

## **Perspective: Endothelial tubes – another window into lymphatic function**

**Kim A. Dora and Dirk F. van Helden**

Email: [kim.dora@pharm.ox.ac.uk](mailto:kim.dora@pharm.ox.ac.uk); [dirk.vanhelden@newcastle.edu.au](mailto:dirk.vanhelden@newcastle.edu.au)

The endothelium isolated as an intact tube of cells offers a powerful approach to study endothelial cell function. The freshly isolated tubes provide significant advantages over isolated endothelial cells or cultured endothelial cells, as the cells remain coupled to each other and as they are native endothelial cells, do not display an altered phenotype. Although in a collapsed state, the constituent endothelial cells retain their spatial arrangement, and remain coupled to each other both physically and electrically. Perhaps most importantly, they are now separated from the surrounding smooth muscle layer. In blood vessels where myo-endothelial gap junctions are present, the flow of current from the smooth muscle otherwise dominates them electrically.

All these considerations mean that endothelial cells represent a homocellular population amenable to study with sharp intracellular microelectrodes, and after loading with fluorescent  $\text{Ca}^{2+}$  indicators, enable studies of both intra- and intercellular signalling. Furthermore, by pooling sufficient numbers of tubes, a pure population of native microvascular endothelial cells can be prepared for qRT-PCR to detect gene expression - in cells isolated in the same manner for studying their signalling properties, and in some examples to date for function in intact arteries and arterioles. All in all, the endothelial tube preparation provides a means to address some key unanswered questions in vascular biology.

This novel approach has now been used for the first time to study isolated endothelial cell tubes from lymphatic vessels. It is known that lymphatic smooth muscle cells have a resting membrane potential near -50mV when unstretched, and with stretch (eg. in response to luminal pressure), the cells depolarize, which thereby increases the frequency of action-potential like spikes (von der Weid *et al.*, 2014). Smooth muscle contraction is sensitive to blockers of L-type voltage-dependent  $\text{Ca}^{2+}$  channels, in a manner not dissimilar to that in resistance arteries and arterioles. It is also known that lymphatic endothelial cells release nitric oxide (NO) to limit contraction of the lymphatic vessels, alongside other roles regarding their permeability (Ohhashi & Takahashi, 1991; von der Weid *et al.*, 1996; Scallan & Davis, 2013).

In common with arteries and arterioles, lymphatic vessels are lined by a monolayer of endothelial cells, but they are also intermittently populated with valves, which might be expected to interrupt the endothelial cell monolayer. The segments between valves, termed lymphangions, often behave as autocratic units, exhibiting synchronized contraction and propulsion within, but not necessarily between lymphangions. This is very different from arterioles where myo-endothelial gap junctions between the endothelium and surrounding smooth muscle cells serve to synchronize vasomotor responses, resulting in a functional syncytium along their entire length.

An immediate question therefore is: What happens when an endothelial cell tube is isolated from lymphatic vessels, often across a valve? The answer is provided by a paper published in the latest issue of J Physiol (Behringer et al). The authors have for the first-time isolated intact endothelial cell tubes from lymphatic vessels to study their electrical properties,  $\text{Ca}^{2+}$  signalling, and gene expression for key membrane ion channels. They present evidence that the lymphatic endothelium of mouse popliteal lymphatics is markedly different from the endothelium of popliteal arteries, in that they lack of expression of  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  channels ( $\text{K}_{\text{Ca}}$ ). These  $\text{K}_{\text{Ca}}$  underlie the endothelium-dependent hyperpolarization and associated dilation to many physiological stimuli in resistance arteries and arterioles. Behringer et al include electrical recordings from the endothelium of intact vessels, supporting findings from guinea pig mesenteric lymphatics – that there is no functional electrical connectivity between the endothelium and smooth muscle and that the agonist acetylcholine increases endothelial  $\text{Ca}^{2+}$  with resultant dominant depolarization (von der Weid & Van Helden, 1997). Yet as in arterioles, stimulation with acetylcholine, acting selectively at endothelial cell muscarinic receptors, leads to increases in nitric oxide (NO) production. The NO serves to reduce contraction of lymphatic vessels (Ohhashi & Takahashi, 1991; von der Weid *et al.*, 1996; Scallan & Davis, 2013). Behringer et al present evidence that agonist-activated TRP channels (primarily TRPV4) conduct  $\text{Ca}^{2+}$  and  $\text{Na}^+$ . While  $\text{Ca}^{2+}$  entry is fundamental to NO production (see also von der Weid *et al.*, 1996), the physiological relevance of depolarization remains to be ascertained.

Another key observation in the study of Behringer et al is the hyperpolarized resting membrane potential of the lymphatic endothelial cells, a finding also consistent with the lymphatic endothelium *in situ* (von der Weid & Van Helden, 1997). This again is very different to arterial endothelial cell tubes, and isolated arterial endothelial cells in general. By way of explanation the current work models lymphatic endothelial cells and predicts a lack of a  $\text{Cl}^-$  current, perhaps due to the absence of the  $\text{Na}^+/\text{K}^+/\text{2Cl}^-$  cotransporter. This electroneutral transporter allows  $\text{Cl}^-$  entry into the endothelium, ready to leave when  $\text{Cl}^-$  channels open. Its absence would suggest that the equilibrium potential for  $\text{Cl}^-$  in lymphatic endothelial cells will be more hyperpolarized. The depolarization of lymphatic endothelium following stimulation with acetylcholine or a TRPV4 channel agonist is instead attributed to cations entering the cells. The model proposed by the authors goes some way to explain these processes in lymphatic endothelium, without yet knowing precisely which other ion channels are expressed nor the intracellular concentrations of ions such as  $\text{Cl}^-$ . Nevertheless, this new approach provides another window to our understanding with the unanswered questions providing scope for much future work.

## References

### Behringer et al – this issue

- Ohhashi T & Takahashi N. (1991). Acetylcholine-induced release of endothelium-derived relaxing factor from lymphatic endothelial cells. *Am J Physiol* **260**, H1172-1178.
- Scallan JP & Davis MJ. (2013). Genetic removal of basal nitric oxide enhances contractile activity in isolated murine collecting lymphatic vessels. *J Physiol* **591**, 2139-2156.
- von der Weid PY, Crowe MJ & Van Helden DF. (1996). Endothelium-dependent modulation of pacemaking in lymphatic vessels of the guinea-pig mesentery. *J Physiol* **493 ( Pt 2)**, 563-575.

- von der Weid PY, Lee S, Imtiaz MS, Zawieja DC & Davis MJ. (2014). Electrophysiological properties of rat mesenteric lymphatic vessels and their regulation by stretch. *Lymphat Res Biol* **12**, 66-75.
- von der Weid PY & Van Helden DF. (1997). Functional electrical properties of the endothelium in lymphatic vessels of the guinea-pig mesentery. *J Physiol* **504 ( Pt 2)**, 439-451.