INTERSTITIAL FLUID FLOW AS A GUIDE FOR ENDOTHELIAL CELL ORGANIZATION IN VITRO

Melody A. Swartz (1,2), Cara-Lynn E. Helm (2), Chee Ping Ng (2), Jeremy Goldman (1), Mihaela Skobe (3), Andreas H. Zisch (4), Jeffrey A. Hubbell (4), and Kendrick C. Boardman (1)

(1) Department of Biomedical Engineering and (2) Chemical Engineering, Northwestern University, Evanston, IL; (3) Donald H. Ruttenberg Cancer Center, Mt. Sinai School of Medicine, New York, NY; (4) Institute for Biomedical Engineering and Department of Materials, Swiss Federal Institute of Technology (ETH) and University of Zurich, Switzerland

INTRODUCTION

A great promise of modern biology is the prospect that cells and tissues may be manipulated so that functional artificial replacement organs may be engineered. One vexing obstacle to the development of artificial tissues is the absence of a blood supply, since cells not served by blood will quickly perish. A number of investigators have made progress in understanding conditions whereby vascular cells live, reproduce, and even form tubular structures in an artificial environment, but the field is still struggling to devise methods to organize clusters of cells into the functional tree-like vascular structures and capillary networks of natural tissues.

On a related problem, we have recently discovered that mechanical stress plays a key organizing role in lymphatic development (1). Our striking observations – namely, that (a) this process relies on fluid channel formation as a precursor to lymphatic vessel formation (Figure 1), (b) lymphatic development occurs in the direction of fluid flow, and (c) reducing interstitial flow inhibits lymphatic development – form a new paradigm for lymphatic development. We now have evidence that lymphatic capillary formation is a highly organized process of creating, organizing, and finally 'endothelializing' fluid channels to enhance interstitial fluid flow. This is in contrast to sprouting angiogenesis and other mechanisms of blood capillary formation.

Therefore, we hypothesize that interstitial fluid flow can be used to guide and organize vascular endothelial cells *in vitro* into vascular beds in artificial tissue constructs, since lymphatic and blood endothelial cells (LECs and BECs, respectively) share a remarkably similar phenotype that may be controlled only by the biochemical and biomechanical environments. To this end, we have begun to understand and utilize the mechanisms underlying flow-induced lymphatic development *in vivo* and then apply those principles to microvascular organization *in vitro*. We have recently developed unique tools for approaching to this problem, including (1) a collagen "window" in the mouse tail skin, (2) a 3-D culture model for interstitial fluid flow, and (3) a matrix-binding form of VEGF-C, a known LEC growth factor. Using these models, we have begun to elucidate how different cell types (e.g. endothelial cells, fibroblasts) respond to their mechanical environment and work together to organize fluid channels into vascular structures and maintain matrix integrity. Specifically, we have examined mechanisms of flow-induced cell migration and organization as well as characterized the differences between LECs and BECs in terms of their responses to growth factors and to interstitial fluid flow. Our future goals are to examine whether self-organized lymphatic capillaries can be used as surrogate blood vessels, and to determine how these capillary beds may be adapted into the normal circuitry of a living animal and whether they are able to carry out their required functions.

METHODS

In order to evaluate the role of interstitial fluid flow on lymphangiogenesis *in vivo*, we established a lymphangiogenesis model in the tail of the mouse whereby a circumferential band of skin is removed and replaced with collagen or fibrin gel (Figure 1). In this model we can visualize lymphatic function and fluid channel formation using microlymphangiography and later sacrifice the mouse and section the skin to correlate molecular events with lymphatic development. We can also modify this model to either (a) include matrix-bound growth factor or (b) reduce interstitial flow rates through the implanted matrix.

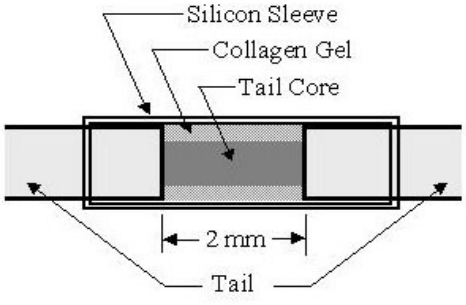

Figure 1: Schematic of mouse tail construct

For *in vitro* studies, our interstitial flow model contains a collagen or fibrin gel seeded with fibroblasts, LECs, BECs, or some combination of cell types (the model is detailed in (2)). Porous polyethylene (PE) is used to anchor the gel in the direction of fluid flow, which is sandwiched between two functionalized glass slides; both serve to anchor the gel and prevent contraction. Medium is perfused through the cell-gel mixture via the PE using a pressure head of 2-10 cm water, which led to flow rates of 30-150 μl/min/ml gel. In static controls, medium readily diffuses through the PE and no differences in cell rounding or death were detected.

VEGF-C was produced recombinantly with a C-terminal factor XIIIa substrate according to similar techniques as described earlier (3). This allowed incorporation into a fibrin matrix during coagulation. An MMP substrate was engineered between the VEGF-C domain and the factor XIIIa substrate domain to permit release by cell-associated MMPs.

RESULTS

The *in vivo* model modification of reduced interstitial flow confirmed that LECs fail to organize into a functional lymphatic network without interstitial fluid flow. Furthermore, the addition of exogenous VEGF-C led to an increase in LEC proliferation but not network formation, and many of the LEC-lined structures were not functionally part of the intact lymphatic network. Together, these results suggest that interstitial flow works synergistically with VEGF-C (among other factors) to control lymphatic capillary network formation.

In the *in vitro* studies, we saw that microvascular LECs, but not BECs, readily formed tube-like networks in all gel types tested. Extensive networking was seen particularly when the LECs were seeded in fibrin gels containing either bound VEGF-A or VEGF-C, even in static controls (Figure 2). With the application of interstitial flow, all cells tended to align perpendicular to the flow, and LEC networking was enhanced and more organized.

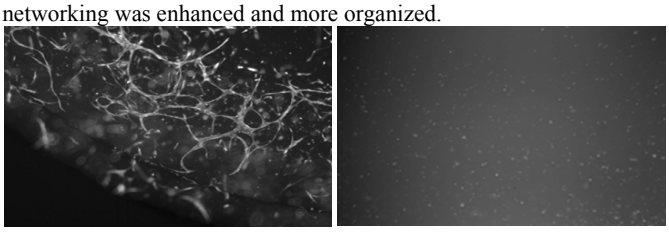

Figure 2. LECs (left) vs. BECs (right) grown 3-D in fibrin containing matrix-bound VEGF under static conditions.

DISCUSSION

Stress management is critical in determining tissue function and organization. In the microcirculation, there has been significant attention on understanding the molecular and mechanical regulation of blood vessel growth (angiogenesis) and functional aspects such as permeability. However, a critical but often ignored part of the microcirculation is the interstitial-lymphatic component through which plasma exudate is transported through the interstitium. Although a minor component of the circulation in terms of volume flow rates compared to blood, the lymphatic system is critical for protein transport to and from cells because it induces convective flow through the interstitium. We have created novel models to study the biology and development of the lymphatic system, and begun to take advantage of its physiological driving force – interstitial flow – towards creating microvascular networks in vitro. Although we are still a long way off from our goal, we have established a foundation for this approach by demonstrating that (1) interstitial fluid flow indeed drives lymphatic network formation and growth, (2) VEGF-C alone is insufficient to drive lymphangiogenesis, and (3) LECs and BECs respond differently in 3-D culture to either growth factors or interstitial flow.

ACKNOWLEDGEMENTS

The authors wish to thank the Beckman Foundation for funding this work and the Whitaker Foundation and the NSF for funding peripheral work that formed the basis of this study.

REFERENCES

(1) K.C. Boardman and M.A. Swartz. Interstitial flow as a guide for lymphangiogenesis. Submitted.

(2) C.P. Ng and M.A. Swartz. Fibroblast alignment under interstitial flow: a unique in vitro model. Submitted.

(3) A.H. Zisch, U. Schenk, J.C. Schenshe, S.E. Sakiyama-Elbert, and J.A. Hubbell. Covalently conjugated VEGF-fibrin matrices for endothelialization. J. Controlled Release 72:101-113, 2001.