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Response of Collagen Matrices Under Pressure and Hydraulic Resistance in Hydrogels

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Abstract

Extracellular matrices in animal tissue are hydrogels mostly made of collagen. In these matrices, collagen fibers are hierarchically assembled and cross-linked to form a porous and elastic material, through which migrating cells can move by either pushing through open matrix pores, or by actively digesting collagen fibers. The influence of matrix mechanical properties on cell behavior is well studied. Less attention has been focused on hydraulic properties of extracellular matrices, and how hydrodynamic flows in these porous hydrogels are influenced by matrix composition and architecture. Here we study the response of collagen hydrogels using rapid changes in the hydraulic pressure within a microfluidic device, and analyze the data using a poroelastic theory. Major poroelastic parameters can be obtained in a single experiment. Results show that depending on the density, porosity, and the degree of geometric confinement, moving micron-sized objects such as cells can experience substantially increased hydraulic resistance (by as much as 10^6 times) when compared to 2D environments. Therefore, in addition to properties such as mechanical stiffness, the fluidic environment of the cell is also likely to impact cell behavior.

1 Introduction

Collagen is the primary extracellular matrix (ECM) protein and the most abundant protein in animal tissue. Collagen and collagen fibers in ECM play important roles in cell adhesion, migration, and differentiation^{1–10}. Mechanical properties of ECM are important in tissue homeostasis, development, and progression of disease. Therefore, tensile and compressive behaviors of collagen ECM have been extensively studied, typically using external mechanical loading. Mechanical parameters associated with stiffness, viscoelasticity, and plasticity, have been quantified^{11–18}. From these studies, it is now clear that the matrix

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Author Contributions

DM, SXS designed the experiments. DM, YL, SXS developed the theoretical model. DM performed the experiments and ran the numerical simulations. YL derived the analytical expressions. DM, YL, YC, SXS wrote the paper.

SI Movies

Movie showing deformation of a 100 μm channel made with 4°C collagen gelation is subjected to ~ 1.2 kPa of pressure change.

architecture is an important factor in determining ECM mechanics. For instance, it is recently reported that calcific aortic valve diseases are associated with layer-specific alterations in collagen architecture¹⁹. The collagen architecture is heterogeneous, and the matrix structure ranges from short-fibered collagen to increased fiber density and width. Different ECM architectures respond to external mechanical perturbations such as compression and shear differently^{20,21}, and some types of ECMs are more likely to expose the epithelium to damaging external mechanical forces²². Indeed, Glioblastoma patients with more organized collagen architecture, characterized by a better degree of fiber alignment, have higher median survival compared to patients with less organized architecture²³. Collagen architecture can be altered during the development of inflammatory disorders, aging, and fibrosis^{24,25}. Such alterations can lead to even more damages to the tissue by making the epithelium more vulnerable²².

Since ECMs are hydrogels, the hydrodynamic properties of the collagen ECM could potentially influence cell mechanics and migration²⁶. Recently, there is increasing recognition that cells may be sensitive to the hydraulic resistance that they experience^{27–29}. How the hydrodynamic variables of the matrix depend on collagen fiber architecture has not been explored. In this paper, we investigate the mechanical response of collagen matrices subjected to sudden changes in hydraulic pressure. We control the gelation temperature to vary collagen architecture³⁰. Furthermore, we modulate the stiffness of the collagen gel by chemical crosslinking³¹. To probe how different matrix architecture responds to mechanical loading, we develop a microfluidic device with a tubular matrix geometry, and apply external hydrostatic pressures (HPs) on the order of a few kPa and record the dynamic response of collagen hydrogels in the tubular configuration. We then use poroelastic theory to explain the observed response. Based on our estimates, we report biot-coefficient³² in the range of 0.94–0.98, a parameter which has been not studied previously while characterizing ECM mechanical properties^{33,34}.

The developed microfluidic device is geometrically similar to the tubular epithelium commonly encountered in mammalian tissues. Therefore, MDCK II (Madin-Darby Canine kidney) epithelial cells are seeded on the inner surface of the collagen channel wall to form a confluent epithelium. We subjected the tubular epithelium to hydrostatic pressure changes and found that the tubular epithelium is much less deformable than the collagen matrix alone. To see how matrix architecture can influence matrix hydraulic properties, we studied hydraulic resistance experienced by a moving object in a 3D matrix based on the experimental parameters obtained above. We found that collagen permeability plays a dominant role in dictating hydraulic resistance experienced by the cell. Geometric confinement of the matrix can also significantly increase the hydraulic resistance due to increased restriction of pressure diffusion and fluid flow. We also found that along with the fluidic viscosity, the matrix shear stress can significantly increase hydraulic resistance on a moving object within confined geometries. The scaling law for the hydraulic resistance remains the same for both a rigid and a viscoelastic object moving in the physiologically-relevant velocity regime. These results suggest that cells in 3D matrices *in vivo* can experience elevated hydraulic resistance, and fluid flow external to the cell is potentially another factor influencing mechanisms of cell motility.

2 Materials and Methods

2.1 Collagen Matrix Preparation

We used 7 mg/ml Rat tail type I collagen (Corning) for all experiments. We choose this concentration to make it as close as to physiologically-relevant values while avoiding collapse of the channel during fabrication. For the final concentration of 7 mg/ml, ~362 ml of the stock solution (9.66 mg/ml) was mixed with ~35 ml of 1N NaOH (Sigma, 795429) and ~103 ml of 10X DMEM (Sigma, D2429)³⁵. Then the collagen was allowed to gel for 1 hr at 37°C, or 24–36 hrs at 4°C.

For matrix cross-linking, we used 0.01 mg/ml (0.04 mM) of genipin (Sigma, G4796), and incubated with an already gelled collagen substrate (which was previously gelled at 37°C) overnight at 37°C. Reflectance confocal images of the matrices are shown in Fig. 1A–C.

2.2 Microfluidic Device Fabrication

A polydimethylsiloxane (PDMS) block with a 1.1 mm × 2 mm × 1 cm rectangular hollow space is created using an aluminum mold, the dimensions of which are much larger than the collagen channel radius. Four holes are punched into the PDMS block after being removed from the aluminum mold and is plasma bonded onto a glass slide (Fig. 1D). A nitinol rod of 100 μ m diameter is inserted into the rectangular channel. Poly-L-lysine coating (~1 hr at room temperature) is used to coat the inner surface of the PDMS channel to allow better adherence of collagen. Rat tail Type I collagen is used to fill the channel. Upon gelation, the interior holes are filled with Sylgard (Dow Corning) 164 (A:B = 10:1) and is allowed to cure for about 1 hr at room temperature. These act as support to the collagen block. The nitinol rod is then pulled out to create a channel of desired diameter. Sylgard 164 (A:B = 10:1) is also used to completely seal the ports located at the two ends of the collagen channel. When MDCK II cells are seeded in the channel, the nitinol rod diameter is increased to 150 μ m.

2.3 Hydraulic Pressure Changes

Two 2.5mm diameter plastic tubes were connected at the outer ports, and were used to perform hydrostatic pressure perturbation. The tubes and the collagen channel were filled with PBS and the pressure was applied by raising the tube ends to height h (Fig. 1E, supplementary movie).

2.4 Cell culture

We cultured cells in Dulbecco's modified Eagle's medium (DMEM) (Corning, 10013CV), 10% fetal bovine serum (FBS) (ATCC 30–2020) and 1% Pen/Strep (Sigma, P0781). The cells were a kind gift from Peter Searson Lab at the Johns Hopkins University. The cells were maintained in an incubator with 5% CO₂ at 37°C. Cells were then seeded into the tubular collagen channel and were allowed to line the inner surface of the channel. They were grown into a confluent epithelium after about a week of adherence.

2.5 Imaging and Image Analysis

We used Differential Interference Contrast (DIC) microscopy for acquiring images. Image acquisition was 14 frames per second. ImageJ and MATLAB were used to trace the

diameters of the channel as a function of time. For imaging the collagen architecture, we used Confocal Reflectance Microscopy (CRM). CRM is a typical optical microscope modality used to visualize biopolymers such as collagen fibers.

3 Mathematical Model of Matrix Response to Perturbations

We use poroelastic theory to model the temporal response of the matrix during pressure perturbation^{36,37}. The governing equation for the solid phase, i.e., the collagen fibers, is

$$G \nabla^2 \mathbf{u} + \frac{G}{1-2\nu} \nabla (\nabla \cdot \mathbf{u}) = \alpha \nabla p, \quad (1)$$

where $\mathbf{u}$, G , ν , and α are the displacement, shear modulus, Poisson's ratio, and the Biot-coefficient, respectively, of the solid phase. The symbol p is the hydrostatic pressure of the fluid phase in the pores. The coupled consolidation equation of the fluid phase is^{36,37}

$$\frac{\partial p}{\partial t} - c \nabla^2 p = - \frac{\gamma}{(1+\nu)} \frac{\partial}{\partial t} (tr \boldsymbol{\sigma} + 4\eta p), \quad (2)$$

where

$$c = \frac{2\kappa\gamma G(1-\nu)}{\mu\alpha^2(1-2\nu)} \quad (3)$$

is the coefficient of consolidation, in which κ is the permeability and μ is the dynamic viscosity of the fluid in the pores; and

$$\gamma = \frac{(\nu_u - \nu)}{(1 - \nu_u)(1 - 2\nu)}, \quad \eta = \frac{\alpha(1 - 2\nu)}{2(1 - \nu)}, \quad (4)$$

where ν_u is the undrained Poisson's ratio and $\boldsymbol{\sigma}$ is the stress in the solid phase. Under plane strain, Eq. 2 becomes

$$\frac{\partial p}{\partial t} - c \nabla^2 p = - \gamma(1 - \nu) \frac{\partial}{\partial t} (tr \boldsymbol{\sigma} + 2\eta p). \quad (5)$$

The constitutive relation is

$$\sigma_{ii} = 2G\varepsilon_{ii} + \frac{2G\nu}{1-2\nu} tr \boldsymbol{\varepsilon} - \alpha p, \quad (\text{no summation}) \quad (6)$$

where $\mathbf{e} = \nabla \mathbf{u}$. The right hand sides of Eqs. 1 and 2 (or 5) show the coupling between the solid and the fluid phases.

Eqs. 1 and 2 (or 5) are solved simultaneously by relevant boundary conditions of each problem. Since the Darcy's Law is implied in Eq. 2 (or 5), i.e.,

$$0 = -\nabla p - \frac{\mu}{\kappa} \mathbf{v}, \quad (7)$$

the fluid velocity can then be post-calculated once p is solved. The full coupled system given by Eqs. 1 and 2 is solved by a finite element solver, COMSOL 5.1©.

3.1 Temporal Response of the Matrix

The collagen channel in Fig. 2A can be treated as a hollow cylinder of inner radius r_i and outer radius r_o (Fig. 1F). The principal stresses are σ_{rr} (radial) and $\sigma_{\theta\theta}$ (tangential). In cylindrical coordinates, the associated constitutive equations are

$$\sigma_{rr} = 2G\varepsilon_{rr} + \frac{2G\nu}{1-2\nu}(\varepsilon_{rr} + \varepsilon_{\theta\theta}) - \alpha p, \quad (8)$$

$$\sigma_{\theta\theta} = 2G\varepsilon_{\theta\theta} + \frac{2G\nu}{1-2\nu}(\varepsilon_{rr} + \varepsilon_{\theta\theta}) - \alpha p, \quad (9)$$

where the strain components of the solid phase are $\varepsilon_{rr} = u_r/r$ and $\varepsilon_{\theta\theta} = u_r/r$. Under plane strain (Eq. 5), the fluid component is

$$\frac{\partial p}{\partial t} - c \left(\frac{1}{r} \frac{\partial p}{\partial r} + \frac{\partial^2 p}{\partial r^2} \right) = -\gamma(1-\nu) \frac{\partial}{\partial t} (\sigma_{rr} + \sigma_{\theta\theta} + 2\eta p). \quad (10)$$

At the inner radius $r = r_i$, the boundary conditions are $\sigma_{rr}|_{r=r_i} = -p_0$ and $p|_{r=r_i} = p_0$, while at the outer radius $r = r_o$, the boundary conditions are $\partial p / \partial r|_{r=r_o} = 0$ and $u_r|_{r=r_o} = 0$. Here, p_0 is the applied hydrostatic pressure in the inner tube.

The parameters ($G, \alpha, \eta, c, \kappa, \nu, \nu_u$) are fitted to the channel diameter response observed in the experiments. μ is taken to be the value of water. Since the process of raising the hydraulic pressure in the tube is not infinitely fast, we assume a time-dependent increment in the pressure, $p_0(t) = p_0 \left(1 - e^{-t/t_0} \right) / \left(1 + e^{-t/t_0} \right)$, where the constant $t_0 = 25$ s was chosen empirically to smoothly introduce a step-wise jump in pressure. The computed peak and plateau deformations are not sensitive to the precise form of this pressure change.

3.2 Hydraulic Resistance Experienced by a Rigid Object Moving in Matrix

Cells moving in 3D matrices generally deform the solid phase of the matrix by exerting contractile forces³⁸. Cells also remodel the matrix by actively digest existing collagen fibers while adding new fibers. To understand the hydraulic effect from the matrix to a moving cell, we first examine the hydraulic resistance experienced by a rigid sphere of constant velocity, v_0 , in the matrix. To simplify the computation, we use cylindrical coordinate with axial symmetry, where the sphere moves along the axial, z , direction. The pore-pressure is governed by Eq. 2, where $\text{tr}\boldsymbol{\sigma} = \sigma_{rr} + \sigma_{\theta\theta} + \sigma_{zz}$.

To solve the solid phase, we take time derivative of Eq. 11, i.e.,

$$G \nabla^2 \mathbf{v} + \frac{G}{1-2\nu} \nabla (\nabla \cdot \mathbf{v}) = \alpha \frac{\partial}{\partial t} \nabla p, \quad (11)$$

where $\mathbf{v} = \mathbf{u}/t$.

The computational domain is bounded by $r = R$ and $z = \pm \ell$. The boundary conditions are set as follows. At the surface of the sphere, non-slip condition for the solid phase is used, i.e., $v_z = v_0$ and $v_r = 0$. For the fluid phase, a non-penetration condition is used, i.e., $\nabla p \cdot \mathbf{n} = 0$, where $\mathbf{n}$ is the unit norm of a surface. We also require that the fluid at the surface of the sphere has the same axial velocity as the moving sphere, i.e., $p/r = -\mu v_0/\kappa$ by Darcy's law. So the non-penetration condition is rewritten as $-z\mu v_0/\kappa + r p/r = 0$. At $r = R$, $\mathbf{v} = 0$ and $p/r = 0$. At $z = \pm \ell$, $p = 0$ and $\mathbf{v} = 0$.

In the simulation, $\ell = 25$ mm is fixed but R varies so that we can examine the effect of geometric confinement on the hydraulic resistance on the moving sphere. Unless otherwise specified or varied in each simulation, the default parameters used in this model are: $\kappa = 10^{-11}$ m², $\mu = 0.001$ Pa·s, $\nu = 0.2$, $\nu_u = 0.39$, $G = 6$ kPa, $\alpha = 0.98$, $\tau_0 = 30$ μ m, $v_0 = 1$ nm/s, and $2R = 5$ mm.

3.3 Hydraulic Resistance Experienced by a Viscoelastic Object Moving in Matrix

Here we extend our study to a viscoelastic object moving in 3D matrices and examine the scaling law of d_g in this case. The geometry setup and the boundary conditions at $r = R$ and $z = \pm \ell$ remains the same as the model described in Sec. 3.2. The interface between the collagen matrix and the object has non-slip condition modeled by the Fluid-Structure-Interaction module in COMSOL 5.1©. Fluid movement in the collagen matrix is governed by the Darcy's Law with permeability κ and dynamic viscosity μ ; the viscoelastic object is described by the Kelvin-Voigt model with elasticity constant E , retardation time τ , and Poisson's ratio ν_s . Unless otherwise specified or varied in each simulation, the default parameters used in this model are: $\kappa = 10^{-11}$ m², $\mu = 0.001$ Pa·s, $G = 1$ kPa, $\tau = 0.1$ sec, $\nu_s = 0.49$, $v_0 = 1$ nm/s, and $2R = 5$ mm.

4 Results and Discussion

4.1 Response of the Matrix to Hydraulic Pressure

To investigate how matrix architecture influence the matrix response to pressure changes, we vary the gelation temperatures and the presence of fiber cross-linking. It is known that increased gelation temperature and cross-linking leads to an increase in the matrix shear modulus^{5,35}. Cross-linking does not cause any significant change in matrix pore size, or hydraulic permeability, as we qualitatively observe in Fig. 1A–C.

To mimic physiological conditions, we adopted a tubular geometry, which is the typical structure of ductal epithelium in organs such as kidney, liver, or lung *in vivo*. We used a nitinol rod to create tubes of 100 μm in diameter as shown in Fig. 1D. This system allows us to employ sudden HP perturbation by raising the height h of the fluid in the connected channel (PBS/media), where the pressure is given by $p_0 = \rho gh$, where g is the gravitational acceleration constant (Fig. 1E). We changed HP incrementally in fixed step size of 1.2 kPa, until reaching 3.6 kPa. A representative image of a typical collagen channel formed in our system is shown in Fig. 2A. We then measure the temporal mechanical response of the channel in terms of diameter changes by tracing the boundaries of the channel (Fig. 2A). The response typically shows a sudden peak in the collagen diameter upon the application of the HP, and a slower gradual relaxation to a final diameter that is different from the initial channel diameter before the application of HP change (Fig. 2B). When the HP is returned to zero, the channel diameter also recovers back to the original value, indicating that the channel is elastic^{33,34}.

To understand the observed response of collagen matrices to HP changes, we mathematically model the system using a linear poroelasticity theory, where the observed deformation peak is a function of shear modulus of the collagen, and the plateau is determined by both the permeability and shear modulus. Higher peak deformations correspond to lower matrix stiffness; lower plateau deformations corresponds to higher hydraulic permeability (Fig. 3A–C). This type of response is explained by poroelasticity which couples fluid and solid phases of the matrix. Upon sudden hydrostatic loading, there is an initial elastic deformation of the channel which corresponds to the observed peak deformation. However, if the hydrostatic load is maintained, there is fluid influx which causes a dilation of the channel, which is the observed plateau (Fig. 2B). We can infer matrix poroelastic properties based on the magnitude of the peak, the magnitude of the plateau, and difference between the peak and the plateau.

The response of the matrix composed of 7 mg/ml of collagen gelled at 37°C is shown in Fig. 3B. The shear modulus is found to be ~5 kPa. From Fig. 1A–C, we observe that the fibers at 37°C gelation are shorter and denser compared to the 4°C gelation. The permeability is found to be $1 \times 10^{-11} \text{ m}^2$. For 4°C gelation, as observed in¹², we observed that stiffness, as indicated by the shear modulus G , was significantly reduced to ~3.5 kPa, while permeability increased by 2.5 times. The effect of crosslinking has been previously studied with various cross-linking agents such as glutaraldehyde³⁹ and genipin³¹. Cross-linking also increased the stiffness of the matrix by 1kPa, however, the permeability was not affected (Fig. 3B–C).

From the poroelastic model, the magnitude of the plateau is determined by shear modulus, G , permeability, κ , and biot-coefficient, α . The drop from peak to plateau indicates that the collagen is less deformed at steady-state due to water permeation into the collagen. Our theoretical work concludes that if $\alpha \rightarrow 1$, the difference between peak and plateau decreases (Fig. 3AB).

4.2 Epithelial Layer Reduces Fluid Flow into the Collagen Matrix

We investigated the temporal response of the collagen matrix with epithelium lining the collagen channel. MDCK II cells were seeded on top of the fibronectin coated collagen channel walls and were allowed to grow to confluency (Fig. 4A,B). Once the epithelium was formed, we applied HP changes. We found that the channel is stiffer with an equivalent stiffness of ~ 9 kPa. Furthermore, the difference between peak and plateau was smaller than channels consisting of only collagen (Fig. 4C), which indicates that the effectively stiffer channel prevents large deformation. Since MDCK cells secrete fibronectin⁴⁰ and remodel the local interfacial matrix, the effect of the enhanced stiffness can be a combined effect of the MDCK epithelial sheet, the exogenous fibronectin, and the fibronectin secreted by MDCK cells and other ECM components. Moreover, we also observe that the permeability by fitting was reduced by an order of magnitude, suggesting that the epithelium is preventing pressure diffusion and fluid flow into the collagen matrix.

4.3 Hydraulic Resistance in the Collagen Matrix

The velocity and morphology of cells in a collagen matrix depend on the mechanical properties of the matrix. While the effects of matrix stiffness, density, alignment, and porosity on cell migration have been studied^{5,6,41–46}, the role of the hydraulic property of the matrix is less understood. Recent work suggested that the hydraulic resistance from the environment acting on cells can influence the mechanism of cell migration and cell speed^{27–29,47}. The hydraulic resistance that the cell experience may be important in morphogenesis and cancer cell metastasis. A direct measurement of the magnitude of the hydraulic resistance in a matrix at cell scales is difficult. Therefore, based on estimated parameters obtained from experiments described above, we utilize numerical simulation to provide insights.

Schematics of a spherical object moving in 3D collagen matrix is shown in Fig. 5A. We examine the hydraulic resistance on objects in both infinite domains and confined channels. In all simulations the total length of the channel is $2\ell = 50$ mm, which is large enough that all relevant fields decay to zero at $z = \pm\ell$. Steady-state is reached in a few hundred seconds; we obtain all results at $t = 1,000$ sec, where the sphere has moved $1 \mu\text{m}$ as we prescribe a constant sphere velocity $v_0 = 1$ nm/s. This velocity is chosen to be the same as typical cell migration velocities observed in matrices.

Fig. 5B shows the computed velocity field of the solid phase (left panel) and the fluid pore pressure field (right panel) surrounding a moving sphere of radius $r_0 = 30 \mu\text{m}$ in a channel of diameter $2R = 5,000 \mu\text{m}$. The solid phase has velocity v_0 on the surface of the sphere and the velocity decays in far field. The magnitude of the velocity is dominated by the velocity in the axial direction, which is about one order of magnitude higher than that in the radial

direction. The fluid pressure, while decays to zero far away from the object, has non-zero values around the sphere. When the channel diameter is reduced to 80 μm , the velocity of the solid phase decays quickly to zero at $r = R$ (Fig. 5C, left panel). There is a column of fluid with non-zero pressure in front of the sphere (Fig. 5C, right panel). The size of the nonzero pressure region is larger for narrower channels because the fluid cannot easily pass around the sphere. The geometric confinement thus affects the hydraulic resistance experienced by the object moving in the matrix.

The coefficient of hydraulic resistance for a moving object, denoted as d_g , is defined as²⁸

$$d_g = \frac{p_*}{v_0}, \quad (12)$$

where p_* is the average fluid pressure acting on the front surface of the object. Simulation results show that d_g varies inversely with the permeability of the matrix, κ , (Fig. 5D). Moreover, we find that d_g is strongly affected by the confinement of the channel: it increases by about two orders of magnitude when the diameter of the channel decreases from 5,000 μm to 80 μm . A 5,000 μm channel is equivalent to the infinite domain limit as further increases in the channel width gives identical d_g . These results indicate that a moving object experiences higher hydraulic resistance in confinement, even when the object does not completely occlude the confinement. This is also consistent with the idea that cell migration in 2D surfaces experiences less hydraulic resistance but when cells are confined in narrow channels, hydrodynamic effects from the environment is no longer negligible²⁸. This is significant because *in vivo*, cells migrating in 3D matrices are often also surrounded by other tissue such as the basement membrane, vessels, bones, and tendons. These objects provide confinement in the sense that they enforce vanishing fluid velocity on the object surface. This geometric confinement changes the pressure field in the matrix and increases the apparent hydraulic resistance by orders of magnitude.

The coefficient of hydraulic resistance, d_g , also increases linearly with the radius of the sphere and the viscosity of the fluid in the media (Fig. 5E). The influence of the shear modulus of the matrix, G , however, depends on the confinement (Fig. 5F). In infinite domains d_g does not vary with G while in confined domains d_g increases with increasing G and saturates at about $G = 1$ kPa. The narrower the channel, the larger the influence of G has on d_g . This result suggests that the stiffness of the collagen matrix affects cell migration not only through adhesion formation but also potentially through hydraulic resistance. This influence is present in confined channels and also in tissue spaces as large as 500 μm in the cross-section. We shall mention that in the simulation we have allowed the collagen matrix to deform up to 1 μm when the object moves. The magnitude of the deformation may vary when the matrix is rapidly dissolved by cells during cell migration, which we will discuss in the next section. When, however, the matrix does deform by a small amount, our simulation implicates non-trivial effect of the matrix stiffness on the hydraulic properties. Future experimental work will uncover the dual role of matrix stiffness on cell migration in 3D collagen matrix.

4.4 Scaling Laws of d_g in Collagen Matrix

Numerical results show that d_g depends on the fluid properties within the matrix as well as the geometry of the moving object and the domain. To understand this further, we derive an analytical approximation of d_g to obtain a scaling law of the dependence of d_g on the matrix properties.

During *in vivo* cell migration, cells secrete matrix metalloproteinases (MMPs)⁴⁸ which dissolve the collagen structure of the matrix. For this reason, the matrix does not deform continuously as cells migrate further. In the limit that MMPs fully dissolve the matrix, no strain or stress is developed within the matrix solid phase. In this case, we can simplify the full system by neglecting Eq. 1 and using an uncoupled version of Eq. 2, i.e.,

$$\frac{\partial p}{\partial t} - c \nabla^2 p = 0. \quad (13)$$

We first derive the scaling law of d_g for a spherical object moving in an infinite domain compared to the size of the object. The front of the spherical object moves at velocity v_0 , which acts as a continuous pressure source in space and time that pushes the fluid in the collagen matrix. To simplify the matter, we may focus on each instantaneous moment where the pressure source is spatially fixed. In this case, the pressure source at the front of the object can be considered as a Dirac delta function in time with an amplitude p_* that serves as a boundary condition for the pore pressure in the matrix. In this case, the pressure field radiating from the object is spherically symmetric. The consolidation equation is best solved in the frequency domain with a time harmonic form, $p = \tilde{p} \exp(i\omega t)$, where the tilde denotes a quantity in the frequency domain. Eq. 13 then becomes

$$\frac{\partial^2}{\partial r^2}(r\tilde{p}) - \frac{i\omega}{c}(r\tilde{p}) = 0. \quad (14)$$

An infinite domain implies that there is no wave reflection. Therefore, the solution to Eq. 14 is

$$\tilde{p} = \tilde{p}_* \frac{r_0}{r} e^{\sqrt{\frac{i\omega}{c}}(r_0 - r)} = \tilde{p}_* \frac{r_0}{r} e^{\sqrt{\frac{\omega}{2c}}(1+i)(r_0 - r)}, \quad (15)$$

where $\tilde{p}_*$ is the pore pressure on the sphere associated with frequency ω . The pressures $\tilde{p}_*$ and p_* have the same value in number since the pressure source is a delta function in time. From the decay rate in Eq. 15, the length scale of the pressure wave propagation is $L_p = \sqrt{2c/\omega}$, which decreases with increasing frequency.

In the spherical coordinate the fluid velocity in the radial direction, v_r , can be solved from the Darcy's Law (Eq. 7) as $v_r = -(\kappa/\mu) \nabla p / r$. The boundary condition of v_r is

$$\nu_r|_{r=r_0} = -\frac{\kappa}{\mu} \frac{\partial p}{\partial r} \bigg|_{r=r_0} = \nu_0, \quad (16)$$

which can also be written in the frequency domain. The combination of Eqs. 15 and 16 gives the coefficient of hydraulic resistance in the frequency domain

$$\tilde{d}_g = \frac{\mu}{\kappa} \left[\frac{1}{r_0} + \sqrt{\frac{\omega}{2c}} (1+i) \right]^{-1}. \quad (17)$$

The dominant ones are those of low frequencies. Indeed, as seen in Eq. 15, components of high frequencies are unable to propagate through long distance. On the first order of approximation, the coefficient of hydraulic resistance in collagen matrix is

$$d_g \approx \frac{\mu r_0}{\kappa}, \quad (18)$$

which provides a clear scaling law for d_g in infinite domains: d_g varies linearly with the viscosity of the fluid in the pores, μ , and the radius of the spherical object, r_0 , and varies inversely with the permeability of the collagen matrix, κ . Using Eq. 18 is equivalent to assuming that the fluid in the pore is incompressible such that the consolidation equation (Eq. 13) is reduced to $\nabla^2 p = 0$. This analytical approximation (Eq. 18) matches well with the numerical simulation in approximately infinite domains (Fig. 5D, E), although some negligible over-estimation is found, which can also be seen from Eq. 17. The agreement of the two solutions also indicates that in infinite domains the mechanics of the solid phase does not play a significant role in setting up d_g , as shown in Fig. 5F.

We can compare to the case that the moving object is maximally confined where $R = r_0$. Here in order for the object to move, it must push the complete column of fluid in front of it. Using Darcy's law, the pressure gradient can be solved by $v_0 = -(\kappa/\mu) \nabla p$. Hence, for a channel of length ℓ at the front of the object, the coefficient of hydraulic resistance is

$$d_g = \frac{\mu \ell}{\kappa}, \quad (19)$$

which is about an order of magnitude higher than that from the $80 \mu\text{m}$ confinement within the poroelastic medium. This solution represents the maximal limit for d_g in confinement.

It is also useful to compare hydraulic resistance of a moving object in pure fluid, the so called Stoke's flow limit (Fig. 5G). A moving sphere with radius r_0 and velocity v_0 in an infinite fluid medium has a total drag force $6\pi\mu r_0 v_0$, in which one-third comes from the pressure drag⁴⁹. By considering the average pressure acting on the front surface of the sphere, the corresponding coefficient of hydraulic resistance is

$$d_g = \frac{\mu}{r_0}, \quad (20)$$

which is inversely proportional to r_0 , as opposed to the linear dependence on r_0 in porous media (Eq. 18). The r_0^{-1} dependence in Stokes flow is confirmed in simulations for a domain with $2R = 5,000 \mu\text{m}$ (Fig. 5H). Unlike the porous medium, the dependence of d_g on r_0 in Stokes flow varies with the confinement. When channels become narrower, d_g starts to increase with increasing r_0 . In the limit that the radius of the sphere is close to the radius of the channel, i.e., $r_0 \approx R$, the coefficient of hydraulic resistance of the maximum confinement case can be solved:

$$d_g = \frac{8\mu\ell}{R^2}. \quad (21)$$

Indeed, when the r_0 approaches R , the d_g from simulations matches Eq. 21 (Fig. 5H, I). Finally, in Stokes flow d_g also increases linearly with the viscosity of the fluid (Fig. 5I) regardless of the confinement (Eq. 20 and 21).

Overall, d_g generally increases when the sphere migrates in a more confined geometry (Fig. 5H, I). This is because a sphere in an infinite domain only generates fluid flow around it, but when a sphere is placed in a channel of the same radius as the sphere, it displaces the entire column of fluid in the channel when moving. The presence of the matrix also can increase the hydraulic resistance by several orders of magnitude. Therefore, the hydraulic resistance is a function of matrix properties as well as the microenvironment geometry.

The above simulations show the scaling law of d_g for a rigid sphere moving either in a poroelastic medium or in Stokes flow. We next examine the scaling law of d_g for a viscoelastic sphere moving in a medium governed by Darcy's Law. For an object moving $v_0 = 1 \text{ nm/s}$, d_g does not depend on the elasticity of the object (Fig. 5J). d_g still scales inversely with the medium permeability (Fig. 5J). It also scales linearly with the radius of the object and the viscosity of the medium (Fig. 5K). However, d_g does not depend on the retardation time nor the Poisson's ratio of the sphere object (Fig. 5L). Collectively, our results suggest a general scaling law of d_g on κ , ν , and r_0 for an object moving at physiologically-relevant velocity in a porous medium.

5 Conclusions

Porous hydrogels such as collagen matrices are important microenvironments where cells can proliferate and migrate. The physical properties of these hydrogels can influence fundamental cell functions. Here we developed a microfluidic method of measuring response of the matrix against changes in hydraulic pressure. Our device is specially suited for studying the tumor microenvironment⁵⁰ which is stiffer than normal tissue matrices and promotes cell migration⁵¹. Using a poroelastic model, our approach is able to obtain

poroelastic parameters such as the permeability, the Biot coefficient, and the shear modulus in a single experiment. This is in contrast with methods that can measure the permeability alone^{33,34}. The method is able to discern differences between matrices of different fibrous architecture. Within the same microfabricated matrix, we also examined fluidic permeability of a confluent epithelial tubule and found that the epithelial cells can resist pressure changes.

In addition to matrix mechanical properties, the matrix hydraulic resistance may also influence cell migration. Here we explore using numerical simulations how the hydraulic resistance in the matrix depends on matrix permeability as well as matrix stiffness. The results show, unsurprisingly, that a moving object experiences higher hydraulic resistance in confined matrices. What is perhaps surprising is the degree of increase: d_g experienced by the cell can be 10^3 times higher in a confined matrix when compared to the d_g in an infinite matrix. This level of increase holds for all values of permeability (Fig. 5D). The range of permeability we have studied, from 10^{-13} m² to 10^{-10} m², are the numbers that are typically reported in literature for collagen of concentrations varying from ~2 mg/ml to ~10 mg/ml^{16,33,34,52,53}. The increase in d_g is even larger (up to 10^7 time higher depending on the matrix permeability) between a cell in a confined matrix and cells in 2D fluidic culture environment (Fig. 5D, H). In addition, increasing matrix shear modulus, which describes a stiffer matrix, can also increase the hydraulic resistance by several fold for small geometric confinement. These findings suggest that matrix mechanical properties may have multiple effects on cell behavior in realistic biological settings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

(A-C) Collagen architecture for different gelation temperatures and levels of cross-linking, imaged using the reflectance mode. (A) 7 mg/ml gelled at 4°C. (B) 7 mg/ml gelled at 37°C. (C) 7 mg/ml gelled at 37°C cross-linked with genipin. The collagen network gelled at 4°C has thicker and longer fibers compared to the collagen gelled at 37°C. The genipin cross-linking does not introduce significant differences in the fiber appearance. Scale Bar = 50 μm . (D) Diagram describing the fabrication of the microfluidic device. After collagen is gelled in the microfluidic channel, a thin rod of diameter 100 μm or 150 μm is pulled from the device, leaving a channel of varying diameter. (E) Experimental setup for changing the hydraulic pressure. Tubes connected to the microchannel are raised and lowered to change the hydrostatic pressure in the channel. (F) Schematics of the channel (created by pulling the Nitinol Rod) filled with PBS with or without MDCK II cells lining in the inner face of the channel. The channel has an inner radius $r_i = 50 \mu\text{m}$ and the boundary of the collagen is modeled by an outer radius $r_o = 1050 \mu\text{m}$. Drawn not to scale.

Fig. 2.

Response of the collagen matrix to hydraulic pressure changes. (A) A DIC image of the collagen channel 100 μm in diameter. See Fig. 1F for a schematic where $2r_i = 100 \mu\text{m}$. The diameter of the channel is monitored in time at 10 Hz resolution. (B) Data from pressure change experiment. The channel pressure is increased by five inches of PBS ($\sim 1.245 \text{ kPa}$) at $t = 1 \text{ sec}$, before which the normalized diameter is always 1. The peak (short time response) and plateau (long term response) are obtained from measuring the channel diameter as a function of time. The channel diameter recovers back to the original value after pressure release.

Fig. 3.

Spatio-temporal response of collagen matrices of different architecture. (A) Peak and plateau deformations for 100 μm diameter channel with 7 mg/ml matrix gelled at 4°C. Best fitting parameters obtained are: $\alpha = 0.97$, $G = 3.5$ kPa, $\nu = 0.2$, $\nu_u = 0.285$, $\kappa = 2.5 \times 10^{-11}$ m², $\mu \approx 10^{-3}$ Pa.s. (B) Peak and plateau deformations for 100 μm channel with 7 mg/ml matrix gelled at 37°C. Best fitting parameters obtained are: $\alpha = 0.98$, $G = 5$ kPa, $\nu = 0.2$, $\nu_u = 0.38$, $\kappa = 10^{-11}$ m². (C) Peak and plateau deformations for 100 μm channel with 7 mg/ml matrix gelled with 1% (w/v) genipin at 37°C. Best fitting parameters obtained are: $\alpha = 0.98$, $G = 6$ kPa, $\nu = 0.2$, $\nu_u = 0.39$, $\kappa = 10^{-11}$ m². The difference between peak and plateau values is significantly larger for matrices gelled at 4°C compared to 37°C and genipin cross-linked collagen. (D) Predicted variations of channel plateau deformation as a function of matrix permeability, κ , and the Biot-coefficient, α . The rest of the parameters are the same as those in panel (A). The pressure change used was five inches of PBD (~ 1.245 kPa). We observe that beyond certain limit of permeability, the plateau is essentially independent of permeability. All error bars are standard errors.

Fig. 4.

Response of a model tubular epithelium in collagen gelled at 37°C. (A) DIC image of a tubular epithelium showing a confluent layer of MDCK II cells in the collagen microchannel. (B) Confocal immunofluorescence images of the confluent MDCK II cells showing F-actin (Phalloidan Red) and nucleus (DAPI, Blue). (C) Peak and plateau deformations of the tubular epithelium of MDCK II cells during hydraulic pressure change. Best fit parameters are: $\alpha = 0.94$, $G = 9$ kPa, $\nu = 0.2$, $\nu_u = 0.4$, $\kappa = 5 \times 10^{-12}$ m². The channel deformation in the presence of epithelium is significantly smaller than that of the matrix without cells. This suggests that the epithelium resists pressure diffusion and fluid flow into the matrix. The error bars are standard errors.

Fig. 5.

Numerical simulations to estimate coefficient of hydraulic resistance, d_g . (A) Schematics of a spherical object moving upwards in axial, z , direction in a 3D poroelastic medium with different levels of confinement. (B, C) Left panels: the velocity of the solid phase from finite element (FE) simulations. Right panels: the fluid pressure field in the matrix pores from simulations. In (B) the radius of the channel is $2,500 \mu\text{m}$ and in (C) the radius is $40 \mu\text{m}$. Both pressure and velocities well decay to zero at the inlets and outlets of the channels. For better visualization, only part of the computational domain is shown (in C, the entire domain is shown). (D) Predicted coefficient of hydraulic resistance, d_g , as a function of κ for different channel confinement. Solid lines: predictions from FE simulations. Dashed line: analytical estimate of an infinite domain given by $d_g = \mu r_0 / \kappa$ (Eq. 18). Dash-and-dotted line: analytical

estimate of maximum confinement $d_g = \mu\ell/\kappa$. (E) Predicted coefficient of hydraulic resistance in the poroelastic medium, d_g , as a function of the radius of the spherical, r_0 , for different fluid viscosities in the matrix, μ . Solid lines: predictions from FE simulation. Dashed lines: analytical estimate $d_g = \mu r_0/\kappa$ (Eq. 18). (F) Predicted coefficient of hydraulic resistance, d_g , as a function of the shear modulus of the solid, G , for different confinement dimensions. $\kappa = 10^{-13} \text{ m}^2$. (G) Schematics of a spherical object moving in a single phase (Stoke's flow) with different levels of confinement. (H) Predicted coefficient of hydraulic resistance, d_g , as a function of the radius of the spherical, r_0 , for different channel confinement in Stoke's flow. Solid lines: predictions from FE simulations for Stoke's flow. Note d_g is significantly lower than in (E) for the same size object. (I) Predicted coefficient of hydraulic resistance, d_g , as a function of the fluid viscosity, μ , for different channel confinement. The solid lines are predictions from FE simulation. and the dashed lines with symbols are from analytical estimates. In (H) and (I), dashed lines with stars are analytical estimates for an infinite domain where $d_g = \mu/r_0$. Circles with or without dashed lines are analytical estimates $d_g = 8\mu\ell/R^2$, calculated from the position of the sphere to the end of the channel. (J–L) Prediction of d_g with a viscoelastic sphere from FE simulations. (J) Predicted d_g as a function of sphere elasticity, E , for different matrix permeability, κ . (K) Predicted d_g as a function of sphere radius, r_0 , for different matrix viscosity, μ . (L) Predicted d_g as a function of sphere retardation time, τ , for different sphere Poisson's ratio, ν_s .