $\hat{L}_B$ for short fibers: The dots represent the mean values, the error bars represent one standard deviation, and the solid line is the theoretical estimate for the mean.

In the next set of simulations, we considered networks formed by long fibers. We selected $\alpha = \pi/64$, $\hat{s} = 0.3$, $f(\varphi) = \pi^{-1}$, and adopted an additional condition of $n_s \hat{s} = \hat{L}_B$. Then Eq. (14) implies $\hat{L}_B = n_f$, so that the network size is proportional to both n_s and n_f . As before, we conducted ten simulations per box size. Simulation results are compared with two theoretical predictions (**Fig. 6**). The first one (dashed line) is obtained by assuming that $f(\varphi) = \pi^{-1}$, i.e.

assuming an isotropic orientation distribution, and therefore $\hat{\mu}_\rho = \pi^{-1}$. The second theoretical prediction is more accurate, as it is obtained from Eq. (10) by integrating the actual, rather than desired ODF. It is clear that the latter prediction is in good agreement with simulation results even for $\hat{L}_B \approx 25$. For $\hat{L}_B \geq 100$ simulation and theoretical results are very close, and the standard deviation for the simulation results becomes small.

Figure 6. The normalized areal intersection density $\hat{\mu}_\rho$ versus the normalized box size $\hat{L}_B$ for long fibers: The circles and the error bars represent the mean values and standard deviations of the simulation results, the crosses represent the mean values derived from Eq. (11) using the actual ODF, and the dashed line represents the mean values derived from Eq. (11) using the uniform ODF.

3.2 - Linear intersection density equation and non-uniform ODFs.

In this section, we focus on the mean linear intersection density and a non-uniform ODF. We selected $\alpha = \pi/60$, $\hat{s} = 0.3$, $n_s = 100$, $n_f = 12,000$, so that Eq. (14) implies $\hat{L}_B = 600$. The ODF was prescribed as

$$f(\varphi) = \frac{30}{\pi} \left(\frac{\varphi}{\pi} \right)^2 \left(1 - \frac{\varphi}{\pi} \right)^2 \quad (17)$$

355 This ODF was assigned to the initial φ_0 and the actual ODF was obtained based on the fibers
 356 constructed via the random walk algorithm (**Fig. 7**). Both ODFs are represented as piecewise
 357 constant functions associated with uniform partitioning the interval $[0, \pi)$ into twenty
 358 subintervals. The plot represents just one realization, but the actual and prescribed ODFs are in
 359 fairly close agreement.

360

361 Figure 7. Prescribed (white) versus actual (black) ODFs.

362 The linear intersection density $\hat{\mu}_\lambda(\varphi)$ predicted from Eq. (11) based on the desired and actual
 363 ODFs was compared with that obtained from the simulation (**Fig. 8**). The plot reveals that Eq.
 364 (11) is an excellent predictor of the simulation results (grey versus white bars). The small
 365 difference between the simulation and theoretical results based on the desired ODF (black
 366 versus white bars) is explained by the fact that the algorithm gives rise to slightly different
 367 prescribed and actual ODFs.

Figure 8. Linear intersection density $\hat{\mu}_\lambda(\varphi)$: simulation (white), theory based on the actual ODF (grey), and theory based on the prescribed ODF (black).

3.3 – Material design

While the algorithm can generate networks with desired properties, the level and type of experimental control over these properties are very much technology dependent. Current electrospun technologies, for example, can be adjusted to produce scaffold structures with a wide range of geometric features, albeit in a trial-and-error approach. One example in the case of electrospun scaffolds is varying the angular velocity of the mandrel to control the degree of anisotropy (Fig. 9).

(a)

(b)

Figure 9. The degree of scaffold anisotropy can be controlled in the electrospinning process by varying the mandrel velocity. (a) Nearly isotropic – 250 RPM. (b) Highly anisotropic – 2300 RPM. [5]

One of the advantages of the current method is that it is fabrication technology independent, so that such geometric features required to obtain the desired macroscopic properties can be determined *a priori* and independent of the choice of a specific fabrication method, either currently available or developed in the future. For example, as shown in **Fig. 10**, one can use the algorithm to generate both isotropic (a) and anisotropic (b) networks.

(a)

(b)

The ODF of the fibers may be controlled by prescribing $f(\varphi_0)$. The network in (a) is isotropic, whereas the network in (b) has a preferred direction, as shown by the arrows. Also, as shown in Fig. 4 one can use the angle α to generate networks with desired tortuosity.

3.4 – Comparison with experimental data.

In this section, we present simulation results for networks whose geometries were generated so as to match the geometries measured from SEM images of actual electrospun scaffold fiber networks. Three different images were chosen, each showing a fiber network having an ODF with a different degree of anisotropy: (i) nearly isotropic, (ii) moderately aligned, (iii) highly aligned. For each of the three images, five analysts were given the task of tracing the fibers in the top layer and marking the associated fiber intersections (**Fig. 11**). Thus we obtained fifteen total observations.

(a)

(b)

(c)

(d)

(e)

(f)

Figure 11. Some of the image traces of the top layer of electro-spun polyester urethane urea (PEUU) scaffolds by one of the analysts. Each image shown is 1/6 of the total image area traced for each scaffold. The degree of anisotropy of the scaffolds was controlled by the angular velocity of the mandrel. (a)-(b) Nearly isotropic – 250 RPM. (c)-(d) Moderately anisotropic – 750 RPM. (e)-(f) Highly anisotropic – 2300 RPM

413 The total length of fiber traced was extracted using a skeletonization plugin for ImageJ [21]. The
414 image height and width, and the ODF were measured using a custom MATLAB program [5].
415 From this raw data, the fiber density ξ , the ODF $f(\varphi)$, and the normalized areal intersection
416 density $\hat{\mu}_\rho$ were derived for all fifteen observations. The five experimentally derived normalized
417 intersection densities $\hat{\mu}_\rho$ are plotted (filled circles) for each of the three images in **Fig. 12**.

(a) – Nearly isotropic ODF

(b) Moderately aligned ODF

(c) Highly aligned ODF

Figure 12. $\hat{\mu}_\rho$ for each analyst: The filled circles are the experimental observations. Unfilled circles and squares are the mean values of the simulations, and the error bars represent one standard deviation. The crosses are values derived from Eq. (11) using the actual ODF, and the line represents the values derived using the experimentally measured ODF.

Looking only at these experimentally-derived results for any one of the images (**Fig. 12(a)**, (b), or(c)), we can see that there is a large degree of variation in the measurements of the analysts. Since all five analysts were given identical copies of the image, we can conclude that this variation is due to differences among the analysts' interpretations of the images.

Next, for each of the three images we used the measured ODF $f(\varphi)$ in Eq. (11) to determine the normalized intersection density $\hat{\mu}_\rho$ predicted by the theory presented in Section 2. The $\hat{\mu}_\rho$ predicted using the measured ODF is plotted (solid line) for each image in **Fig. 12**. The first conclusion that may be drawn is that the predicted $\hat{\mu}_\rho$ decreases with increasing alignment, as can be seen by comparing **Fig. 12(a)**, (b), and (c). Also, by comparing the predicted $\hat{\mu}_\rho$ (solid line) and measured values (filled circles), we notice that in addition to the large degree of random variation between the measurements of the different analysts, there is also a systematic deviation between the measured and predicted values.

In order to determine whether part of this discrepancy may be attributed to specimen size, we performed simulations using large and small specimens. The measured values of ξ and $f(\varphi)$ were used as inputs to the algorithm developed in Section 2 to generate the simulated geometry. We selected $\alpha = \pi/60$, $\hat{s} = 0.3$, and $n_s = 100$ so that Eq. (14) implies $\hat{L}_B^2 = 30n_f$. For each of the fifteen architectures, we generated two sets of ten realizations. For the first set of realizations, we used the very large specimen size $\hat{L}_B = 300$. For the second set, we used much smaller sizes, which were chosen to match the specimen sizes of the experimentally measured images. These smaller specimen sizes fell in the range $16 \leq \hat{L}_B \leq 46$. The mean and standard deviation of $\hat{\mu}_\rho$ from the large-specimen (unfilled squares) and small-specimen (unfilled circles) sets of realizations are shown in **Fig. 12**. The error bars represent one standard deviation for those samples. For each microstructure, we also used Eq. (11) to calculate $\hat{\mu}_\rho$ predicted by the theory using the $f(\varphi)$ actually generated by the algorithm, rather than the measured $f(\varphi)$ that the algorithm was targeting. The means of these predicted values are shown for the large-specimen (x's) and small-specimen (crosses) sets of realizations in **Fig. 12**.

For the large specimen simulations, the mean $\hat{\mu}_\rho$ values (open squares) coincide almost exactly with the theoretically predicted value (solid line), and the error bars are so small that they are barely visible. We can thus assume that this size is sufficient for treating the specimens as RVEs. For the small specimen simulations, there is a large degree of variation in the mean $\hat{\mu}_\rho$ values (open circles), and the error bars are large. This variation can be understood by noticing that the mean values coincide almost exactly with theoretical predictions based on the ODF actually produced by the algorithm (crosses). Therefore, the specimen-to-specimen variation in $\hat{\mu}_\rho$ can be explained by the inability of the algorithm to correctly reproduce the measured ODF when the specimen size is small. However, the systematic deviation between the measurements (filled circles) and the theoretical prediction (solid line) cannot be attributed to the small specimen size causing a mismatched ODF, since the *measured* ODF was used in Eq. (11) to make the prediction. This suggests that the systematic deviation is likely caused by other factors.

4. DISCUSSION

4.1 – Overview.

The future state of the art in biomaterial fabrication could allow a degree of finely controllable **microstructural** design not possible with current non-woven elastomeric biomaterials. Thus, the development of computational frameworks that allow for the design of macroscopic **properties** based on microscopic features represents a positive departure from what has mostly been an empirically driven field. Central to this objective is elucidating how the unique structural characteristics exhibited by these materials translate to distinct macroscopic mechanical **properties**, especially in relation to duplicating specific biomechanical tissue **properties**. It should be noted that while the elastomeric fiber networks of electrospun scaffold materials are of particular interest to the biomaterials community, the applicability of the theory and algorithm presented here is not restricted to the elastomeric case. The elastomeric case, in which the material experiences large deformations at the macroscale, is one of the more difficult to work with since the fibers will experience large rotations at the microscale. A wide variety of important biomaterials may be modeled, such as collagen fiber networks in skin

and connective tissue, cellular stress fibers, and basement membranes. The current approach is well suited to any layered material composed of long intersecting fibers.

The current work presents approaches for characterizing and simulating layered fibrous materials, based on theoretical estimates for the mean linear and areal intersection densities for two-dimensional fibrous networks. It builds on the experience with periodic elastic lattices [22, 23], which share some geometric and mechanical features with fibrous networks. The macroscopic elastic properties of a periodic elastic lattice are derived from a unit cell analysis based on classical Euler-Bernoulli beam theory. Networks generated using the algorithm described in Section 2 can be used as the mesh for finite element simulations of multiscale mechanical behavior of layered fibrous materials. The current theoretical results are valuable because they allow one to estimate the difficult-to-measure intersection densities, which have a significant effect on bulk mechanical behavior, in terms of the easy-to-measure linear fiber density ξ and the ODF. The algorithm is valuable because it can be adopted for micromechanical simulations of electrospun scaffolds and other layered elastomeric fibrous biomaterials. The theoretical and numerical results compare favorably with each other for sufficiently large networks, which can be characterized as RVEs. For the mean areal intersection density it is sufficient to use specimens with $L_b \geq 100\xi^{-1}$. The mean linear intersection density function requires higher values; $L_b \geq 600\xi^{-1}$ for the example presented in Section 3.

We note that the current theoretical development and algorithm are restricted to a single 2-D layer. Moreover, these theoretical and algorithmic developments are not easily extended to three dimensions. The key difficulty is that the intersection densities in three dimensions depend not only on the fiber length but also fiber diameter. However, the current algorithm may be easily extended to generate 3-D geometries that consist of multiple connected layers of 2-D networks. In this case, each sequential layer is generated in a plane that is located one fiber diameter above the previous layer. During the random walk process, intersections are generated not only with segments within the same layer, but also with segments in adjacent layers. This extended algorithm may be used to generate realistic geometry for a broad range of layered fibrous network materials, including the electrospun structures considered in the present work. The algorithm may also be improved in order to

attain an even more accurate approximation of desired ODFs. The improvements could include adaptivity, so that the initial angles φ_0 are not chosen *a priori* but rather using the current ODFs for the fibers that have been already generated.

The comparison between theoretical estimates with experimental measurements shows that measuring network intersection density μ_p unambiguously is very difficult (**Fig. 12**). The large systematic and random deviations from the predicted values are not all that surprising, even under ideal lab conditions. In real images of fiber networks, in which fibers have non-zero diameter and weave over and under each other, the determination of “what counts as an intersection” and “which fibers are on the top layer” become open to individual interpretation. In contrast, the simulation results are very consistent because the geometry is idealized as a single layer of zero-thickness curves, so there is no ambiguity related to fiber intersections or layers. While the linear and areal intersection densities of real networks are difficult to measure directly, these quantities may be determined with relative ease using the relationships between microscopic and macroscopic quantities derived in this paper. In contrast with intersection density μ_p , the fiber density ξ and ODF $f(\varphi)$ of a network are macroscopic quantities and may be measured with high accuracy using images or approximated using knowledge of the manufacturing conditions. Subsequently, these quantities may be used in Eq. (6) and Eq. (9) - Eq. (11) to determine the intersection densities of these networks.

4.2 – Limitations.

The fibrous network geometry generated using the proposed algorithm is a good idealization of many materials of interest to the biomaterials community and represents a significant advance in the fidelity of geometric representations of such materials. However, there are some limitations to the model. One limitation is that the fiber orientation in the initial configuration generated using the proposed algorithm is not permitted have any component perpendicular to the plane is a limitation of our approach. This is not a significant limitation, however, since the proposed stacked layer approach is a very good approximation for electrospun scaffolds, as well as many tissues such as basement membranes. In such scaffolds, most fiber out-of-plane undulation amplitudes are not more than a few fiber diameters. In our

approach, fibers form intersections with fibers in adjacent layers, which are separated by a distance of one fiber diameter, so this is a reasonable, albeit imperfect representation of the real material. Additionally, within each layer, the fibers are represented as 1-D curves that reside strictly in the 2-D plane, so each fiber intersection contains a single point that is shared by two curves. Of course, a real fiber have non-zero diameter and cannot pass through other fibers in the plane, but must weave above and below them at a centerline-to-centerline distance of at least one fiber diameter. Finally, we note that another characteristic of the proposed algorithm is that there are only a few input parameters to select, which makes the algorithm relatively easy to implement and use. However, this could lead to a situation where one has less control over the geometric characteristics of the resulting network than could be gained by using a more complex algorithm with more user inputs.

4.3 – Relations to biomaterial scaffold micromechanics.

The context of the usefulness of the present findings can be best understood in the context of an improved appreciation of how electrospun scaffolds function across multiple length scales. Fiber tortuosity, a measure of how much a fiber deviates from being straight in the unloaded scaffold, was observed to be dependent on both mandrel velocity during production and orientation. As the scaffold underwent planar biaxial modes of deformation, fiber tortuosity was extinguished and substantial fiber rotational kinematics was observed contributing to an intricate fiber recruitment process [24]. Electrospun constructs were observed to follow gross affine fiber transformations. Interestingly, some fibers were observed to rotate or change their direction of orientation during deformation, while as a population, no net change was measured. Furthermore, it was observed that neighboring fibers were well attached where they overlapped or intersected. These attachments impede translation of fibers with respect to one another but do not appear to inhibit rotational fiber kinematics about these points of intersection. The overall strain behavior tends to become increasingly more homogenous as the scale of interest approaches the tissue level. Yet, in a recent study, elastomeric electrospun construct fabrication conditions were evaluated in regards to their

effect on fiber geometry and biaxial mechanical behaviors [11]. It was determined that fiber alignment alone was insufficient to predict mechanical response.

Simulation clearly plays an important role in understanding these phenomena [25]. The underlying models must be based on a necessary subset of morphology descriptors, such as material porosity, fiber density, fiber alignment distribution, fiber connectivity distribution, and fiber diameter, which may be measured through image analyses. The need for rigorously defined material structural descriptions has been extensively highlighted in recent literature where the model capability relies prevalently on the accuracy of the network topology [26-30]. The present approach presents a method to not only simplify such studies by defining a limited set of parameters, but also by defining such parameters that are easily determined experimentally.

4.4 – Conclusions and future work.

The present algorithm can greatly inform the development of layered fibrous network materials. While maturing, current efforts in the field of tissue engineering continue to be confounded by a lack of understanding of the intricate multi-scale hierarchical arrangements and interactions typically found in native tissues. The current state of the art in biomaterial processing enables a degree of controllable microstructure that can be used for the development of model systems to deduce fundamental biological implications of scaffold matrix morphologies on cell function. From our initial finite element simulations, one of the more interesting phenomena we have observed is the emergence of long-range fiber structure [25]. The precise relations between initial network geometry and the resulting fiber kinematics under loading remain to be elucidated. The work of understanding these relationships using finite element simulations with meshes generated using the algorithm presented here is the next phase in progressing toward the ultimate goal of designing better materials. The first step will be to validate the model by attempting to accurately reproduce macroscopic experimental stress-stretch curves of scaffolds under in-plane biaxial tension. Next, we will try to quantify the relationships between the geometric quantities proposed here to characterize the fiber network, and the shapes of the in-plane stress-stress curves. It may also be necessary to define

598 additional geometric quantities to characterize the mesoscale structures that emerge during
599 loading. The 3-D nature of our simulated networks will then allow us to investigate out-of-plane
600 shear, bending, and twisting load cases. Other future work in the area of tissue engineering will
601 include the adding cellular and extracellular matrix (ECM) phases to the model and using
602 parallelization and multiscale modeling approaches to model an entire heart valve leaflet.
603 Ultimately, the development of computational frameworks that enable the simulation of
604 experimentally derived observations represents a positive departure from what has mostly
605 been an empirically driven field.

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