A blood-vessel-on-a-chip for cavitation enhanced endothelial permeability

Giulia Silvani[a,b], Chiara Scognamiglio[b], Davide Caprini[b], Giulia Grisanti[a], Luca Marino[a], Giorgia Sinibaldi[a], Giovanna Peruzzi[b], Carlo Massimo Casciola[a,b]

[a] Dep. of Mechanical and Aerospace Eng., Sapienza University, via Eudossiana 18, 00184, Roma, Italy
[b] CNLS@Sapienza of Istituto Italiano Tecnologia, via Regina Elena 291, 00161, Roma,. Italy

e-mail: carlomassimo.casciola@uniroma1.it

Keywords: microfluidics, endothelium, cavitation, barrier permeability

In drug delivery, therapeutical agents are most often injected systemically in the circulation raising concern for uptake efficiency at target tissues. Indeed, large molecule permeation through the endothelial barrier lining the blood vessels is a necessary and difficult step [1]. It is known that ultrasound irradiated microbubbles (MBs) succeed in increasing endothelium permeability (see the upper part of the sketch in Figure 1). Here we report on our recent multidisciplinary efforts to fabricate a proof-of-concept microfluidic chip (ERC grant 779751) endowed with a living endothelial layer (blood-vessel-on-a-chip) to study cavitation enhanced endothelial permeability. The mechanics through which irradiated bubbles lead to the endothelium permeabilization is illustrated in Figure 2 showing the two typical cavitation regimes, namely stable and inertial cavitation. In our artificial blood vessel, the endothelium is grown under physiological shear stress conditions and let maturate under flow in order to form well developed junctions between neighboring cells [2]. A biological barrier that prevents diffusion of large solutes from the vessel to the central tissue compartment is then formed, left part of Figure 3 showing a brightfield composite image of the vascular channel with the confluent endothelium (HVEC, human vein endothelial cells). To permeabilize the barrier, stabilized microbubbles (SonoVue contrast agent) are injected in the vessel and irradiated with ultrasound (US), Figure 1. The state of the endothelial junctions at different stages of the experiment (i.e. maturation, ultrasound excitation, MB irradiation and eventual recovery) was assessed by fluorescence imaging following VE-Cadherin protein, a crucial component of inter endothelial junctions. In addition, actin filaments rearrangement was also monitored. Combined with MBs, the irradiation protocol results in the transient formation of gaps between cells that temporarily increases the permeability of the endothelium. Crucial for clinical applications, after irradiation a compact endothelium reforms, recovering the barrier biological functionality. The right part of Figure 3 indeed shows histograms of the number of gap openings just after and 40 minutes after the end of the ultrasound irradiation (units in pixel, 1 px = 0.225 x 0.225 μm^2). In the talk fabrication, bio-functionalisation issues and the related microfluidics will be addressed, discussing the mechanics of cavitation bubble-cell junction interaction and putting the present blood-blood-vessel-on-a-chip in the context of the so-called organs-on-a-chip expected to pave new avenues for high precision medicine.

Acknowledgement: This work was supported by the European Research Council under the ERC-2017-PoC Grant Agreement no. 779751

[1] Perspectives on Cavitation Enhanced Endothelial Layer Permeability, G. Peruzzi, G. Sinibaldi, G. Silvani, G. Ruocco, C.M. Casciola, , Colloids and Interfaces B: biointerf. 2018.
[2] A novel dynamic neonatal brain-blood barrier on a chip, S.P. Deosarkar, B: Prabhakarpandian, B. Wang, J.B. Sheffield, B. Krynska, M.F. Kiani PloS One, 10(11), 2015.
[3] Reversible USMB-induced junctional opening in an artificial endothelial layer, G. Silvani, C. Scognamiglio, D. Caprini, L. Marino, M. Chinappi, M.F. Kiani, G. Sinibaldi, G. Peruzzi, C.M. Casciola, submitted 2019.

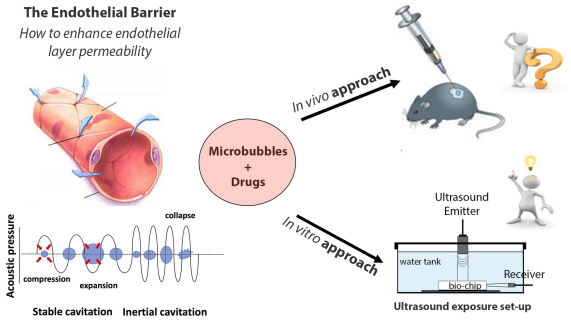

Figure 1. In vivo vs in vitro study of cavitation enchanted endothelial permeability.

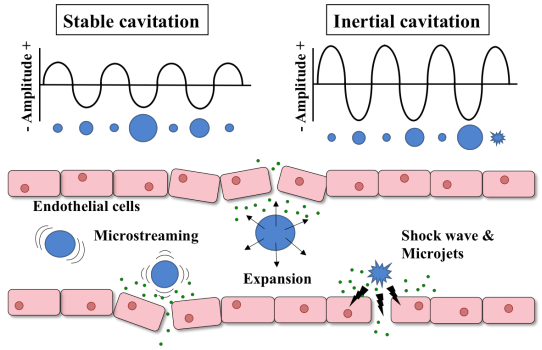

Figure 2. Sketch of the mechanisms through which stable and inertial cavitation affects cell junctions.

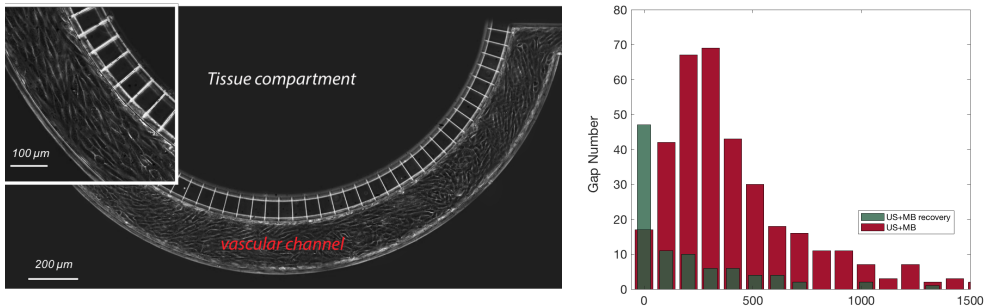

Figure 3. Left, artificial vascular channel with endothelial layer. Right, gap opening under USMB irradiation and recovery 40 min after irradiation (area in px units).