Computational Analysis and Optimization of Geometric Parameters for Fibrous Scaffold Design

Authors

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1. **Cell coordinate system**

   We generated the cell coordinate system using an approach similar to the one described by Uchida and Delp.\(^1\) First, we defined the position of the cell origin relative to the scaffold frame by computing the position of the geometric cell centroid. We computed the position of the centroid by taking the average x-, y-, and z-positions of the four cell attachment points in the scaffold coordinate frame. The position of the cell origin is denoted as \( C_C \) in Figure S1A. Next, we computed the orientation of the axes of the cell system relative to the scaffold frame based on the positioning and orientation of the cell.

We constructed the z-axis of the cell frame such that it was parallel to the longest edge of the cell tetrahedron, which is highlighted with a red dashed line in Figure S1B. To do so, the algorithm computed the length of all six edges of the cell tetrahedron by taking the difference between the positions of each pair of cell attachment points (the vertices of the tetrahedron), then

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*Figure S1: Construction of the cell coordinate frame. A) Position of the cell centroid and the origin of the cell coordinate system. B) The orientation of the z-axis in the cell coordinate frame is parallel to the longest edge of the cell tetrahedron (red dashed line). C) The orientation of the x-axis in the cell coordinate frame is mutually perpendicular to the longest edge of the cell and the edge opposite of the longest edge (red dashed lines). D) The orientation y-axis of the cell coordinate frame is selected to create a right-handed coordinate system with the other two existing axes.*
taking the root-sum-squares of the differences. The algorithm identified longest vector and normalized it to create the unit vector for the z-axis of the cell coordinate system relative to the scaffold coordinate system.

We constructed the x-axis of the cell frame such that it was mutually perpendicular to the longest edge of the cell and the opposite edge of the cell, which are highlighted with red dashed lines in Figure S1C. We first identified the two vertices that were connected by the longest edge of the cell. Next, we identified the other two cell vertices and connected them to define the opposite edge. Once we constructed vectors for the longest edge of the cell and the opposite edge, we computed their cross product to construct a vector mutually perpendicular to both edges, as depicted in Figure S1C. We normalized the result of the cross product to create a unit vector that expressed the direction of the x-axis of the cell frame relative to the scaffold coordinate system.

Finally, we constructed the unit vector for the y-axis by taking the cross product of the z- and x- unit vectors (Figure S1D), then used the unit vectors and the origin position to construct transformation matrices to the cell frame from the scaffold frame and vice versa. We transformed the positions of the cell vertices to the cell coordinate system to compute the volume and aspect ratio of the cell, as described in the main text.

2. Fiber orientation

Each fiber was initially centered at the origin of the scaffold coordinate system with the long axis aligned with the z-direction of the scaffold frame (Figure S2A). The fiber was first rotated about the z-axis of the scaffold coordinate system to randomly orient the radial fiber attachment points on each fiber. This rotation is described by the rotation matrix from the scaffold frame to the first intermediate coordinate system shown in Figure S2B. The rotation about the z-axis of the scaffold frame was specified by the rotation matrix $^1R_s$ such that

\[
^1R_s = \begin{bmatrix}
\cos (\phi r_1) & \sin (\phi r_1) & 0 \\
-\sin (\phi r_1) & \cos (\phi r_1) & 0 \\
0 & 0 & 1
\end{bmatrix}
\]

where $r_1$ is a uniformly distributed random number from interval [0,1) and $\phi$ is $2\pi$. 
After rotation about the z-axis of the scaffold frame, the fiber was then rotated randomly about the y-axis of the first intermediate frame within a range of angles to create the second intermediate coordinate frame (Figure S2C). The rotation about the y-axis of the first intermediate frame was specified by the rotation matrix \( R_1 \) such that

\[
R_1 = \begin{bmatrix}
\cos(\theta_f) & 0 & -\sin(\theta_f) \\
0 & 1 & 0 \\
\sin(\theta_f) & 0 & \cos(\theta_f)
\end{bmatrix},
\]

\( f = 0.5 - r_2, \) \( (S2) \)

where \( f \) is a value on the interval [-0.5, 0.5), which is calculated from another random number, \( r_2 \). For simulations where the fibers were aligned, \( \theta \) was equal to 0. For simulations with randomly oriented fibers, \( \theta \) was equal to \( \pi \).

Next, the fibers were rotated randomly about the x-axis of the second intermediate frame to the fiber frame within a range of angles (Figure S2D). This rotation was specified by the rotation matrix \( R_2 \) such that

\[
fR_2 = \begin{bmatrix}
1 & 0 & 0 \\
0 & \cos(\theta_f) & \sin(\theta_f) \\
0 & -\sin(\theta_f) & \cos(\theta_f)
\end{bmatrix},
\]

\( f = 0.5 - r_3, \) \( (S5) \)
where \( f \) is calculated with the same formula above but using a newly selected random number, \( r_3 \). To rotate from the scaffold frame to the fiber frame, the elementary rotation matrices were multiplied

\[
f^1 R_s = f^1 R_2^{-1} R_1^{-1} R_s.
\]

Following the sequential rotations, the fiber was translated along the three coordinate axes of the scaffold frame. The \( x \)- and \( y \)-translations were selected to generate hexagonal packing of the fiber centers, as noted in the methodology of the main text. The translation in the \( z \)-direction is described by the equation

\[
z_{tr} = (0.5 - r_4) * (z_{max} - z_{min})
\]

where \( r_4 \) is a uniformly distributed random number, \( z_{max} \) is the largest \( z \)-value in the simulation box and \( z_{min} \) is the smallest \( z \)-value in the simulation box (in the scaffold coordinate system). These values were restricted to the maximum and minimum \( z \)-values specified by the periodic boundary, -37.5 \( \mu \)m to 37.5 \( \mu \)m.

### 3. Volume fraction as a function of fiber spacing

The volume fraction is defined as

\[
v = \frac{V_{fiber}}{V_{total}}
\]

where \( V_{fiber} \) is the total volume of fibers in a hexagonal unit cell and \( V_{total} \) is the total volume of the hexagonal unit cell. The volume of fibers in each unit cell of hexagonally packed cylinders is

\[
V_{fiber} = \frac{3 \pi d^2 l_z}{4}
\]

where \( d \) is the diameter of the fibers, and \( l_z \) is the length of each fiber. The volume of each unit cell (hexagonal prism) is

\[
V_{total} = \frac{3 \sqrt{3}(d + s)^2 l_z}{2}
\]

where \( s \) is the spacing between fibers. Substituting,
\[ v = \frac{3\pi d^2 l_z}{4} = \frac{\pi d^2}{2\sqrt{3}(d + s)^2} \]  
\[ v - \frac{\pi d^2}{2(d + s)^2\sqrt{3}} = 0 \]  

Equation (S12) above was solved for \( s \) using volume fractions of 0.13 and 0.21, the experimentally reported range. With \( v = 0.13 \), \( s = 1.97 \mu m \). With \( v = 0.21 \), \( s = 1.29 \mu m \).

4. Aspect ratio, volume, and length relationships

Meehan and Nain reported cell lengths between 80 \( \mu m \) and 100 \( \mu m \) when cells were suspended on single fibers. In the present work, the optimization procedure resulted in aspect ratios of up to 29, which are higher than the aspect ratios in some experimental reports. To determine if the cell lengths were reasonable at these high aspect ratios, the cell lengths were computed based on the volume (V) and aspect ratio (AR) equations.

\[ V = \pi \frac{dx_c}{2} \frac{dy_c}{2} \frac{dz_c}{2} \]  
\[ AR = \frac{\text{length of longest edge}}{\text{average length of other edges}} = \frac{2 * dz_c}{dx_c + dy_c} \]

To derive the length of the cell, the z-axis of the cell was assumed to be the longest edge and the x- and y- axes were assumed to be equal, resulting the equations

\[ V = \pi \frac{dx_c^2}{4} \frac{dz_c}{2} \]  
\[ AR = \frac{dz_c}{dx_c} \]

From these equations, the experimentally reported volumes, and the maximum aspect ratios from the optimization, the maximum cell lengths can be computed via substitution.

\[ AR * dx_c = dz_c \]  
\[ V = \pi \frac{dx_c^2}{4} \frac{AR * dx_c}{2} = \frac{AR * \pi}{8} * dx_c^3 \]
\[ dx_c = \left( \frac{V * 8}{AR * \pi} \right)^{\frac{1}{3}} \]  

(S19)

For a volume of 500 $\mu m^3$ and an aspect ratio of 29, $dx_c = 3.52 \mu m$ and $dz_c = 102 \mu m$. For a volume of 1000 $\mu m^3$ and an aspect ratio of 29, $dx_c = 4.44 \mu m$ and $dz_c = 129 \mu m$. These lengths, while higher than the experimental results, seem to be reasonably close to experimentally observed cell lengths.\(^3\)

5. Supplemental References

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