FiberCell Systems Inc.
a better way to grow cells

Culture of Endothelial Cells in Hollow Fiber Systems

By John J. S. Cadwell

Hollow fibers are small, cylindrical filters, shaped like drinking straws, and as small in diameter as a human hair (200 µm) (see *Figure 1*). The pore size or molecular weight cut off (MWCO) can be controlled in manufacturing to be from .1µm to 5kd.

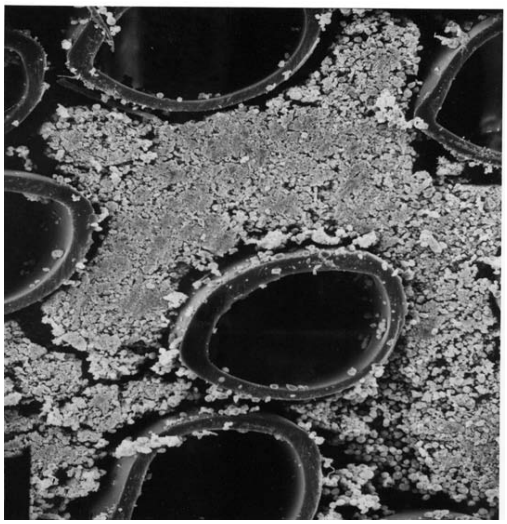

Figure 1 Cross-section of hollow fiber bioreactor growing lymphocytes at high density.

Large bundles of these fibers can be potted into cylindrical housings in such a way that any liquid entering from the ends of the cartridges (end ports) will go through the insides of the fibers, while access to the area outside the fiber (extracapillary space, or ECS) is provided by side ports on the outside of the housing. In general, cells are placed on the outsides of the fiber where they can attach and grow, while cell culture medium is continuously circulated through the interior of the fibers to provide nutrients and oxygenation. Because of the characteristics of the filter, smaller molecules such as glucose and lactate can cross the fiber freely, while larger molecules such as proteins cannot cross the fiber. Antibody production, protein expression, and the generation of conditioned medium are some of the classic applications of hollow-fiber bioreactors.

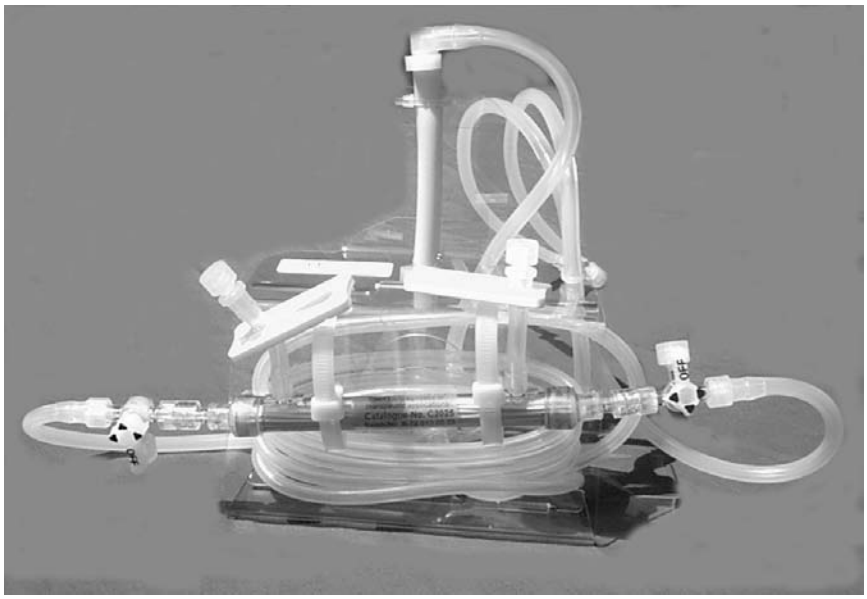

Figure 2 FiberCell Systems PS+ cartridge

Endothelial cell culture

Endothelial cells cultured in standard cell culture flasks grow in an active state and continuously divide without expressing tight junctions and other indicators of normal physiology. Unlike the techniques described above where the cell are seeded onto the outer surface of the fiber endothelial cells can be seeded onto the inside of the fiber wall and medium circulated over them at a specific flow rate. The flow rate of medium over the endothelial cells will produce a defined amount of shear stress expressed in dynes/cm^2. The FiberCell hollow fiber cartridge for endothelial cell culture under shear contains 20 PS+ fibers with an internal diameter of 700µm and an active length of 10cm for a total surface area of 80cm^2. Approximately 100µg of total RNA can be extracted from each cartridge. Additionally, the Polysulfone Plus (PS+) fiber (**FiberCell Systems**) permits the attachment of proteins, cytokines, antibodies, or other proteinaceous materials to the fiber surface. This fiber enables the study of surface biochemistry on long-term cultures of endothelial cells.

Depending upon the matrix used the cells can be removed intact and either re-plated into static culture or run through a flow cytometer as shown below. Endothelial cells cultured under chronic shear stress respond in a physiologic manner and form a monolayer, stop dividing, orient to the flow of medium and form tight junctions. Gene expression patterns are affected, as are protein expression patterns.

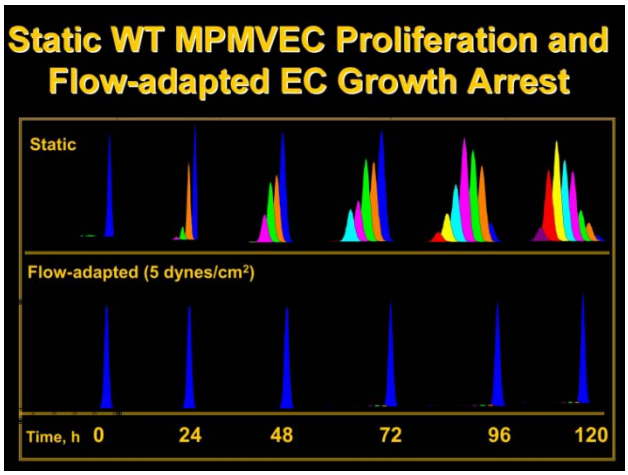

Figure 3 Proliferation assay demonstrating the lack of cell division for endothelial cells when grown under conditions of shear stress.

Morphological changes can be induced in endothelial cells responding to differing amounts of shear stress. Figure 5 demonstrates the effect of low shear (5 dynes/cm^2) versus high shear (15 dynes/cm^2) on human pulmonary endothelial cells. The low shear photograph (left) depicts a monolayer of cells formed on the surface of the fiber. At high shear stress, the cells pile on top of one another in a plexiform lesion (right). This is also observed in vivo but not in flask culture.

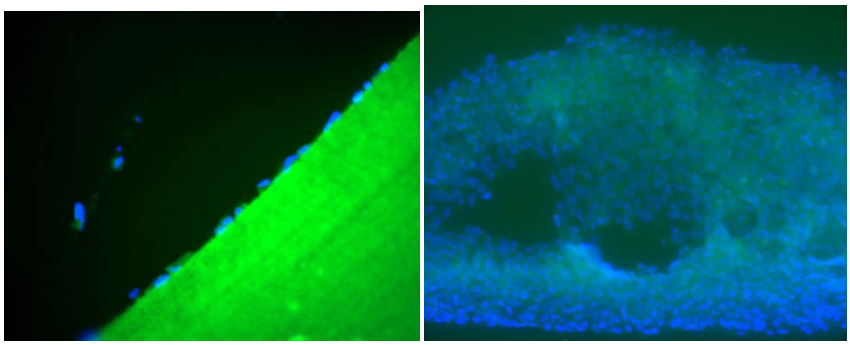

Figure 5 Pulmonary endothelial cells grown under high shear stress

It is also possible to culture a second cell type on the outside of the fiber while endothelial cells are cultured on the inside as shown in Figure 6. Endothelial cells on the inside of the fiber co-cultured with vascular smooth muscle cells on the outside of the fiber demonstrated that as the flow rate was changed, G-protein formation and endothelin receptor expression in the smooth muscle were directly affected. The endothelial cells secreted a factor that crossed the fiber and changed the physiology of the smooth muscle cells.

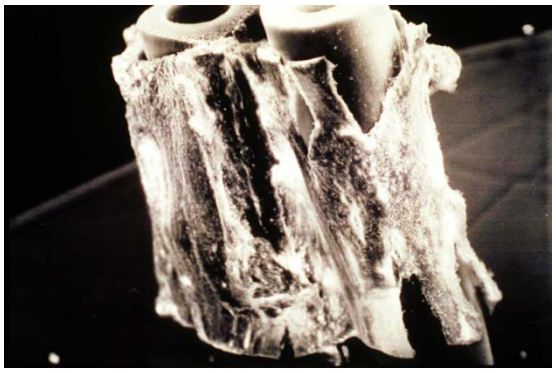

Figure 6 Bovine aortic endothelial cells co-cultured with vascular smooth muscle cells on the outside of the fiber.

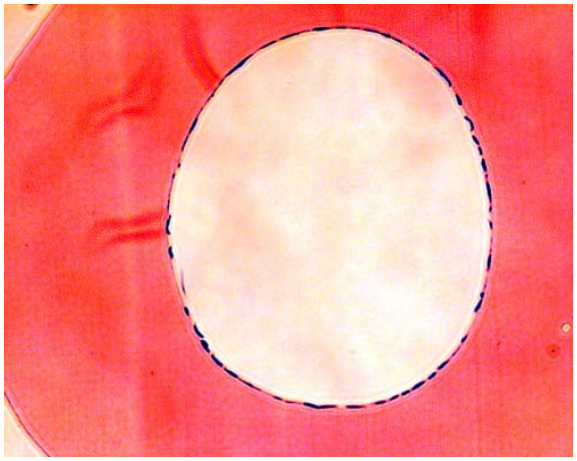

Figure 7 Human coronary endothelial cells Guerkan Sengoelge, Medical University of Vienna

The PS+ fiber permits the study of extra cellular matrix effects on the long-term culture of different cell types. The figure above shows a cross-section of a PS+ fiber with human umbilical cord vein endothelial cells (HUVECS) seeded onto a fiber that has been coated with standard collagen. In diabetes and aging collagen in extra cellular matrices becomes heavily glycosylated. Using the PS+ fiber it will be possible to observe the effects of this highly glycosylated collagen vs. normal collagen on gene and protein expression during long-term culture (2 weeks or more).

Other possible applications for the fiber include the attachment of specific antibodies for lymphocyte stimulation, attachment of specific ligands to promote growth of hepatocytes or pancreatic islets, and study of the effects of extra cellular matrix on long-term cell growth and differentiation. The

fiber permits studies on growth and differentiation that were not previously possible with conventional cell culture systems.

Although the technology is not new, significant advances in fiber materials have resulted in hollow-fiber bioreactor systems with improved productivity and ease of use. The culture of endothelial cells on the inside wall of PS+ permits their culture for extended periods of time under unique conditions of shear stress and extra-cellular matrix.

References

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