Vessel-on-a-chip: advanced *in vitro* model for endothelial function analysis

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INTRODUCTION

Cardiovascular diseases, in particular, those **developed by atherosclerosis** are **leading causes of death**. Atherosclerosis is a chronic **inflammatory disease** of the **arterial vessels** characterized by the formation of intimal **lesions in the vasculature** and it lies behind of the **main vascular pathologies** as myocardial **infarction and stroke.** New models are needed to reproduce the atherosclerosis features:

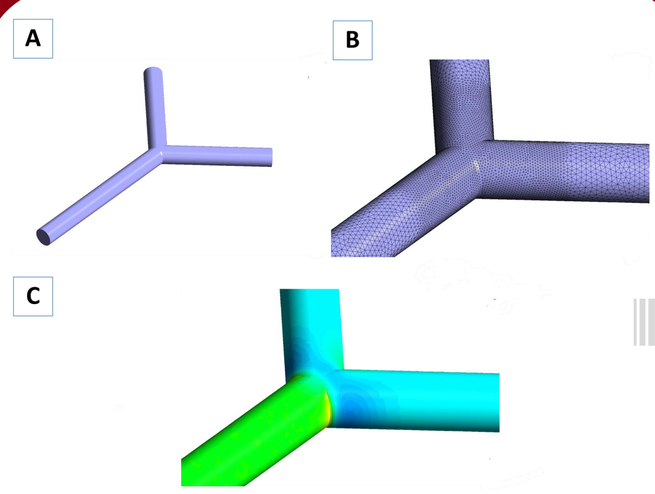
- **Atherosclerosis** is a complex multifactorial **process beginning** by **endothelial dysfunction**.
- **Atherosclerotic plaques** form in **discrete locations** (near arterial bifurcations, branch ostia and curvatures).
- **Disturbed** blood **flow** (low shear stress) near these specific locations **is associated with altered endothelial gene expression, cytoskeletal arrangement, leukocyte adhesion, oxidative stress and inflammation.**

Vessel-on-a-chip are **advanced *in vitro* models** to **study** endothelial dysfunction **mechano-physiological mechanisms.**

METHODS

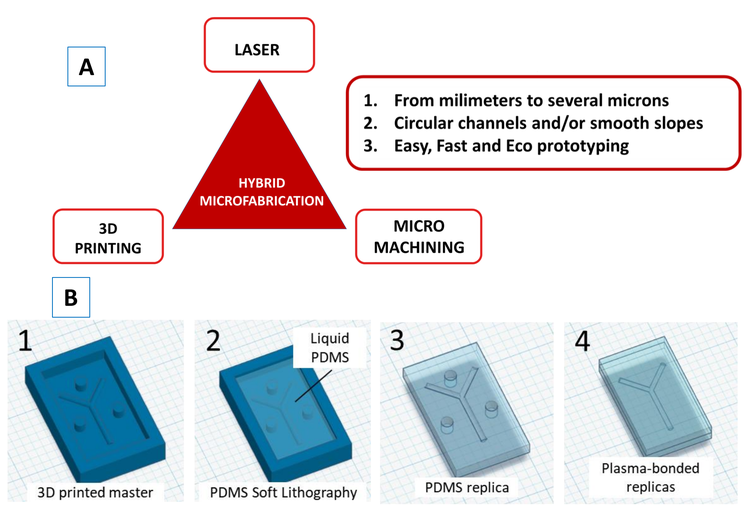
Vessel-on-a-chip manufacturing steps: 1) numerical simulation are used to select a proper geometry, 2) hybrid technology is used to manufacture the PDMS chips and 3) endothelial cells can be cultured to mimic 3D cell culture structure and the associated physical factors on the endothelium.

1) Numeric Simulation



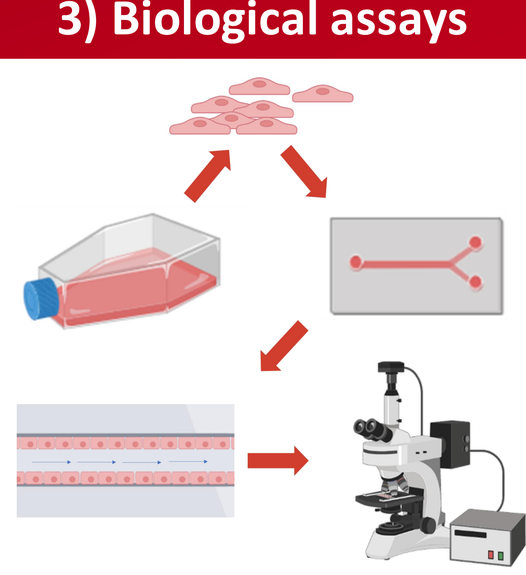
A) 3D CAD representation of a bifurcated blood vessel. B) 3D triangular superficial mesh used for numerical simulation. C) Example of the simulation of the wall shear stress with a rainbow scale.

2) Manufacturing



A) Manufacturing protocols combining laser ablation, 3D stereolithographic printing and micromachining, B) Soft Lithography of PDMS from a micromanufactured master to obtain Vessel-on-a-chip.

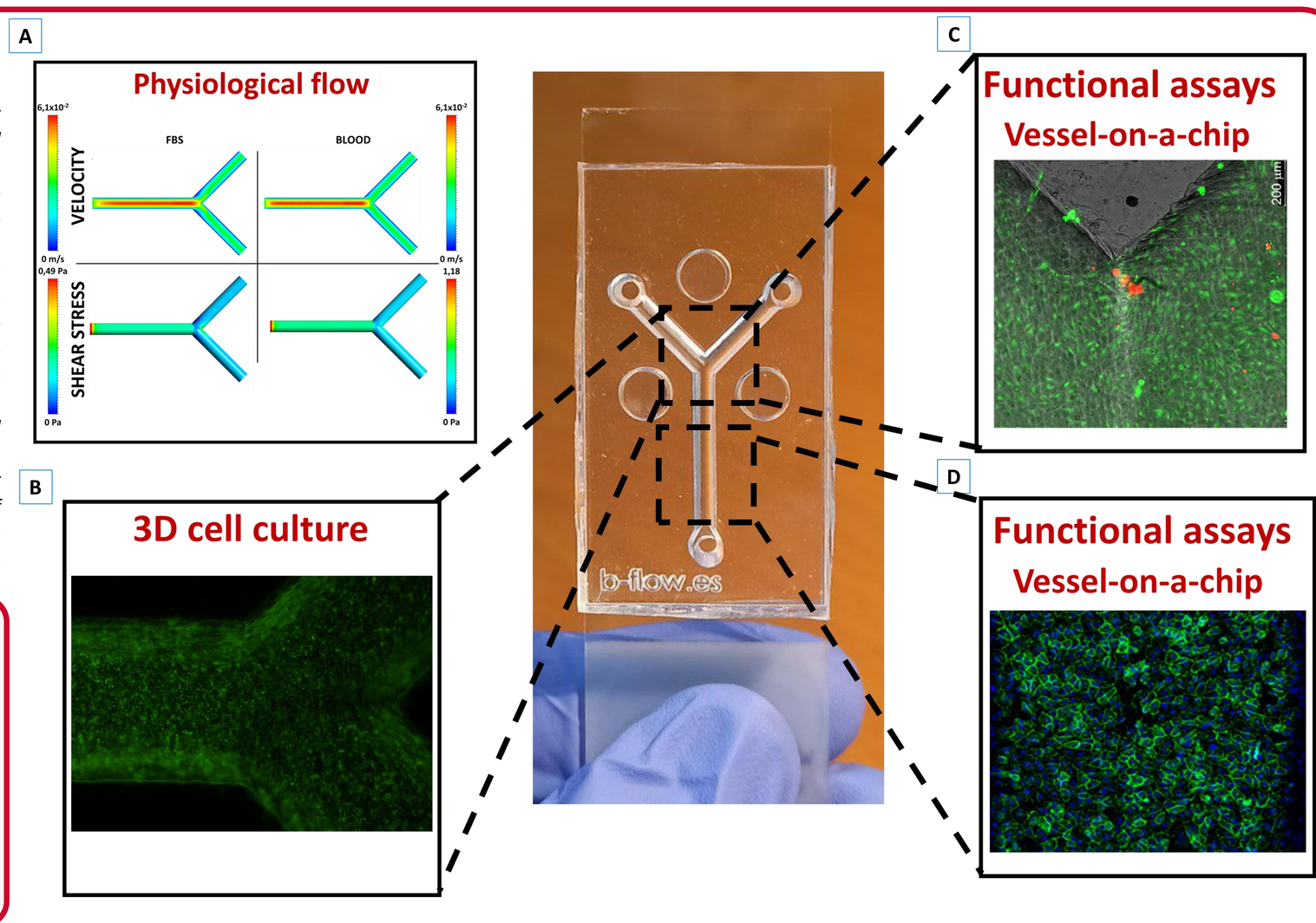
3) Biological assays



Human umbilical vein endothelial cells (HUVEC) are trypsinized and cultured on the channels. These cells are cultured under controlled flow conditions and analyzed by microscopic techniques

RESULTS

A) Numerical simulations allow to reproduce the physiological flow conditions into the microfluidic channels. In this case, the flow inside of a 2mm circular channel with a 90º bifurcation is simulated, indicating the velocity and the wall shear stress. The simulation include two different fluids, cells culture medium with 10% of Fetal Bovine Serum (FBS) and human blood. Local effects are produced by the geometry, allowing to obtain two interesting regions in the Vessel-on-a-chip: Low and high shear stress. B) HUVEC 3D cell culture is stablished into the chip under controlled flow conditions, allowing to reproduce the physiological conditions better than the widely used 2D cell cultures with static stimulations. This is especially relevant for endothelial cells, since these are affected by the geometry and flow conditions. C) HUVEC growing in these conditions allow to perform functional assays, such as the interaction of the endothelial cells with circulating cells. In this example, circulating-tumour cells (in red) interact with the carina of the bifurcations, because of the local physical conditions of the flow and the characteristics of the endothelial layer (in green). D) In addition, HUVEC in physiological conditions can be used to test toxicological condition such as drug-induced vascular injury (DIVI). Viability of the endothelial layer can be assayed with fluorescent viability probes such as Calcein-AM, and permeability by measuring vascular endothelial-cadherin in the intercellular junctions.



CONCLUSIONS

- *Multidisciplinary collaboration allowed to develop a new technology such as Organ-on-a-chip.*
- *The vessel-on-a-chip model showed robustness and reproducibility, physiological biomimicry, appropriate fluidic behavior and good optical characteristics.*
- *Organ-on-a-chip technology aims to optimize the use of animal models and to provide advance knowledge of the human physiology and pathological mechanisms.*

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