

Effects of extracellular fiber architecture on cell membrane shear stress in a 3D fibrous matrix

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Abstract

Interstitial fluid flow has been shown to affect the organization and behavior of cells in 3D environments in vivo and in vitro, yet the forces driving such responses are not clear. Due to the complex architecture of the extracellular matrix (ECM) and the difficulty of measuring fluid flow near cells embedded in it, the levels of shear stress experienced by cells in this environment are typically estimated using bulk-averaged matrix parameters such as hydraulic permeability. While this is useful for estimating average stresses, it cannot yield insight into how local matrix fiber architecture—which is cell-controlled in the immediate pericellular environment—affects the local stresses imposed on the cell surface. To address this, we used computational fluid dynamics to study flow through an idealized mesh constructed of a cubic lattice of fibers simulating a typical in vitro collagen gel. We found that, in such high porosity matrices, the fibers strongly affect the flow fields near the cell, with peak shear stresses up to five times higher than those predicted by the Brinkman equation. We also found that minor remodeling of the fibers near the cell surface had major effects on the shear stress profile on the cell. These findings demonstrate the importance of fiber architecture to the fluid forces on a cell embedded in a 3D matrix, and also show how small modifications in the local ECM can lead to large changes in the mechanical environment of the cell.

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1. Introduction

Interstitial flow is a ubiquitous slow fluid flow through the extracellular matrix (ECM). It has been shown to play important roles in the growth and remodeling of many tissues including bone (You et al., 2001; Knothe Tate, 2003), the arterial wall (Kim et al., 2004), cartilage (Grodzinsky et al., 2000), and lymphatics (Boardman and Swartz, 2003), and in vitro it can drive the organization of endothelial cells (Ng et al.,

2004; Helm et al., 2005; Semino et al., 2006), align fibroblasts and cause them to differentiate into myofibroblasts (Ng and Swartz, 2003; Ng et al., 2005) and alter smooth muscle cell behavior (Wang and Tarbell, 1995, 2000). Despite its importance, the mechanical environment imposed on a cell by interstitial flow is not well understood.

The architecture of ECM fibers is important in determining the efficiency of strain transmission through the solid phase of the matrix (Agoram and Barocas, 2001; Sawhney and Howard, 2002; Chandran and Barocas, 2004), but it has not been considered in relation to the shear forces imposed by the fluid phase moving through it. Most mathematical models of interstitial flow through soft tissues to date represent the tissue as a continuum with a flow resistance

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Nomenclature

K	hydraulic permeability
$K^{1/2}$	Brinkman screening length, also estimated average pore size of a medium with permeability K
a	cell radius
d	fiber diameter
s	fiber spacing

τ	shear stress
τ^*	dimensionless shear stress ($= \tau/U_0\mu/a$)
μ	viscosity
ρ	fluid density
σ	dimensionless permeability
μ'	effective viscosity of a porous medium
U_0	free stream velocity, also superficial velocity
U_i	average interstitial velocity
ε	porosity
ϕ_s	solid volume fraction ($= 1-\varepsilon$)

parameter (hydraulic permeability, K) and are concerned with bulk-averaged fluid stresses (Mow et al., 1980; Wang and Tarbell, 1995, 2000; Tada and Tarbell, 2002). Such models cannot predict the maximum or range of shear stresses experienced by the cell, which depend on the details of the matrix architecture.

Here, we take a computational fluid dynamics (CFD) approach to investigate, on a first-order scale, the following questions: (1) how do fiber size and spacing affect the fluid profile around a cell embedded within it? and (2) to what degree can local pericellular matrix contraction change that flow profile? We are particularly interested in sparse in vitro matrices such as reconstituted collagen and fibrin gels, where the fiber spacing can be within an order of magnitude of the cell diameter (Pedersen and Swartz, 2005). Recently, other investigators have begun studying flow within 3D constructs and through bioreactors using CFD simulations (Raimondi et al., 2002; Boschetti et al., 2006; Cioffi et al., 2006); in those cases, the cells were adherent to the side of a large pore or large fiber rather than being suspended within a mesh of smaller fibers, and fluid did not flow around the cell but rather past the exposed side. We show here that the local flow field perturbations caused by the presence of ECM fibers near the cell can cause significant changes in shear stress magnitude on the surface of the cell. This is relevant for evaluating the pericellular mechanical environment in 3D reconstituted fibrous matrices where imposed interstitial fluid flow drives cell response.

2. Materials and methods

2.1. CFD model construction and meshing

The 3D model of the fluid flow domain was constructed using Rhinoceros (v2.0, Robert McNeel & Assoc; Seattle, WA). The domain was centered around a spherical cell of radius a (7.5 μm), embedded in a cubic lattice of fibers of diameter d and spacing s (Fig. 1). The extent of the domain was a minimum of $6a$ in the flow direction and $3a$ perpendicular to flow. In all three

directions, the domain extent was additionally constrained to be an integer multiple of s , to ensure the edges of the domain coincided with fiber centerlines. We designed and built two sets of models. The models in group A were designed to study the influence of fiber size and spacing on the shear stress (see Table 1). In the group B models, extra fibers were added near the cell (B2, B3, see Figs. 6c and d), or the size of existing fibers was increased (B4), to determine the extent to which local fiber contraction might shield the cell from fluid shear stress. The decrease in porosity near the cell was identical for matrices B2–B4, and when the symmetry boundary conditions are considered, the total number of additional fibers is identical for B2 and B3.

The 3D models were exported in ACIS format and loaded into Gambit (v2.2, Fluent Inc; Lebanon, NH) for meshing. The fluid flow domain was meshed using triangular face meshes and tetrahedral/hybrid volume elements, at meshing intervals that varied between models. The domains were meshed on a Pentium M/Windows XP machine, and were limited to $\sim 2.8 \times 10^6$ mesh volumes. A grid convergence study (Roache, 1997) showed that models meshed using intervals of $0.1a$ or smaller were within the asymptotic convergence region for both average and maximum shear stress.

2.2. CFD simulation, boundary conditions and assumptions

The commercial finite-volume CFD software FLUENT (v6.2, Fluent, Inc; Lebanon, NH) was used to simulate fluid flow and analyze the results. No-slip boundary conditions were applied to the surface of the cell and the fibers, which were considered rigid and impermeable. A null gauge pressure condition was set at the outlet plane, and symmetry conditions were set on the four planes parallel to the flow direction (Fig. 1). The two surfaces passing through the sphere's center are true symmetry surfaces, and the size of the domain was selected to ensure that the edges of the domain were far enough from the cell that the theoretical velocity magnitude on the other two boundaries was within

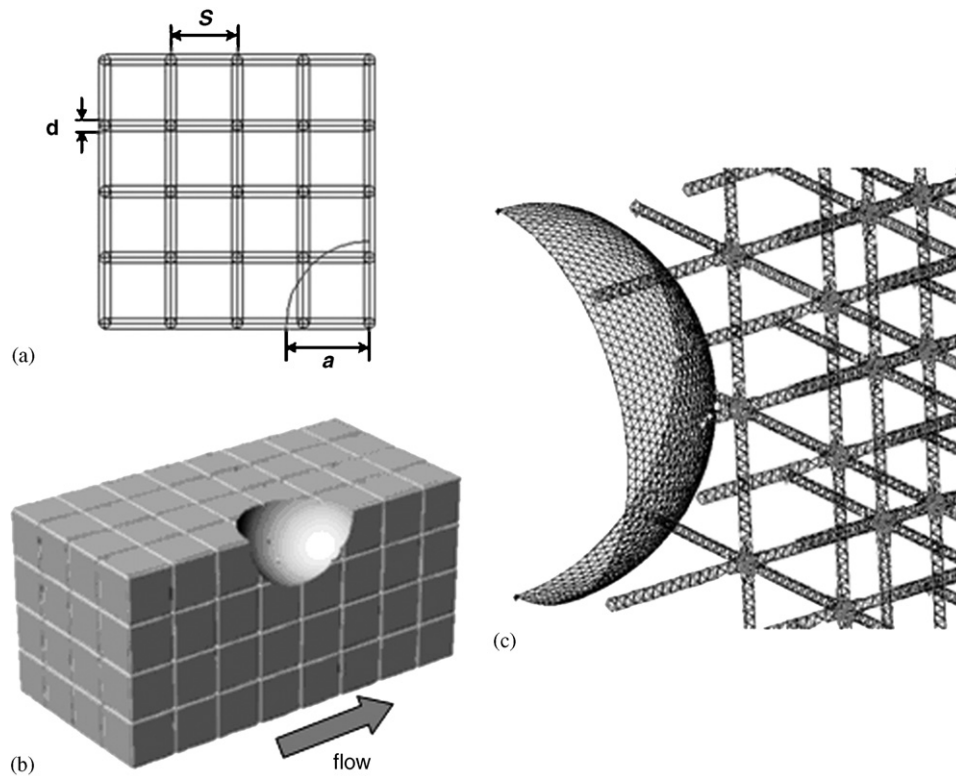


Fig. 1. CFD model setup. (a) Definition of the geometrical parameters of the model. (b) 3D rendering of the flow domain. (c) Detail of the mesh on the cell surface and nearby fibers.

Table 1
Summary of computational model parameters

Group A—Homogenous cubic lattices

	d (nm)	s (μm)	Notes	ε
A1	700	4	$K = 2.23 \times 10^{-8} \text{ cm}^2$	0.934
A2	1245	5	$K = 2.23 \times 10^{-8} \text{ cm}^2$	0.872
7 other models	500–2000	4,5,6	K varies from 1.9×10^{-8} to $9.5 \times 10^{-8} \text{ cm}^2$	Varies from 0.78 to 0.98

Group B—Shielding study models

	d (nm)	s (μm)	Notes	ε (bulk/near cell)
B1	750	6	Homogenous lattice	0.966/0.966
B2	750	6*	*Extra fibers parallel to flow	0.966/0.953
B3	750	6*	*Extra fibers perpendicular to flow	0.966/0.953
B4	750*	6	*Larger fibers parallel to flow	0.966/0.953

5% of the free stream value (Ganapathy, 1997). In most cases, a mass-flow boundary condition was applied at the inlet of the domain to deliver a superficial fluid velocity U_0 of $4.2 \mu\text{m/s}$, which was the mean of the velocity range we used in other studies to drive fibroblast differentiation and capillary morphogenesis in collagen and fibrin gels (Ng et al., 2004, 2005; Helm et al., 2005). For models B2 and B3, the mass flow inlet boundary condition was replaced by a pressure gradient of $1.79 \times 10^{-3} \text{ Pa}/\mu\text{m}$, which was calculated by inducing a superficial fluid velocity of $4.2 \mu\text{m/s}$ through model B1.

The interstitial fluid was modeled as an incompressible Newtonian fluid with a density ρ of 1 g/cm^3 and the viscosity μ of 37°C culture medium used for recent in vitro experiments, 0.748 cP (Helm et al., 2005). Steady-state solutions of the fluid momentum equation and continuity equation were sought, and solutions were iterated until the continuity residual was 1×10^{-7} ; the velocity variable residuals were always smaller than the continuity residual. Simulations were run on either a Pentium M/Windows XP machine with 1.0 GB RAM or an SGI IRIX Origin 2000 8-processor machine.

2.3. Non-dimensionalization of shear stress in the CFD model

The flow in the sparse fibrous matrices modeled here is well within the low Reynolds number (Re) regime— Re based on the cell diameter ($Re = 2a\rho U_0/\mu$), fiber diameter ($= d\rho U_0/\mu$), and mean pore size ($= 4V_p/S_p\rho U_0/\mu$), where V_p is the pore volume and S_p is the pore surface area (Dullien, 1979)) were approximately 8×10^{-5} , 4×10^{-6} , and 1×10^{-4} , respectively. Therefore, our solution is in a linear regime where the shear stresses scale linearly with the superficial fluid velocity through the matrix, for fluid speeds less than 500 $\mu\text{m/s}$. We thus non-dimensionalized the shear stress results as

$$\tau^* = \tau / \left(\frac{U_0\mu}{a} \right). \quad (1)$$

3. Comparison to continuum theory

Traditionally, flow through fibrous media has been modeled using continuum approximations such as the Darcy and Brinkman equations, where the properties of the fibrous matrix are represented by a hydraulic permeability K that averages the flow resistance offered by the porous media across the entire flow domain. The Brinkman equation (Eq. (2)) was originally introduced as an interpolation between Darcy flow and Stokes flow (Brinkman, 1947), but it has since been validated using more rigorous approaches based on the Navier–Stokes equation (Lundgren, 1972; Chernyakov, 1998).

$$\vec{\nabla} P = -\frac{\mu}{K} \vec{v} + \mu' \nabla^2 \vec{v}. \quad (2)$$

We derived an analytical expression for the shear stress, τ , on a sphere of radius a in Brinkman flow from the solution of the flow field (Ganapathy, 1997). With the exception of the term containing σ , the dimensionless permeability, this result is identical to the shear stress on a sphere in Stokes flow; thus, the effect of the porous media is to globally amplify the shear stress at the surface of the sphere:

$$\tau = \frac{3}{2} \frac{U_0\mu}{a} \sin \theta \left(1 + \frac{1}{\sigma} \right) \quad (3)$$

and

$$\sigma = \sqrt{\frac{K}{a^2} \left(1 + \frac{5}{2} (1 - \varepsilon) \right)}, \quad (4)$$

where ε is the porosity of the medium and θ the angle between the direction of U_0 and the position on the sphere where τ is being measured. The average shear stress is then

$$\tau_{\text{avg}} = \frac{3\pi}{8} \frac{U_0\mu}{a} \left(1 + \frac{1}{\sigma} \right). \quad (5)$$

The maximum shear stress, occurring when $\sin \theta = 1$, is

$$\tau_{\text{max}} = \frac{3}{2} \frac{U_0\mu}{a} \left(1 + \frac{1}{\sigma} \right). \quad (6)$$

The shear stress gradient, with respect to the arclength l ($= \theta a$) is

$$\frac{d\tau}{dl} = \frac{3}{2} \frac{U_0\mu}{a^2} \left(1 + \frac{1}{\sigma} \right) \cos \theta. \quad (7)$$

The maximum shear stress gradient occurs at the stagnation point on the sphere, where $\theta = 0$.

Our interest focuses on the effects of ECM fibers on the flow around cells embedded in highly hydrated fibrous matrices. Since it is not possible to directly measure flow velocities around or shear stresses on such cells experimentally, we will compare our CFD results to the benchmark predicted by the Brinkman equation using Eqs. (3)–(7).

4. Results

4.1. Permeability of in vitro matrices

Reducing the complex structure of fibrous matrices to a single parameter (K) introduces an ambiguity into analyses of flow through porous media, predicting that two matrices of different architectures can have identical fluid flow properties. To illustrate this ambiguity, K was calculated for each of a series of cubic fiber lattices with d ranging from 300 to 1500 nm, and s ranging from 2–10 μm using a Carman–Kozeny-based permeability relationship ((Happel, 1959); see Appendix A for details). K ranged from approximately 1×10^{-9} to $4 \times 10^{-7} \text{ cm}^2$ for these matrices (Fig. 2), which compares well with previously measured values for reconstituted collagen matrices (Wang and Tarbell, 2000; Ng and Swartz, 2003). Our choice of the range of d was informed by recent investigations showing that fiber diameters of reconstituted collagen matrices are larger than previous electron microscopy images indicated (summarized in (Pedersen and Swartz, 2005)). As shown, matrix geometry and permeability have a nonunique relationship—some matrices with small, more closely spaced fibers can yield the same K as other matrices with larger fibers spaced more widely apart.

4.2. Comparison of CFD shear stress results to Brinkman predictions

For two specific matrices, models A1 (“thin fiber”) and A2 (“thick fiber”), CFD simulation shows that the maximum shear stresses were elevated at least $3 \times$ above that predicted by the Brinkman equation (using $\sigma = 0.22$), and average shear stresses were elevated twofold (Fig. 3). Both the thin and thick fiber matrices

had a K of $2.23 \times 10^{-8} \text{ cm}^2$, and a σ of 0.22 and 0.23 despite having different porosities ε of 93.4% and 87.2%, respectively. The maximum and average dimensionless shear stress, τ^* ($= \tau/(U_0\mu/a)$), predicted by the Brinkman equation was 8.32 and 6.53, while for the thin fiber matrix these were 27.9 and 11.6, and for the thick fiber matrix they were 35.6 and 13.7, respectively. Therefore, both matrices yielded results quite different from the Brinkman prediction, and the thick fiber matrix had τ_{max} and τ_{avg} that were 28% and 18% higher than those of the thin matrix, respectively. Furthermore, the locations of highest shear stresses are not confined to the portions of the cell surface tangent to the flow

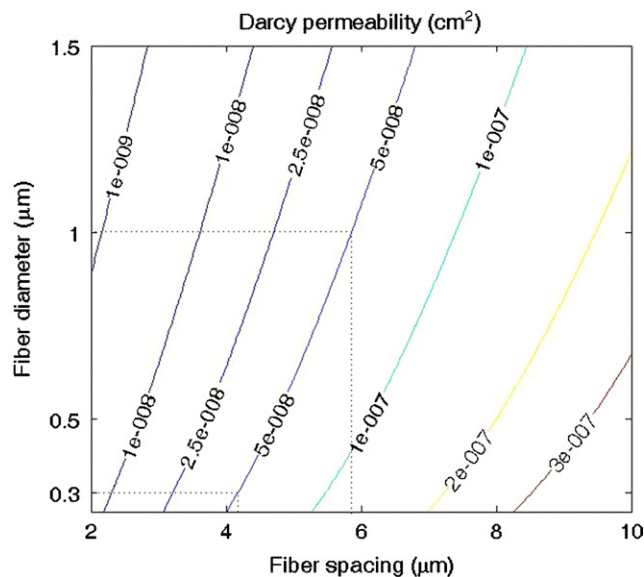


Fig. 2. Contour plot of Carman–Kozeny hydraulic permeabilities for cubic-lattice matrices. Note that a matrix with 300 nm fibers spaced 4.17 μm apart has the same permeability as one with 1 μm fibers spaced 5.85 μm apart.

direction, as would be predicted by Brinkman (Eq. (3)), but correspond to regions where fibers have focused higher speed flows near the cell surface. The location and relative magnitude of these shear stress “hot spots” depends on the specific fiber architecture and the relative location of the cell with respect to the fibers nearest the cell surface.

Fig. 4 shows a summary of the maximum and average τ^* results for both simulations (data points) compared to the Brinkman solutions (lines), with results grouped by fiber spacing. For all matrices tested, CFD simulations consistently yield average shear stresses that are $>75\%$ above Brinkman predictions, and in some cases τ_{max} is more than five-fold higher. All simulation results followed the general trend predicted by the Brinkman model: that is, as the porosity of the matrix decreases (σ decreasing), τ_{max} and τ_{avg} increase for a given fixed mass flow rate. The CFD-estimated τ_{avg} was approximately twice the Brinkman values for all tested values of σ and s . However, for matrices with $s = 6 \mu\text{m}$, τ_{max} was three times the Brinkman value for $\sigma = 0.42$ and 5 times the Brinkman value for $\sigma = 0.23$ (Fig. 7a). This trend of τ_{max} increasing more than what the Brinkman equation predicts for decreasing values of σ was also seen in the matrices with $s = 3$ and $4 \mu\text{m}$. Maximum shear stress gradients are 5–14 times greater those predicted by Brinkman using Happel’s permeability relationship (Fig. 5), with the greatest differences being seen again in matrices with the highest flow resistance (low σ).

Simulation results were also compared to the Brinkman prediction using K from a recent computational model of permeability in cubic fiber lattices (Higdon and Ford, 1996). For a given matrix, and therefore a given d , s and ε , Higdon and Ford’s permeability relationship yields a K that is approximately 4x lower than the Happel relationship. Reducing K by a factor of 4 with a

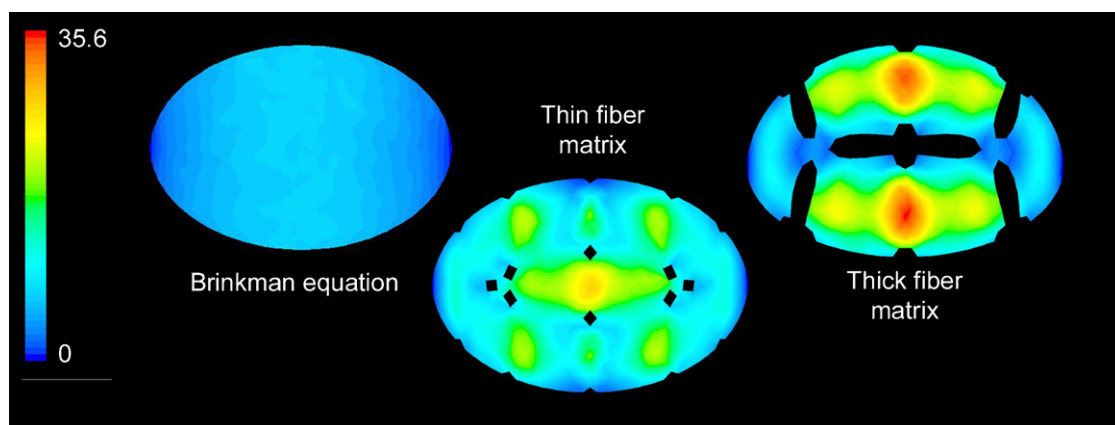


Fig. 3. Comparison of shear stress results from the Brinkman equation and two CFD simulations of matrices with $K = 2.23 \times 10^{-8} \text{ cm}^2$. Color contours of τ^* ($= \tau/(U_0\mu/a)$) show that the presence of fibers in the flow field substantially increases the maximum τ^* for matrices with both thinner, more closely spaced fibers ($d = 700 \text{ nm}$, $s = 4 \mu\text{m}$) and thicker, more broadly spaced fibers ($d = 1245 \text{ nm}$, $s = 5 \mu\text{m}$) above that predicted by the Brinkman equation for a porous medium with the same K . CFD results show average τ^* approximately twice as high as the Brinkman prediction. For these simulations, $U_0\mu/a = 4.19 \times 10^{-4} \text{ Pa}$.

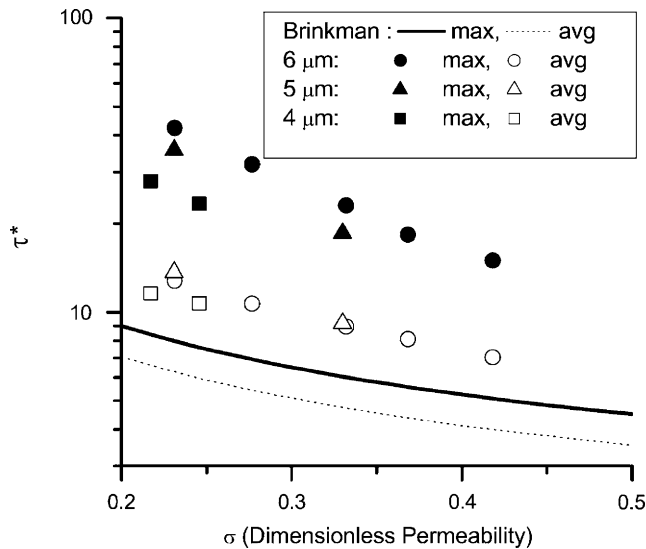


Fig. 4. Maximum and average shear stress on the cell surface increases as the matrix becomes more restricted. Here, τ^* ($= \tau/(U_0\mu/a)$) is the dimensionless shear stress, and σ is the dimensionless permeability ($= (K/a^2 (1-5/2(1-\epsilon)))^{1/2}$). As σ decreases, the expected $\tau_{\max}^*$ (solid line) and τ_{avg}^* (dashed line) predicted by the Brinkman equation increase slightly. Our computational results show considerably elevated $\tau_{\max}^*$ (solid markers) and τ_{avg}^* (open markers) for matrices with fiber spacings of 6 μm (circles), 5 μm (triangles) and 4 μm (squares). Matrices with widely spaced large fibers (circles with low σ) show the largest increase in $\tau_{\max}^*$, while τ_{avg}^* is approximately twice that predicted by the Brinkman equation for all values of σ tested here.

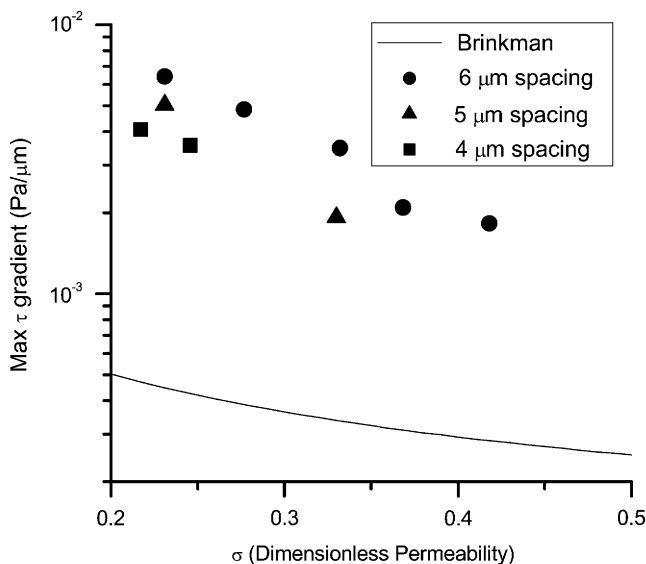


Fig. 5. The presence of fibers significantly affects the shear stress gradients on the cell surface. As matrix permeability decreases, the amplification of the maximum shear stress gradient over the maximum Brinkman value increases for all 3 matrix families.

and ϵ constant reduces σ by 50% (Eq. (4)), and increases the Brinkman-predicted τ by about 76% (Eqs. (5) and (6)) with no change in U_0 , a or μ (Fig. 7(b)). Experimental data linking flow through fibrous matrices

with structural information on those matrices has considerable scatter (Jackson and James, 1986), but regardless of which relationship is used, we find that the Brinkman approximation consistently underestimates $\tau_{\max}$, and that the amount of $\tau_{\max}$ underestimated increases with decreasing σ (see Fig. 7).

4.3. Shear stress shielding via addition of fibers around a cell

Using models from group B, we found that the addition of a few fibers near the cell can substantially reduce the shear stress on the cell surface without significantly affecting matrix porosity or K . In the models with extra fibers near the cell, the “extra parallel” (model B2) and the “extra perpendicular” (model B3), the porosity near the cell was decreased from 96.6% to 95.3%. When the pressure gradient calculated for model B1 was applied to model B2, $\tau_{\max}$ decreased by 34%, and τ_{avg} decreased by 24%. $\tau_{\max}$ and τ_{avg} also decreased for model B3, by 29% and 12%, respectively (Figs. 6a and b). The average superficial inlet velocity for models B2 and B3 decreased from 4.2 to 3.8 and 3.7 $\mu\text{m/s}$, respectively. When holding the superficial velocity constant (as in the group A models) instead of the pressure gradient, the shielding effect was reduced for model B2, but it was almost eliminated for model B3 (Fig. 6b). Model B4, under both the pressure gradient and superficial velocity boundary conditions, yielded $\tau_{\max}$ and τ_{avg} within 1% of the unmodified model B1 (data not shown).

5. Discussion

Our results demonstrate that the local architecture of the ECM strongly affects the shear stress on a cell undergoing interstitial flow, and that these shear stresses are substantially underestimated by the Brinkman equation. These differences appear to result from different mechanisms for shear stress amplification as the matrix becomes more restricted. In the CFD matrices with a constant superficial velocity condition, decreasing the porosity (and with it K and σ) forces the fluid into smaller spaces, resulting in higher maximum flow speeds, which in turn result in higher τ . In the Brinkman equation, all of these obstacles to flow are averaged into K , and the superficial velocity boundary condition remains unaffected. Therefore, there are no high speed flow regions: the shear stress amplification arises from the shrinking of the viscous boundary layer around the cell, which is approximately $K^{1/2}$ in size. Our CFD analysis shows that the effect of the high-speed flows increases τ on the cell surface more quickly than the amplification factor in the Brinkman equation, $1/\sigma$, would predict (Eqs. (3), (5) and (6)). We attempt to

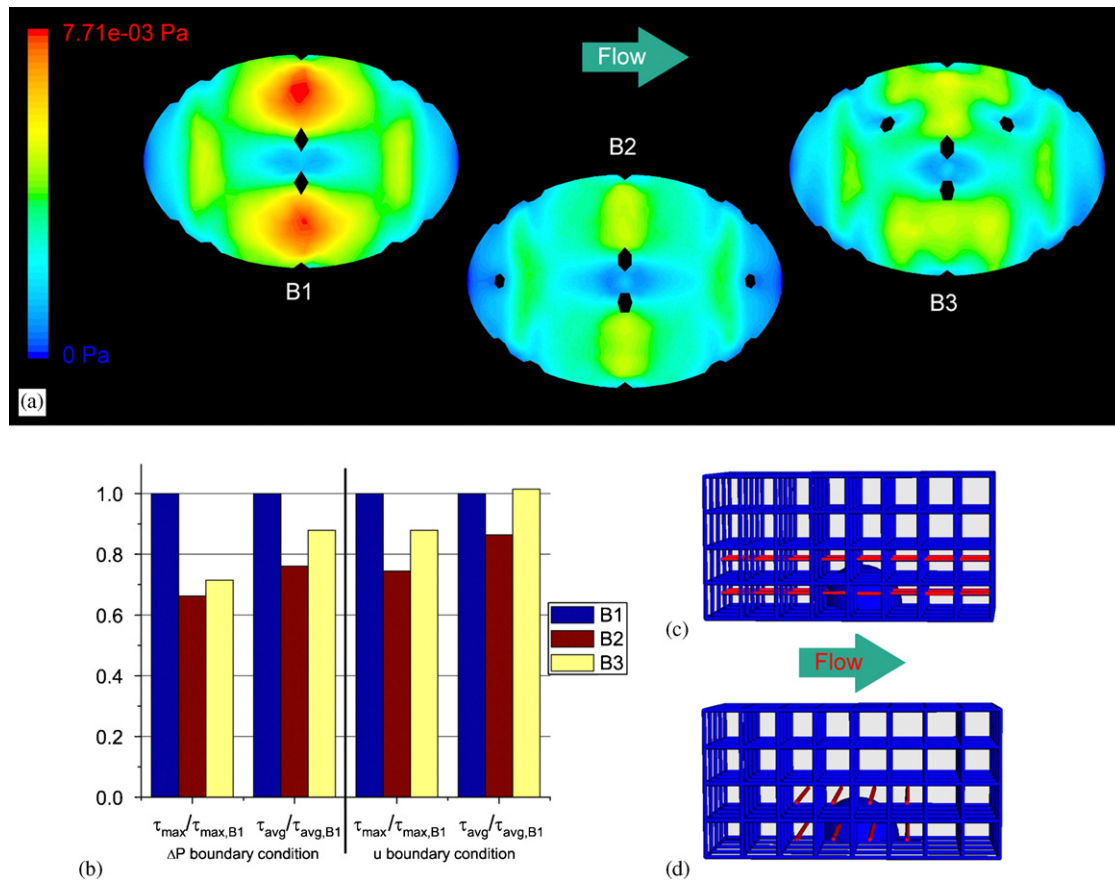


Fig. 6. Addition of even a few fibers near the cell dramatically reduces shear stress on that cell. (a) Contour plot of τ on a cell in an unmodified cubic lattice ("homogeneous matrix," case B1), and on cells modified with additional fibers parallel to (B2) or perpendicular to (B3) the direction of flow. (b) The reduction in $\tau_{\max}$ and τ_{avg} for models B2 and B3 compared to B1 is dramatic even with minimal shielding, as measured by pericellular porosity ε_{ca} divided by bulk porosity ε_{bm} for the ΔP boundary condition. Shielding remains somewhat effective for the constant U_0 condition for the case of extra parallel fibers (B2), but not for the case of extra fibers perpendicular to flow (B3). (c, d) The modified matrices are shown with extra fibers (red) either (c) parallel to or (d) perpendicular to flow.

correct for this effect by also using average interstitial velocity, $U_i (= U_0/\varepsilon)$, rather than U_0 in the Brinkman equation. This brings the CFD results and the Brinkman prediction closer together by a factor of $(1/\varepsilon - 1)$ (Fig. 7b), but as our matrices are highly porous this only explains a maximum of 30% of the difference between the two models. For ε very near 1, this correction is negligible, so our results continue to show a 3-fold difference between the Brinkman predicted τ and our CFD result. Our CFD models cover much of the expected range of σ for typical reconstituted in vitro collagen and fibrin matrices—approximately 0.2–0.6, as calculated from recent observations of reconstituted collagen matrix dimensions (see Pedersen and Swartz (2005) for a summary), and indicate that the Brinkman equation considerably underestimates cell membrane shear stress, particularly $\tau_{\max}$. Our results are somewhat sensitive to the choice of permeability relationships, as seen in Fig. 7(b), but the trend of $\tau_{\max}$ increasing more for decreasing σ than the Brinkman relationship prediction remains whether the Happel relationship or

the Higdon and Ford relationship is used, as does the result that $\tau_{\max}$ is underpredicted by the Brinkman equation. However, the results of our analysis cannot be extrapolated for all interstitial tissues, particularly those with $<90\%$ porosity or permeabilities lower than 10^{-10} cm^2 . For in vivo tissues with K very small (e.g., 10^{-14} cm^2), $K^{1/2}$ will be of the order of nanometers, making the continuum approximation much more appropriate. However, if the matrix solid volume fraction is too large, theoretical work indicates that the predictive accuracy of the Brinkman equation will decrease in this case as well (Durlafsky and Brady, 1987).

The tissue remodeling resulting from matrix contraction likely serves to shield cells from shear stress. The shear stress shielding resulting from the addition of only a few extra fibers near the cell surface demonstrates the importance of the pericellular matrix architecture to the fluid flow near the cell surface. This result cannot be completely explained by an increase in solid volume fraction ϕ_s near the cell, since the model with increased

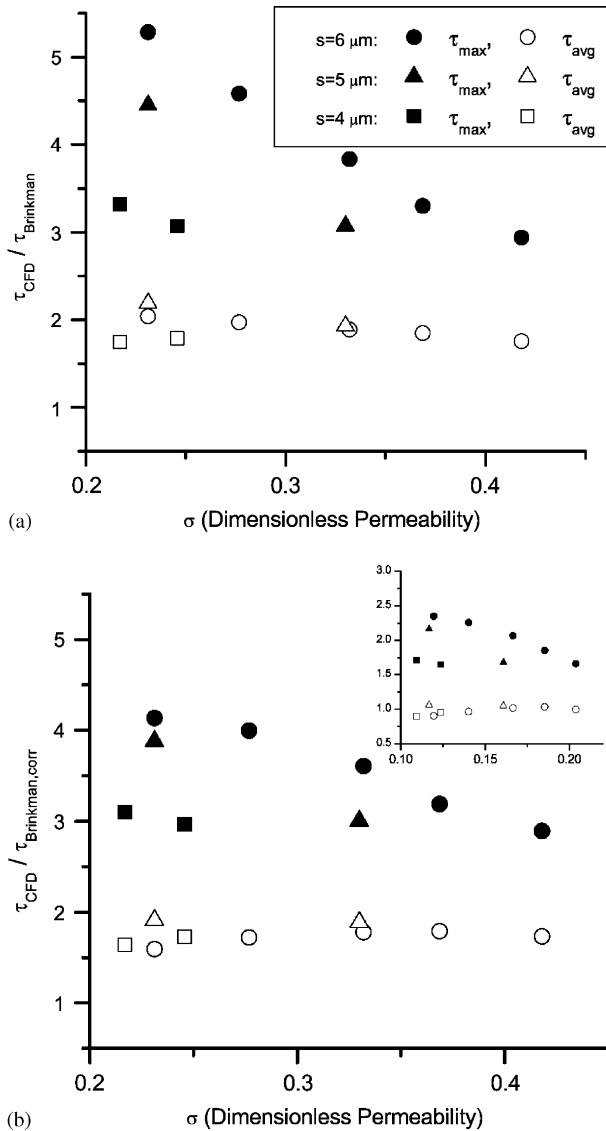


Fig. 7. Magnification of CFD shear stress over Brinkman shear stress is not an artifact of the boundary conditions. (a) As the matrix becomes more restricted (σ decreasing), our CFD shear stress results are increasingly amplified vs. the Brinkman prediction. (b) When the Brinkman prediction is recalculated using the average interstitial velocity ($U_i = U_0/\varepsilon$) rather than the superficial velocity, the effect seen in panel (a) remains, albeit slightly reduced for matrices with the lowest ε ($s = 6 \mu\text{m}$, σ small). Inset: Using the Higdon and Ford permeability relation changes the amount the CFD τ is amplified with respect to the Brinkman prediction, but our CFD results continue to show that τ_{max} increases faster than predicted by Brinkman.

ϕ_s but no additional fibers (B4) displayed no shielding qualities at all. We find that shielding will be more effective in systems driven by constant pressure gradients than in systems with constant flow (or systems in which the matrix itself does not dominate interstitial resistance). Our results also show that fibers oriented along the flow direction shield the cell more effectively from shear stress while decreasing the superficial velocity to a lesser extent, which demonstrates the

importance of fiber location and orientation near the cell.

The permeability, K , is a good predictor of the flow resistance of matrix on a large scale, but our results with matrices of identical K and different architectures demonstrate that when predicting shear stress on the level of a single cell, differences in matrix architecture do matter. Matrices in vitro clearly lack the structural order of our cubic lattice model, but recent numerical work found that the permeability of high-porosity matrices constructed from either ordered fiber lattices or random dispersions of spheroids were not substantially different (Higdon and Ford, 1996), suggesting that the cubic lattice is an acceptable model for sparse random matrices. Finally, our study shows that interstitial fluid flow may generate low levels of physiologically relevant shear stress on cells embedded in sparse fibrous matrices, but also shows that even a small amount of matrix remodeling around interstitial cells can shield the cells from this direct fluid force.

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Appendix A

A.1. Relationship between permeability and matrix structure

Multiple models exist to convert structural information about a porous material into an expected hydraulic permeability, including the Carman–Kozeny relation and recent computational work by Higdon and Ford (Carman, 1956; Higdon and Ford, 1996). Estimating K from geometric parameters is not necessarily straightforward, and no single theoretical approach is valid across all types of porous media (Scheidegger, 1974; Dullien, 1979). We use the following form of the Carman–Kozeny relation to estimate K for our matrices, given d and s :

$$K = \varepsilon m^2 / k. \quad (\text{A.1})$$

Here, m is the hydraulic radius, $m = (s^2 - d^2)/4d$. The Kozeny constant k depends on the type of matrix being modeled. We use Happel's relationship for k for arrays of cylinders perpendicular and parallel to flow (Happel,

1959; Happel and Brenner, 1965) as seen below:

$$k = (2/3)k_{\text{perp}} + (1/3)k_{\text{par}}, \quad (\text{A.2})$$

$$k_{\text{perp}} = \frac{2\varepsilon^3}{(1-\varepsilon)[\ln\{1/(1-\varepsilon)\} - \{1 - (1-\varepsilon)^2\}/\{1 + (1-\varepsilon)^2\}]}, \quad (\text{A.3})$$

$$k_{\text{par}} = \frac{2\varepsilon^3}{(1-\varepsilon)[2 \ln\{1/(1-\varepsilon)\} - 3 + 4(1-\varepsilon) - (1-\varepsilon)^2]}. \quad (\text{A.4})$$

Finally, for our cubic fiber lattice, ε is related to d and s by

$$\varepsilon = 1 - \left(\frac{3\pi d^2}{4s^2} - 2(2 - \sqrt{2}) \frac{d^3}{s^3} \right). \quad (\text{A.5})$$

For the Higdon and Ford calculations, we used MATLAB's cubic spline interpolation scheme to compute the dimensionless permeabilities of our computational models from the tabular data presented in their 1996 paper.

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