Cavitation enhanced permeability in a bio-inspired micro device

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Methods combining focused ultrasounds and microbubbles offer the unique capability of non-invasively, locally e transiently open the endothelial barrier. In general, cavitation is already exploited to locally and transiently open biological barriers in *In vivo* animal models with the advantage to allow studies of therapeutics effects in natural environments [1][2]. Nevertheless, these studies are expensive, time-consuming and difficult to perform. In this context, the need to develop a physiological *in-vitro* model takes place as a necessary and effective clinical trials.

Here, we present a bio-inspired micro-device, specially designed, that enables to investigate the effects of ultrasound-driven microbubbles cavitation. The expected effect is a temporary increase of the barrier permeability due to the increase of inter cellular spaces of the endothelium, allowing drugs to extravasate into tissues of interest.

The bio-inspired micro device consists of a PDMS microfluidic network, see Fig. 1a, with a central circular tissue compartment (1575 µm width, 100 µm height) enclosed by two independent vascular channels (200µm width, 100µm height), mimicking the three-dimensional morphology, size and flow characteristics of micro-vessels *in vivo* [3]. An interface with a series of 3 µm pores, spaced every 50 µm, separates the vascular channels from the tissue chamber. The unique features of the device are the three-dimensional geometry of the vascular channels with realistic size, the correct perfusion rate, the correct physiological shear stress intensity and the ability to reproduce biochemical interactions between different kind of tissues. Moreover, the optically clear microfluidic chip allows for visualization and real time measurements of the dynamic interactions occurring in vascular channels and tissue compartment [3].

It has been developed a reliable and reproducible experimental procedure to culture endothelial cells (HUVECs) within the artificial vessels in physiological conditions (fig.1b). A continuum flow of cellular growth medium, kept at typical blood flow rate and body temperature, is injected to force the cell to take the typical elongated shape in the stream-wise direction, see Fig.1c.

The endothelial membrane permeability is evaluated through a dedicated experimental procedure. Measurements of fluorescent dye diffusion through the pores membrane will be carried out with a 2-hours of time-lapse acquisition, under a confocal microscope operated in epi-fluorescence mode. An image analysis on the intensity change due to fluorescent accumulation in the tissue compartment is performed to obtain to quantify of permeability, see Fig.2a,b.

The same experimental procedure will be adopted to quantify the effect of ultrasound-induced cavitation on permeability. The acoustic setup will consist of an ultrasound transducer driven by a function generator through a power amplifier. Commercial microbubbles (SonoVue®), typically used as contrast agent, will be used. The change of permeability will be obtained in different steps of the experiment, i.e. the free-cell device, the HUVECs cultured device and HUVECs cultured device with microbubbles cavitation.

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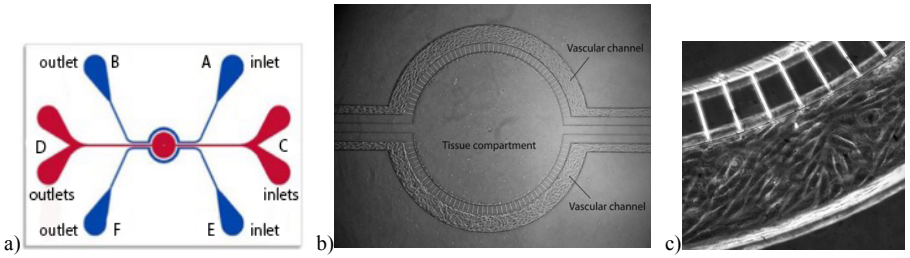

Figure 1 – (a) Sketch of the SynVivo blood-vessel-on-a-chip (b) Bright-field image of the bio-inspired chip cultured with HUVECs. (c) Reconstructed bright-field image: section of the vascular channel with HUVECs. The porous interface is also visible.

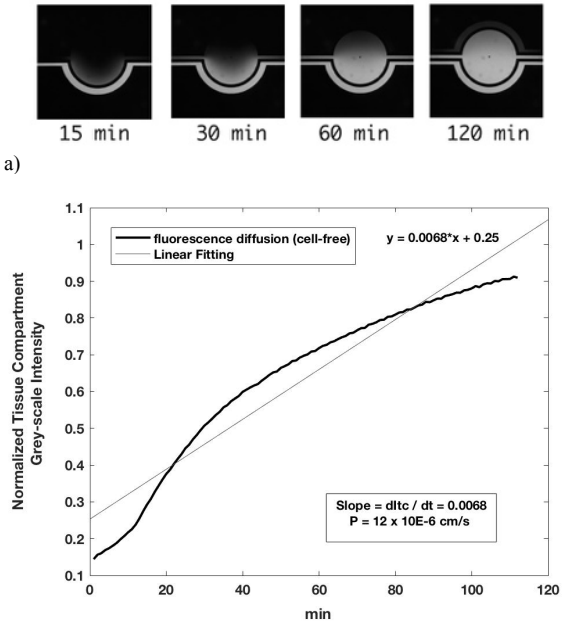

Figure 2 -(a) The time-lapse setting is 1 photo per minute, for a total of 120 photos captured at an exposure time of 90 ms. (b) Quantification of permeability using MATLAB.The slope of the line dI_t/dt is used to calculate the permeability (P).