Supplemental Material

Vessel Distribution in E– and E+I-Circulated Constructs

Hematoxylin and eosin sections of E- (a and b) and E+I-circulated constructs have been provided. In E-circulated constructs cut perpendicular to the lumen (a), few vessels are found near the artificial lumen (L). If sections are cut parallel to the artificial lumen, many sprouting, bifurcating fragments are visible (b, arrows). In E+I-circulated constructs, very few sprouting vessel fragments are presented (c). (Supplemental Fig. S1, available online at www.liebertonline.com/ten).

Example Images of Sprout Scoring from Dynamic *In Vitro* Perfusion Chamber Regions of Interests

Example fluorescent images from each regions of interest in E- and E+I-circulated constructs are provided. Within these images, viable fragments are green, nonviable are red, the scale bar is 1 mm. In the figure, 0, 1, and 2 represent example images of the scoring system used to evaluate construct sprouting. (Supplemental Fig. S2, available online at www.liebertonline.com/ten).

Calculations for Variables within Dynamic *In Vitro* Perfusion Chamber

Oxygen concentration

The equation for transient mass diffusion is

$$\frac{\partial \varphi}{\partial t} = D\nabla^2\varphi,$$

where φ is the concentration and D is the diffusion coefficient. This served as a viable constitutive model. The perfusion system employed had a very low flow rate of 1.5 mL/min; therefore, convection and advection were considered negligible.

Henry's law was used to calculate oxygen concentration:

$$k = P/C,$$

where P is the partial pressure of the gas, C is the equilibrated concentration of the gas in the solution, and k is a constant. Polymerized collagen was assumed to have the same solubility properties as water, corresponding to $k = 769.23$ L-atm/mol. The partial pressure, P, for oxygen in air is 0.21 atm. These values result in a calculated oxygen concentration of 8.736×10^{-9} g/mm^3.

Oxygen diffusion coefficient

The Stokes–Einstein equation was used to calculate the diffusion coefficient of oxygen:

$$D = \frac{k_B T}{6\pi\mu R_h},$$

where k_B is Boltzmann's constant (1.38×10^{-23} J/K), T is temperature (310 K), μ is the dynamic viscosity of water (6.531×10^{-4} Pa-s), and R_h is the hydrodynamic radius of oxygen (152×10^{-9} mm). This resulted in a value of $D = 2.286\times10^{-3}$ mm^2/s.[1]

Oxygen consumption of vascular cells

To approximate oxygen consumption of microvessel fragments within the dynamic *in vitro* perfusion (DIP) chamber, we assumed that each fragment is composed approximately 50 cells. Endothelial and smooth muscle cells consume approximately 2×10^{-9} mol O_2 per minute per 10^6 cells.[1] Within 3 mL of the DIP chamber, there are approximately 60,000 rat fat microvessel fragment (RFMF)$\times$50 cells/RFMF = 3,000,000 cells. Approximately 80% of fragments remain viable after the isolation. This corresponds to oxygen consumption of approximately 5×10^{-9} mol O_2/minute. Initially, there is approximately 26×10^{-6} g O_2/DIP chamber, corresponding to approximately 1.6×10^{-6} mol of oxygen. Therefore, oxygen consumption is relatively small compared with the available oxygen within the collagen volume.

Oxygen concentration at collagen–medium interface

Our assumption of a constant oxygen concentration on the surface of the tube that delivered medium to the culture was based on the relevant Peclet number. The relevant processes are the rate at which oxygen diffuses out of the tube into the collagen gel and the rate at which oxygen is replenished at the boundary of the tube and collagen gel due to flow in the tube. The Peclet number, Pe, is the ratio of these two processes, namely, the ratio of rate of advection to the rate of diffusion:

$$\mathrm{Pe} = \frac{LV}{D},$$

where L is a characteristic length, V is a characteristic velocity, and D is the diffusivity. For the present case, we take L = length of tube, V = velocity in the tube, and D = diffusivity of oxygen in the collagen gel. The length of the tube was 14 mm. The average velocity in the tube is

$$V = (1.5\ \mathrm{ml/min}) \times (1\ \mathrm{min}/60\ \mathrm{s}) \times \left[\pi(0.95\ \mathrm{mm})^2\right] = 0.07\ \mathrm{mm/s}$$

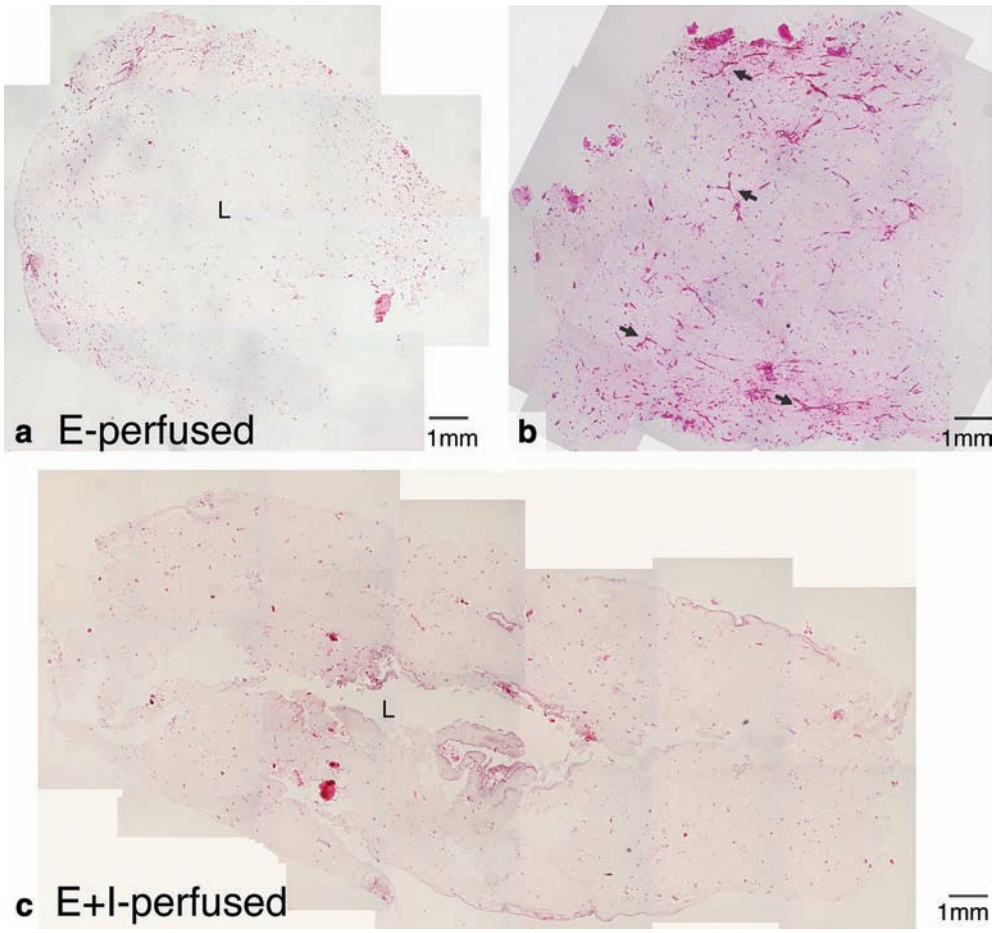
The Peclet number is then

$$\mathrm{Pe} = \frac{(14\ \mathrm{mm})(0.07\ \mathrm{mm/s})}{2.286\times10^{-3}\ \mathrm{mm^2/s}} = 428.0$$

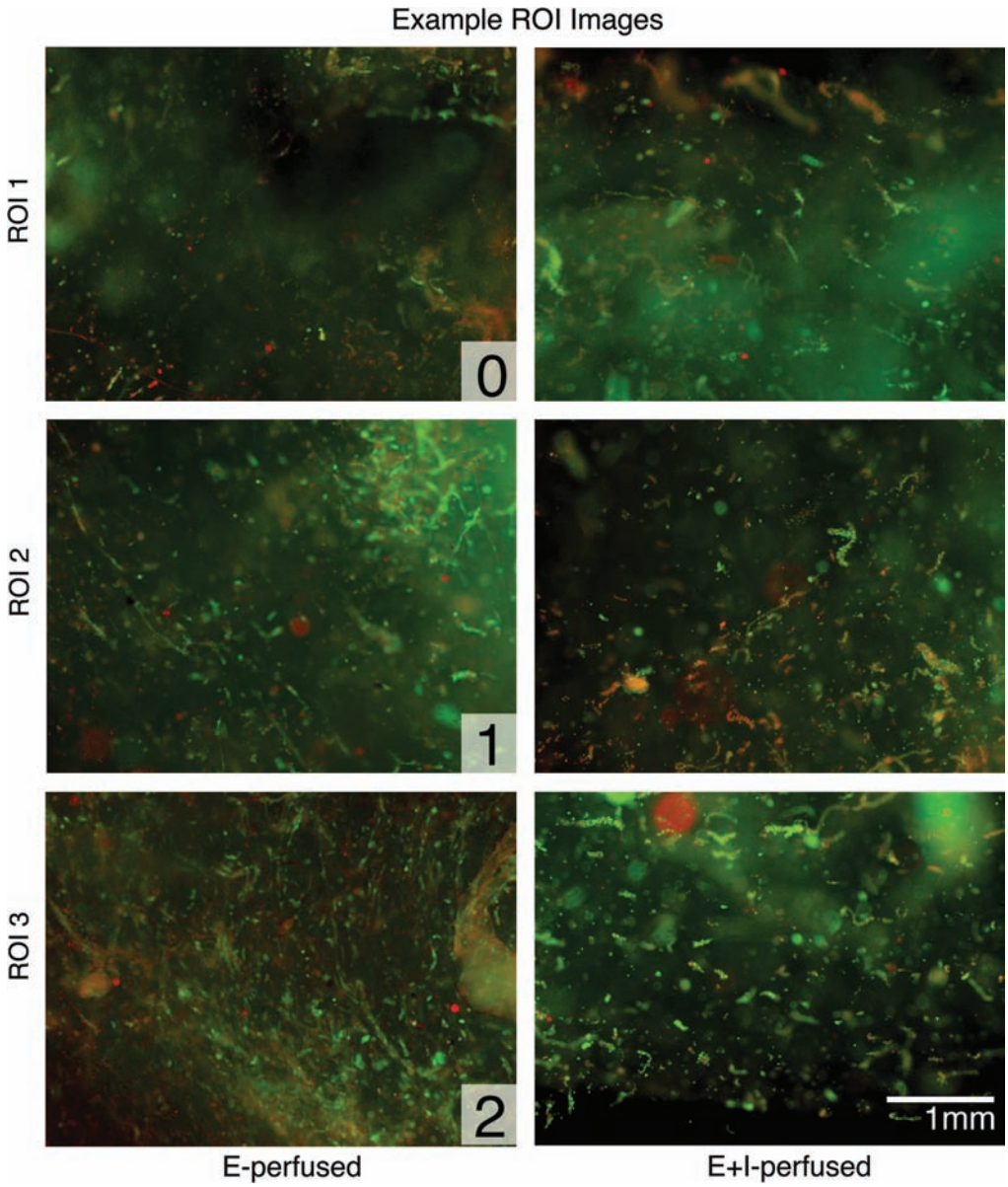
Thus, advection will dominate diffusion at the interface of the tube and the gel, and downstream locations on the surface of the tube will be replenished with oxygen much faster than it can be depleted due to diffusion into the collagen gel.

Reference

1. Ramanujan, S., Pluen, A., McKee, T.D., Brown, E.B., Boucher, Y., and Jain, R.K. Diffusion and convection in collagen gels: implications for transport in the tumor interstitium. Biophys J **83,** 1650, 2002.



SUPPLEMENTAL FIG. S1. Example hematoxylin and eosin stained sections from E-perfused (**a, b**) and E+I perfused (**c**) DIP chamber conditioned samples. Arrows in **b** represent endothelial sprouts that have bifurcated.



SUPPLEMENTAL FIG. S2. Fluorescent images of E-perfused and E+I perfused conditioned constructs. These representative samples provide examples of the scoring system used to evaluate endothelial cell sprouting. 0 = no sprouting, 1 = small, scattered sprouts, 2 = extensive sprouting. In these images, green = viable fragments, red = nonviable fragments.