THE EFFECTS OF INTERSTITIAL FLOW ON FIBROBLAST ORIENTATION *IN VITRO*

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INTRODUCTION

Fluid movement through the tissue extracellular matrix, or interstitial flow, constitutes an important component of the microcirculation. It provides convective transport of large molecules and proteins through the interstitial space, which is crucial in maintaining the health of cells and tissues. Aside from its role in transport, interstitial flow imposes mechanical stresses to the interstitial cells and can play an important role in determining interstitial organization and architecture. It can affect cells both directly by fluid shear forces and indirectly by extracellular matrix strain caused by fluid shear on the ECM fibers to which the cells are attached. This strain can in turn prompt integrin-mediated cell responses, which may, for example, cause the cells to reorganize and remodel the matrix. There is abundant evidence in the literature that mechanical forces help govern the architecture of tissues, but the mechanical role of interstitial fluid flow in cell organization and matrix architecture remains poorly understood, in part due to a lack of experimental models for *in vitro* studies.

We have developed a novel model to directly examine the effects of interstitial flow on cell organization in soft tissue cultures, where we can (i) specifically control the flow environment, (ii) observe cell organization microscopically, and (iii) measure the mechanical properties (e.g. hydraulic conductivity) in real time. Using the model, we observe that fibroblasts seeded within collagen gels align perpendicular to the direction of interstitial flow.

METHODS

Our model, which delivers interstitial flow through a matrix in a radial direction and perpendicular to the plane of view (Figure 1), consists of a cell-populated collagen gel anchored by acid-treated porous polyethylene (PE) inlet rod and outlet ring sandwiched between functionalized glass coverslips. The flow chamber was assembled under sterile conditions with silicone glue.

Human dermal fibroblasts (CCD1079sk, ATCC, Manassas, VA) were added at a density of 10^5 cells/ml to a 0.35% type I rat tail collagen solution. The cell-gel mixture was then injected into the chamber and incubated at 37°C for 30 minutes to allow the gel to polymerize and react with activated glass surfaces. The system was further incubated in media overnight to allow cell adhesion to the matrix. To induce flow, the chamber was connected to a sterile media reservoir via a peristaltic pump and manometer. A static control was set up exactly as the flow chamber, except that it was not connected to the flow delivery apparatus.

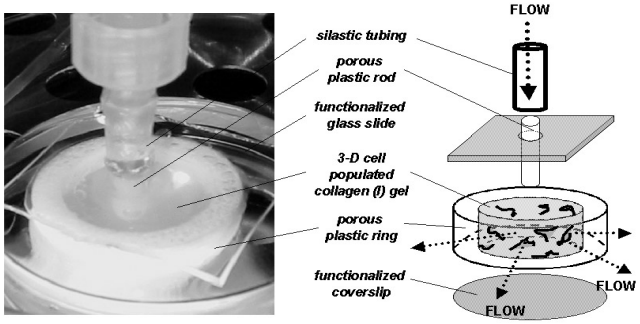

Figure 1. Interstitial Flow Model Set-up

One unique feature of the model is that we can track the average bulk hydraulic conductivity of the matrix (K) *in situ*. We measured the pressure gradient ΔP across the gel under an imposed constant volumetric flow rate Q at various time points t and calculated K using Darcy's equation for 1-D radial flow:

$$K = \frac{Q}{2\pi h \Delta P} \ln \frac{r_o}{r_i} \qquad (1)$$

where h was the gel thickness, r_o and r_i were the outer and inner radii of the gel respectively.

RESULTS

The fibroblasts aligned perpendicularly to the radial direction of flow and had a more noticeable spindle-shaped morphology than those

in static conditions (Figure 2). In contrast, the cells in static cultures retained a random orientation and appeared more branched in shape. This result was reproducible at flow rates of 0.01ml/min, whereas at lower flow rates the cells remained randomly oriented and at higher flow rates they rounded up.

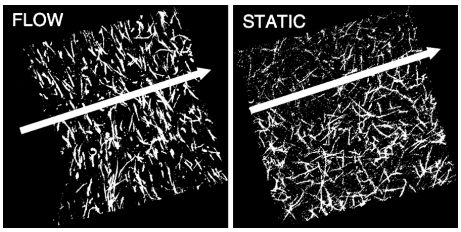

Figure 2. Cell orientation (arrow shows radial direction)

Our unique model also allowed us to directly examine the dependence of cell orientation on velocity due to the radial variations of the model (Figure 3). Consistent with our observations at higher flow rates, we observed that cells subjected to interstitial flow became more randomly oriented with increasing radial distance (and thus decreasing velocity).

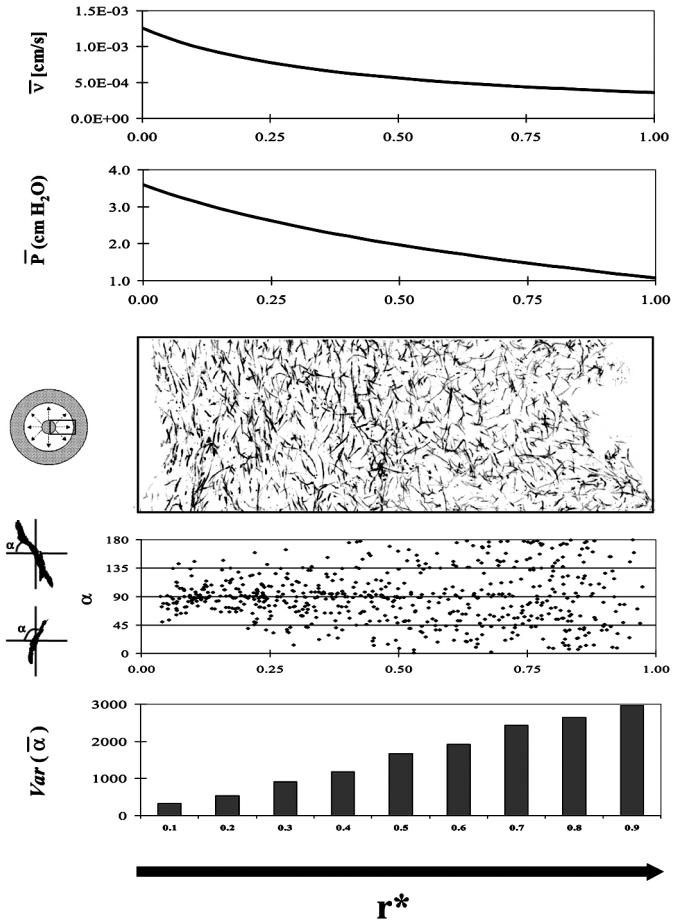

Figure 3. Velocity, pressure, inverted confocal image, angle scatter plot and variance of average cell alignment profiles in a radial section of fibroblast-populated gel subjected to interstitial flow

The hydraulic conductivity measurements were consistently found within the narrow range of 0.5 to 2.5 x 10^{-4} $cm^2/s/cm\ H_2O$. For the acellular gel, *K* initially increased and leveled off at 3.5 x 10^{-4} $cm^2/s/cm\ H_2O$ under interstitial flow.

DISCUSSION

The results establish interstitial flow as having distinct effects on cells and strongly suggest that interstitial flow can affect cell organization in a matrix, although the mechanisms of this interesting phenomenon have yet to be elucidated. It is likely that this realignment may help to improve the mechanical strength of the gel, as seen in matrix remodeling in other tissues in response to mechanical stress. The circumferential alignment of the fibroblasts was dependent on the bulk velocity *v*. The fibroblasts became less aligned and more randomly oriented as *v* decreased (Figure 3). Lastly, it should be noted that this perpendicular cell alignment under interstitial flow is different from other kinds of mechanical loading of fibroblast-populated collagen gel where parallel alignment of the cells to the direction of the applied load is typically observed [1].

The narrow range of the hydraulic conductivity data suggested that cells could affect the hydraulic conductivity of the gel but they did not elicit drastic changes. The average *K* of 1 x 10^{-4} $cm^2/s/cm\ H_2O$ agreed with the data obtained previously for type I collagen [2] but this value was at least 2 orders of magnitude higher than that found in mammalian tissues, whose range varied from 10^{-6} to 10^{-10} $cm^2/s/cm\ H_2O$ [3]. The difference is expected since the collagen concentration of the tissue culture system here is very small (about 3.5 mg/ml), and reconstituted collagen gels do not have any specialized architecture and are not incorporated with other ECM molecules such as proteoglycans (which contribute to *K* significantly).

CONCLUSION

We have developed a unique model to directly examine the effects of interstitial flow on cell organization in soft tissue cultures. Using the *in vitro* model, we observed that fibroblasts aligned perpendicularly to the direction of flow. The results established the distinct effects of interstitial flow on cell orientation and strongly suggest that interstitial flow can affect cell and matrix organization, although the mechanisms of this interesting phenomenon remains to be elucidated.

ACKNOWLEDGEMENTS

The authors wish to thank the National Science Foundation and Whitaker Foundation for funding this project.

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