A MODEL FOR THE MECHANICS OF PRIMARY LYMPHATIC VALVES AND THE INTERACTION WITH THE SECONDARY VALVES

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INTRODUCTION

The mechanisms for transport from the tissue into the lymphatics remains an important unresolved problems in many areas of medicine and biology. Lymphatics can be conveniently divided into initial lymphatics without smooth muscle media which feed into collecting lymphatics with smooth muscle media and spontaneous contractions. We recently presented evidence that initial lymphatics have two valve systems, a *primary valve* system at the level of the initial lymphatic endothelium, and a *secondary valve* system that is positioned in the lumen of the lymphatics [1]. Microspheres deposited in the immediate proximity of an initial lymphatic channel readily enter into the initial lymphatics, but once inside the lymphatic channel are not able to return back into the interstitial space without rupture of the lymphatic wall. Fluid inside the lymphatic channel requires the secondary valve system for unidirectional transport towards the lymph nodes and the thoracic ducts. The structure and mechanics of the secondary valves is well-recognized [2]. But no analysis of the mechanics of the primary valve system is available.

We present a model for fluid flow through a primary valve at the junction between two overlapping lymphatic endothelial cells. The fluid is assumed to be Newtonian with constant viscosity and inertia forces are neglected. We assume one cell extension is attached to the adjacent interstitial fibers while the other is in part unattached and free to bend into the lymphatic lumen when the lymphatic pressures is lower than the adjacent interstitial fluid pressure. In contrast, when the lymphatic pressure becomes higher than the adjacent interstitial fluid pressure, the cell extensions are pushed against each other and the cell junction is closed. Based on scanning electron microscopy evidence for the magnitude of the cell overlap and previous measurements of the mechanical properties of endothelial cells, we present a mechanical model of the primary valves.

THEORY AND METHODS

Endothelial cells in initial lymphatics are highly attenuated and form a thin lining. The lymphatic endothelial cells lack the inter-endothelial junctions seen in vascular endothelium and they have a discontinuous endothelial basement membrane with incomplete junction protein components. The cells are attached to the surrounding extracellular matrix via a set of anchoring filaments so that the two neighboring endothelial cells are firmly attached via the basement membrane to the interstitial tissue matrix. One cell, however, is assumed to have a cytoplasmic extension without anchoring filaments or interendothelial adhesion molecules so that it is free to bend into the lymphatic lumen.

In line with direct measurements on endothelial cells [3] the mechanical properties of lymphatic endothelial cells are assumed to deform elastically during the relative short term periods of lymphatic pumping (about 1 Hz) [4]. The endothelial cells in normal lymphatics are assumed to be impermeable to fluid flow during lymph pumping so that fluid passage across the endothelial sheet is only possible via a fluid gap between endothelial cells. This assumption is consistent with the observation that lymphatic fluid tracers do not leave the initial lymphatics once inside, even after application of a negative fluid pressure outside the initial lymphatics adjacent to the endothelial cells [1]. Fluid is free to percolate by convection through the interstitial space between solid fibers on which the endothelial cells are resting.

Interstitial fluid and lymphatic fluid is similar to plasma but with lower levels of plasma proteins. We assume constant mass density and Newtonian properties with a coefficient of viscosity $\mu = 1$ centipoise. The Reynolds number for the fluid flow in the fluid gap between the two cells is of the order of 10^{-4}. Therefore inertial fluid forces can be neglected and the Stokes approximation for the equation of motion governs the flow through the endothelial gap. On the surface of the endothelial cell membranes the no-slip condition is assumed. Possible mechanotransduction mechanisms are ignored. The equations governing the bending of the endothelial cell extensions are solved simultaneously with the fluid flow in the junction as a coupled solid-fluid problem.

RESULTS

Lymphatic pumping requires periodic expansion and compression of the initial lymphatics. During compression, the lymphatic transendothelial pressure drop from the lymphatic lumen into the interstitial space is positive and therefore leads to closure of the cell junction with zero flow rate back from the lymphatic lumen into the interstitial space. In contrast, when the lymphatic pressure during expansion of an initial lymphatic channels falls below the adjacent tissue fluid pressure, the unattached endothelial cell extension is deformed (bend into the lumen of the lymphatic) by the fluid pressures in the junction. Interstitial fluid flows across the junction into the initial lymphatic. Thus, the current model operates effectively as a valve for fluid flow *into* the initial lymphatics but *no return* of fluid into the tissue.

To permit opening of a fluid gap between the cells by the fluid pressure, it is necessary in this model that a segment of cell extension is not attached to the connective tissue. The flow rate through the junction is a non-linear function of the pressure drop from the interstitial space to the lymphatic lumen. The flow rate through a lymphatic endothelial junction is sensitive with respect to the exact location of the attachment of the endothelial cells, and thus the location of the cell extensions that are not attached and free to deform under the action of fluid pressure.

DISCUSSION AND CONCLUSION

The current analysis indicates that the overlap between neighboring lymphatic endothelial cells in initial lymphatics may serve as the primary valve system required for unidirectional transport from the tissue into the lymphatics and lymph nodes.

We estimated typical dimensions of the cell junctions by means of scanning electron microscopic images. For this purpose, muscle specimens were fixed during active lymph pumping so that primary valves can be encountered in an open state [5]. These measurements indicate a length of the cell junction reaching into the lumen of the order of 1 μm.

The unidirectional transport from the interstitium into the initial and collecting lymphatics requires two valve systems. The combined action of the primary and secondary valves may be illustrated as follows (Figure 1). During the expansion phase of the initial lymphatics, the primary lymphatic valves are open since the intra-lymphatic pressure is lower than the interstitial fluid pressure, while the secondary valves are closed preventing backflow inside the contractile lymphatics towards the initial lymphatics. In contrast, during a *compression* phase, the primary valves are closed since the lymphatic pressure exceeds the adjacent tissue fluid pressure. Consequently the lymphatic fluid already inside the initial lymphatics is pushed into the contractile lymphatic through open secondary valves. This extraordinary simple mechanism works for any fluid composition in the lymphatics.

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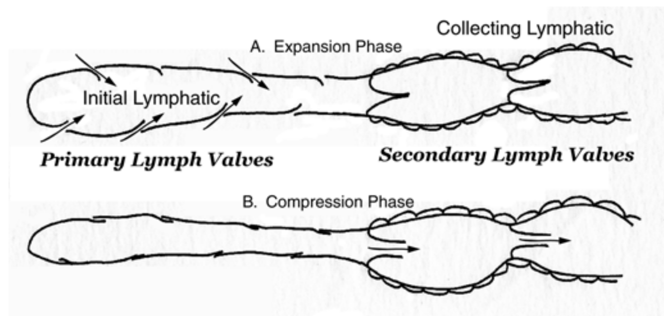

Figure 1. Schematic diagram of primary and secondary valve opening during an (A) expansion and (B) compression phase of an initial lymphatic channel. (A) The primary valves in the initial lymphatic are opened during the filling phase (expansion), facilitating interstitial fluid to enter the initial lymphatic. But the secondary (intralymphatic) valves are closed to prevent reflux inside the lymphatic. (B) During the compression phase the primary valves are closed to prevent reflux into the interstitial space while the secondary valves are open to facilitate lymph discharge towards the collecting lymphatics and lymph nodes.