Effect of Fluid Flow on Smooth Muscle Cells in a 3-Dimensional Collagen Gel Model

Su Wang, John M. Tarbell

Abstract—A 3D collagen gel model was developed to simulate interstitial fluid flow and to assess the importance of this flow on the biochemical production rates of vascular smooth muscle cells (SMCs). Rat aortic SMCs were suspended in type I collagen, and the gel was supported by nylon fibers that allowed a 9-cm length of the SMC-gel model to withstand 90 cm H₂O differential pressure over a 6-hour period without significant compaction. Up to 1 dyne/cm² shear stress on the suspended SMCs could be induced by the pressure-driven interstitial flow. The suspended SMCs were globular, had a diameter of ≈10 µm, and were distributed uniformly throughout the gel. The collagen fibers formed a network that was connected randomly with the surface of SMCs and nylon fibers. The diameter of the collagen fibers was ≈100 nm, and the concentration of collagen was 2.5 mg/mL. Using these parameters, fiber matrix theory predicted a Darcy permeability coefficient ($K_p$) of $1.22 \times 10^{-8}$ cm², which was close to the measured value of $K_p$. The production rates of prostaglandin (PG) I₂ and PGE₂ were used as markers of biochemical responsiveness of SMCs to fluid shear stress. Both PGI₂ and PGE₂ production rates under 1 dyne/cm² shear stress were significantly elevated relative to static (no-flow) controls. The production rates, however, were ≈10 times lower than observed when the same cells were plated on collagen-treated glass slides (2D model) and exposed to the same level of shear stress by use of a rotating disk apparatus. The results indicate that interstitial flow can affect SMC biology and that SMCs are more quiescent in 3D cultures than in 2D cultures. The 3D collagen gel model should be useful for future studies of interstitial flow effects on SMC function. (Arterioscler Thromb Vase Biol. 2000;20:2220-2225.)

Key Words: shear stress • smooth muscle cell • collagen gel • prostaglandin

Blood flow exposes endothelial cells to fluid shear stress, which affects their physiological function and is believed by many to play a role in the localization of atherosclerotic plaques in arteries. Smooth muscle cells (SMCs) are not normally exposed directly to the fluid shear stresses of blood flow, because they lie beneath the intact intima. In the case of injury to the intima, as occurs, for example, in angioplasty, near the anastomoses of vascular grafts, and in atherosclerotic disease, the superficial layers of SMCs are exposed directly to blood flow shear stresses (order of magnitude, 10 dyne/cm²).

Several groups have studied the effects of fluid shear stress on SMCs using cultured cells grown in monolayers on impermeable substrates. Using these 2D models, they have shown that vascular SMCs are responsive to shear stress in the range 1 to 25 dyne/cm² and increase their synthesis of transforming growth factor-β and tissue plasminogen activator, heme oxygenase-1, and nitric oxide. Protease-activated receptor-1 and tissue plasminogen activator gene expression are also regulated by shear stress on SMCs.

A more subtle mechanism by which SMCs are exposed to fluid shear stress derives from the transmural pressure gradient (typically 100 mm Hg in an artery), which drives transmural interstitial flow across the vessel wall. Using the Brinkman model of porous medium flow, Wang and Tarbell estimated that for an artery with an intact intima at normal physiological pressure, the transmural flow would induce a shear stress on the SMCs in the tunica media on the order of 1 dyne/cm². Damage to the intima or an increase in arterial pressure would elevate the interstitial flow shear stress on SMCs.

The normal configuration of SMCs in the tunica media is a 3D network of cells embedded in a fiber matrix of collagen and proteoglycans. In the present article, we describe an experimental system in which rat aortic SMCs are suspended in a 3D collagen gel and exposed to pressure-driven interstitial flow with a calculated shear stress of 1 dyne/cm². The production rates of prostaglandins by SMCs in response to this simulated interstitial flow are observed to be upregulated significantly. This represents the first demonstration that SMCs are responsive to fluid shear stress in a 3D configuration that simulates their normal physiological environment more closely than a 2D (monolayer) model.

Methods

Cell Culture
Rat aortic SMCs were enzymatically isolated from the thoracic aortas of male Sprague-Dawley rats (10 weeks old, ≈250 g) after...
The SMC-gel reactor and flow delivery apparatus (Figure 1) was fabricated from acrylic sheet. The SMC-gel reactor (Figure 1) was placed at the downstream end of the tube to retain the gel under flow conditions. The tube, nylon mesh, and filter were then sterilized before addition of the SMC-gel mixture. An equilibrium volume (in ice water) of mixture of SMCs suspended in acid-dissolved collagen (type I, derived from rat tail tendon, 3.75 mg/mL, Collaborative Biomedical) and DMEM containing 10% FBS was neutralized to pH 7.0 by addition of 0.1N sodium hydroxide. The collagen concentration of the mixed solution was 2.5 mg/mL, and the SMC density in the solution was 1.0×10^6 cells/mL. The mixture of SMCs and collagen was then poured into the silicon tube and placed in the incubator (37°C, 5% CO_2/95% air) for 30 minutes to complete the gelation process, then incubated further in DMEM with 10% FBS for an additional 2.5 hours. For static (no-flow) control experiments, the SMC-gel mixture was poured into a 25-mm-diameter silicon tube used for flow experiments. The bubble displacement was converted to fluid volumetric flow rate (J_v) by the formula J_v=(Δd/Δt)(F), where Δd/Δt is the bubble displacement per unit time and F represents the volume of fluid contained in a known length of tubing. The calibrated fluid flow then passed through the 3D SMC-gel model, where it imposed interstitial flow shear stress on the surface of the SMCs. Samples of the effluent (~1.5 mL) from the 3D model were collected at 1-hour time intervals for 6 hours and stored in a freezer (~20°C) for subsequent chemical assay. For the static (no-flow) control experiments that were conducted in the incubator, 1.5-mL samples of media were removed from the top of the SMC-gel mixture in the Transwell chamber at 1-hour time intervals and were replaced by the same volume of fresh medium.

Darcy Permeability and Shear Stress

The Darcy permeability (K_v) of the SMC-gel mixture was determined from the definition

\[ K_v = \frac{\mu (J_v/A)}{(ΔP/L)} \]

where \( \mu \) is the viscosity of the perfusing medium (1.2 cp), \( J_v \) is the measured volumetric flow rate, \( A \) is the cross-sectional area of the tube, and \( ΔP \) is the imposed pressure drop over the length (L) of the SMC-gel reactor.

The average shear stress over the SMC surface imposed by the interstitial flow has been estimated assuming cylindrical cells by Wang and Tarbell^7 and for flow around spheres by Brinkman. For both cell geometries, the average shear stress can be expressed as follows:

\[ \tau = \frac{B}{4/\pi} \]

where

\[ B = 4/\pi \] for cylinders

\[ B = 3/\pi \] for spheres.

Because \( B≈1 \), we will report values of \( τ \) assuming \( B=1 \).

PGI_2 and PGE_2 Production Rates

The concentrations of prostaglandin I_2 (PGI_2) and prostaglandin E_2 (PGE_2) in the effluent stream from the SMC-gel reactor were determined by measuring the concentration of the stable products 6-keto-PGF_1α and PGE_2 by use of enzyme immunoassay systems (ELISA, Amersham). The production rates of these species were determined from a mass balance over the SMC-gel reactor assuming a uniform distribution of SMCs in the gel and a constant reaction rate depending only on the shear stress level. The resulting expression for the production rate is

\[ R = \frac{J_v C_i}{V N_i} \]

where \( C_i \) is the concentration (mass/volume) of product in the effluent from the reactor, \( V \) is the volume of the SMC-gel reactor, and \( N_i \) is the number density of cells in the gel (number/volume). Several additional assumptions that are necessary to obtain Equation 3 are justified in the Appendix. It should also be noted that Equation 3 provides an accurate estimate of the production rate only after the initial concentration of products in the reactor before the initiation of flow have been pushed out of the tube. Because the velocity profile in the reactor is expected to be flat (see Appendix), it takes 1 tube residence time (tube length/flow velocity) for the initial contents to be cleared. At the higher flow rate, this amounts to 19 minutes and at the lower flow rate, 137 minutes. Therefore, the production rates given by Equation 3 may be overestimated in the first hour at the higher flow rate and in the first 2 hours at the lower flow rate.

For the static experiments, Transwell filter holders (25 mm; Costar) were sealed with glass slides with silicon elastomer (Dow Corning) and sterilized under UV light overnight. The SMC-gel...
mixture was added to the chamber, and the gelation process was completed. Then 1.5 mL of medium was added to the chamber, and a sample was removed for concentration assay every hour and replaced by an equal volume of fresh medium. The production rate was calculated from the expression

\[
R = \frac{V \Delta C_i}{N \Delta t}
\]

where \(\Delta C_i\) is the change in concentration of the product over the previous hour (\(\Delta t\)), \(V\) is the volume of the system (gel + media), and \(N\) is the total number of cells in the Transwell chamber. The use of Equation 4 to determine the rate in static cultures is based on the assumption that the concentration of prostaglandin in the sampled medium is equal to that in the gel. This assumption is justified because the diffusion time for a planar slab \((L^2/4D)\), where \(L\) is the gel layer thickness (1.4 mm) and \(D\) is the solute diffusivity in the gel, which is close to the free diffusivity \((7 \times 10^{-6} \text{ cm}^2/\text{s})\), is \(\approx 5\) minutes, which is short compared with the time frame of the experiments (6 hours).

**Morphological Studies: Electron Microscopy**

For transmission electron microscopy, samples of SMCs in collagen gels were first fixed in 2.5% glutaraldehyde at 4°C for 2 hours and washed 3 times with 0.1 mol/L cacodylate buffer at room temperature for 5 minutes. The samples were then postfixed in 1% OsO4 at room temperature for 1 hour, dehydrated in a graded series of ethanol solutions that were replaced with acetone, and embedded in epoxy resin (Epon 812). Ultrathin sections were stained with 2% uranyl acetate and 0.2% lead citrate and examined with a JEM 1200EXII electron microscope (JEOL).

The samples for scanning electron microscopy were fixed in 2.5% glutaraldehyde at 4°C for 2 hours and washed with 0.1 mol/L cacodylate buffer, then postfixed in 1% OsO4 at room temperature for 1 hour and dehydrated in a graded series of ethanol solutions that were replaced with acetone and embedded in epoxy resin. The samples were support gelation coated on the glass slide for 20 minutes at 37°C overnight. Type I collagen (1 mL; same material as in the 3D experiments) at a low concentration (0.05 mg/mL) that would not support gelation was coated on the glass slide for 20 minutes at 37°C and then aspirated off. Cells were then seeded \((4 \times 10^3 \text{ cells/cm}^2)\) on the slide and incubated for 4 days \((37°C, 5% \text{ CO}_2/95\% \text{ air})\) to allow SMCs to reach confluence.

A rotating disk apparatus that has been described previously was used to impose a defined shear stress on the cells. The system consisted of a cylindrical disk of radius \(r\), rotating at a frequency \(f\), in fluid of viscosity \(\mu\), at a separation distance from the SMC surface of \(h\). The shear stress varied linearly with distance from zero at the center of the disk to a maximum shear stress \(\tau\) given by

\[
\tau = \mu \alpha \frac{r}{h}.
\]

The average shear stress over the disk was two thirds the maximum value. All subsequent values of shear stress will be given as the maximum shear stress.

The SMCs were subjected to 0 (control), 1 dyne/cm², or 2 dyne/cm² shear stress for 6 hours in 2 mL of experimental medium (DMEM/10% FBS). Samples (500 \(\mu\)L) were taken from the chamber every hour and replaced with an equal volume of fresh medium. The samples were assayed as described previously, and cumulative concentrations were determined taking into account the dilution factor associated with fresh medium replacement. The production rate at a given hour was calculated from Equation 4, where in this application \(V\) represents the volume of medium in the well and \(N\) is the number of cells on the slide.

**Data Presentation and Statistical Analysis**

Data are presented as mean ± SEM. In graphical presentations, standard error bars are shown. Two-sample \(t\) tests at selected time points were conducted to determine statistical differences between treatments. Differences were considered statistically significant if \(P < 0.05\).

**Results**

**Morphological Characteristics of the 3D Model**

The collagen formed a gel from solution after 30 minutes at 37°C, and the gel (supported by nylon fibers) was strong enough to bear pressure differentials of 90 cm H₂O that were imposed on it for 6 hours. Under the scanning electron microscope, the SMCs appeared globular, and their diameter was typically \(\approx 10 \mu\text{m}\) (Figure 2). The SMCs were distributed uniformly throughout the collagen gel at a low volume fraction (<0.5%). The collagen fibers formed a random network that was integrated into the surface of the SMCs (Figure 2) and the nylon fibers (not shown). After 6 hours of exposure to 1 dyne/cm² shear stress, the collagen fibers had a tendency to align with the flow direction, while still remaining connected in a network (Figure 2). Collagen fibers in gels not exposed to flow did not display a preferred orientation. The collagen fibers had a rope-like structure under transmission electron microscopy (\(\times 20000\)), and the diameter of the collagen fibers was determined to be 100.9 ± 93.4 nm (\(n = 182\)). The nylon mesh fibers were much larger, and their diameter was determined from scanning electron microscope photomicrographs to be 17.5 ± 1.81 μm (\(n = 20\)).

**Flow Characteristics of the 3D Model**

At a pressure drop of 90 cm H₂O, the initial volumetric flow \((L/A)\) through the SMC-gel model was \(7.74 \times 10^{-3} ± 6.2 \times 10^{-4} \text{ cm}^3/\text{s}\). The Darcy permeability at this pressure was \(K_v = 9.48 \times 10^{-9} ± 7.5 \times 10^{-10} \text{ cm}^2/\text{s}\) (Equation 1), and the average shear stress on SMCs was \(\approx 1 \text{ dyne/cm}^2\) (0.95 ± 0.27 dyne/cm²; Equation 2). There was a gradual reduction of flow through the gel over time by \(\approx 20\%\) after 6 hours. At a pressure drop of 13 cm H₂O, the initial volumetric flow through the gel was 1.09 ± 0.15 cm³/10⁻⁶ cm²/s. The Darcy permeability at this pressure was \(K_v = 9.24 \times 10^{-10} \text{ cm}^2/\text{s}\) (Equation 2).
10^{-6} \pm 7 \times 10^{-11} \text{ cm}^2$, and the average shear stress was $\approx 0.15 \text{ dyne/cm}^2$ (0.14 ± 0.01 dyne/cm). There was no perceptible reduction of flow through the gel over time at this lower pressure drop condition.

Production of Prostaglandins in the 3D Model
PGF$_{1\alpha}$ production (Figure 3) appeared to be highly upregulated by 1 dyne/cm$^2$ shear stress in the first hour, but this apparent burst in production was very likely the result of the initial washout of product accumulated in the reactor before the initiation of flow. The production rate, however, was significantly upregulated relative to the static controls for the next 5 hours, by as much as 7 times. Even the low shear stress of 0.15 dyne/cm$^2$ elicited a significant elevation in production rate relative to static controls at 3 hours and at later times.

The pattern of PGE$_2$ production at 1 dyne/cm$^2$ shear stress was similar to the PGF$_{1\alpha}$ production (Figure 4). Although the production rate during the first hour is uncertain, elevated production continued throughout the 6-hour period, with the highest production rate observed after 6 hours. Unlike the case of PGF$_{1\alpha}$, PGE$_2$ production was not enhanced by the low shear stress of 0.15 dyne/cm$^2$ at any time point.

Production of Prostaglandins in the 2D Model
PGF$_{1\alpha}$ production was significantly upregulated by 1 dyne/cm$^2$ and 20 dyne/cm$^2$ shear stress after 2 hours of exposure to flow (Figure 5). There was no significant difference between the production rates at 1 dyne/cm$^2$ and 20 dyne/cm$^2$ at any time point. The production rates were approximately constant for hours 3 through 6, reaching a level that was 20 times control at hour 6. It is striking to note that the steady-state production rate at 1 dyne/cm$^2$ shear stress in the 2D model is $\approx 10$ times the production rate at 1 dyne/cm$^2$ shear stress in the 3D model (compare Figures 3 and 5).

The pattern of PGE$_2$ production was similar to the PGF$_{1\alpha}$ production (Figure 6). PGE$_2$ production was significantly upregulated by 20 dyne/cm$^2$ shear stress after 2 hours of exposure to flow and by 1 dyne/cm$^2$ shear stress after 3 hours. There was no significant difference between the production rates at 1 dyne/cm$^2$ and 20 dyne/cm$^2$ for hours 3 through 6. As in the case of PGF$_{1\alpha}$, the production rate of PGE$_2$ at 1 dyne/cm$^2$ shear stress in the 2D model is $\approx 10$ times the production rate at 1 dyne/cm$^2$ shear stress in the 3D model (compare Figures 4 and 6).

Discussion
We developed a 3D collagen gel model to simulate interstitial flow on SMCs. The fluid shear stress on SMCs in the model was computed to be on the same order of magnitude as expected for SMCs in the tunica media of an artery (1 dyne/cm$^2$). Although other studies have shown that SMCs are biochemically responsive to fluid shear stress in 2D (monolayer) models, this was the first study to demonstrate biochemical responsiveness to shear stress in a more physiological 3D model.
The SMCs in the 3D gels were globular and not elongated as they typically appear in an artery. The globular shape was associated with the fact that the cells were in the gel for <10 hours total elapsed time from preparation until termination of experimentation. It typically takes 48 hours for SMCs to contract a collagen gel and assume an elongated morphology. We avoided significant gel contraction to maintain a good seal of the gel within the flow tube so that flow channeling would not alter the shear stress distribution on cells.

It is interesting to note that the biochemical production rates in the 3D model (Figures 3 and 4) were an order of magnitude lower than in the 2D model (Figures 5 and 6), even though in both the 2D and 3D models the cells were cultured in serum and were presumably in a synthetic (as opposed to contractile) phenotype. This large difference in production rates is consistent with a study of endothelial cell production of thrombospondin in 2D and 3D collagen-based cultures (no flow) in which the production rates were observed to be much higher in 2 than in 3 dimensions. A related study of rabbit arterial SMCs in 2D and 3D collagen-based cultures (no flow) showed that the cell growth rate was greatly inhibited in 3D cultures relative to 2D cultures. It seems that cells in 3 dimensions are in a more quiescent state than cells in 2 dimensions in terms of both growth rate and biochemical activity.

Our calculation of the shear stress on SMCs suspended in a collagen gel (Equation 2) depends on an accurate estimate of the Darcy permeability coefficient (Kp) of the fibrous medium and the assumption of spatial uniformity of this property over the entire volume of the SMC-gel reactor. For the particular case of uniform, highly fibrous materials of solid volume fraction φ and fiber radius a, the predictions of several theoretical models are well approximated by

\[
K_p = 0.319 \cdot a^2 \cdot \varphi^{-1.17}
\]

The measured radius of collagen fibers in our in vitro model was 50 nm. The volume fraction of collagen fibers must account for the dry collagen (density 1.36 g/cm³) plus the intrafibrillar water, which ranges from 0.0 to 0.7 times the mass of dry collagen, depending on the osmotic swelling of the collagen. The volume fraction of collagen fibers, which was not measured directly in our experiments, therefore lies in the range of \(\varphi = 0.0025/1.36 \) to \(\varphi = 0.0025 (1/1.36+0.7)\). This range of \(\varphi = 0.00184 \) to \(0.00359\) leads to a prediction (Equation 4) of \(K_p\) in the range of \(5.79 \times 10^9\) to \(1.27 \times 10^8\) cm². Our experimental value based on pressure drop and flow rate measurements (\(K_p = 9.48 \times 10^8\) cm²) lies within the predicted range. The close agreement between measured and theoretical values of \(K_p\) supports our assumption of a uniform gel over the length of the SMC-gel reactor and the validity of the computed shear stress on SMCs.

The 20% reduction in flow over the 6-hour experiment observed at the highest flow rate was primarily the result of gel compaction induced by flow and not gel contraction induced by the presence of SMCs. This conclusion is based on our observation of similar flow reductions in cell-free gels at the highest flow rate.

Our studies provide evidence that interstitial fluid flow can stimulate vascular SMCs. Interstitial fluid flow can therefore be thought of as a signal communicating information from the blood vessel lumen (blood pressure and blood flow rate) to the underlying SMCs. Clearly, a change in blood pressure will have a direct effect on the transmural pressure gradient, which drives interstitial flow and in turn shear stress on SMCs. A change in blood flow rate will alter the shear stress on endothelial cells, and this has been shown to affect the hydraulic conductivity of the endothelial layer. A change in endothelial hydraulic conductivity leads to a corresponding change in interstitial flow resulting in altered shear stress on SMCs.

It has also been observed that leaky endothelial junctions associated with dying or mitotic cells can lead to punctate regions of enhanced permeability and water flux in arteries. This could produce higher local fluid stress on SMCs near leaky endothelial cells than on cells near intact endothelial junctions. It should also be realized that transmural flow is distributed to the medial layer of an artery through pores in the internal elastic lamina. This funneling of flow through small pores can produce a highly nonuniform distribution of shear stress on the SMC layer closest to the internal elastic lamina.

Studies of the effects of fluid shear stress on SMCs in 2D cell culture models have shown that shear stress affects SMC proliferation rates and SMC contraction states. Thus, it is plausible that changes in blood pressure and flow can affect both local vascular control (SMC tone) and vascular remodeling (SMC proliferation) through alterations in interstitial flow shear stress on SMCs. Further studies of SMC contraction and proliferation in response to flow in 3D models and in vivo will be necessary to assess this hypothesis.

**Appendix**

Equation 3 in the text is used to compute the production rate, \(R\). Several assumptions are necessary to justify this simple equation, as outlined in this Appendix. If we assume that (1) convective transport dominates diffusive transport in the flow direction and that (2) there is negligible fluid-phase resistance to mass transfer of prostaglandins from the SMCs to the medium perfusing the gel, then the following differential mass balance defines the transport processes:

\[
\frac{dC}{dz} = RN_v,
\]

where \(v_z\) is the axial velocity, \(C\) is the prostaglandin concentration, \(z\) is the tube axis direction, \(R\) is the production rate on a cell basis, and \(N_v\) is the volume density of cells. Furthermore, if we assume that (3) \(v_z\) is uniform (does not vary with radial position in the tube), then we can integrate Equation 7 from the entrance of the tube (\(z = 0\)) to the exit of the tube (\(z = L\)), where the concentration is \(C_{z=0}\) and find

\[
C_{z=L} = \frac{RN_v L}{v_z}.
\]

Noting that \(v_z = J/A\), where \(A\) is the cross-sectional area of the tube, and that \(V = AL\) is the total volume of the tube, we see that

\[
\frac{J}{C_{z=L}} = \frac{RN_v}{VN_v},
\]

which is Equation 3 in the text. The three assumptions required to arrive at Equation 9 must be justified.

**Assumption 1: Convection Dominates Diffusion**

The Peclet number, \(N_p\), which characterizes the relative importance of convection and diffusion, is defined as follows:

\[
N_p = \frac{v_z L}{D}
\]
This definition neglects the very small volume fraction of cells and collagen fibers in the tube. Using values for the volume flux, tube length, and prostaglandin diffusivity defined previously in the text, we find $N_{pe} = 5950$ for the 1 dyne/cm² case, and $N_{pe} = 1400$ for the 0.15 dyne/cm² case. Convection is clearly dominant in both cases.

**Assumption 2: Negligible Fluid-Phase Transport Resistance**

If we consider the mass balance on a single SMC in the reactor, then the rate of production of prostaglandin ($R$) is equal to the rate of its transport from the cell surface (concentration $C_s$) to the bulk of the reactor fluid (concentration $C_b$) as expressed by the equation

$$k_L A_p (C_s - C_b) = R,$$

where $k_L$ is the fluid-phase mass transfer coefficient and $A_p$ is the surface area of an SMC. Equation 11 can be rearranged as

$$C_s/C_b = 1 + \frac{R/C_b}{k_L A_p}.$$

If the dimensionless quantity on the right side of Equation 12 is small ($<< 1$), then we can conclude that there is negligible fluid-phase transport resistance ($C_s = C_b$). To estimate this quantity, we will take $C_s \approx C_b$ (note that $C_s$ lies between 0 and $C_b$) and use Equation 3 to obtain

$$R/C_b = (J_v/A)/L N_v.$$

To estimate $k_L A_p$, we will use a well-known result for diffusive transport from a sphere:

$$N_{sh} = k_L r/D = 1,$$

where $N_{sh}$ is the Sherwood number and $r$ is the sphere (cell) radius. This estimate for $k_L$ gives the smallest possible value because it neglects convective contributions, which happen to be negligible for the system under consideration. Finally, noting that $A_p = 4\pi r^2$, we arrive at

$$R/C_b = \frac{J_v/A}{4\pi r LN_v D} = 0.02,$$

where we have taken the highest volume flux ($J_v/A = 7.74 \times 10^{-3}$ cm/s), an SMC radius of $r = 5 \mu m$, and used previously defined parameters to make the estimate. This calculation supports assumption (2).

**Assumption 3: Uniform (Flat) Velocity Profile**

It is shown elsewhere that when the diameter of a gel-filled tube ($d_t$) is much greater than $\sqrt{d_t}$, the velocity profile is flat. For our system, $d_t/\sqrt{d_t} \approx 2.8 \times 10^4$, and the assumption is justified.

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**References**


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