THE LIVER SINUSOID WITHIN A MICROFLUIDIC CHAMBER: A NEW TOOL FOR VASCULAR BIOLOGY RESEARCH.

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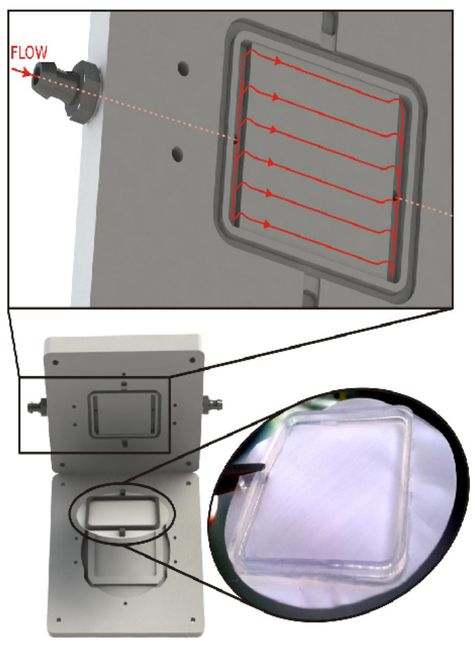
INTRODUCTION / AIMS

- The liver sinusoid plays a key role in the development of liver diseases, including cirrhosis and portal hypertension. However, there are no *in vitro* research tools that correctly mimic the unique features of the sinusoid: adequate spatial distribution of cells, biomechanical stimulation of the endothelial lineage and free paracrine interactions.

- The aims of the present study were to design, fabricate and validate a three-dimensional co-culture chamber with microfluidics that mimics the hepatic sinusoid.

METHODS I – *DESIGN*

A transparent PMMA chamber composed by 3 growing areas of 9.7 cm^2 arranged at different heights and separated by 0.5 mm was manufactured. The higher level integrated a microfluidic system allowing the application of homogeneous shear stress on a reinforced porous membrane where the endothelium is grown. The middle level had a second culture membrane, and on the lower level, the growing area was the base of the camera.



Schematic of the two-plate bioreactor integrating the home-modified membrane, with detail of the upper plate where the flow paths are shown in red (top).

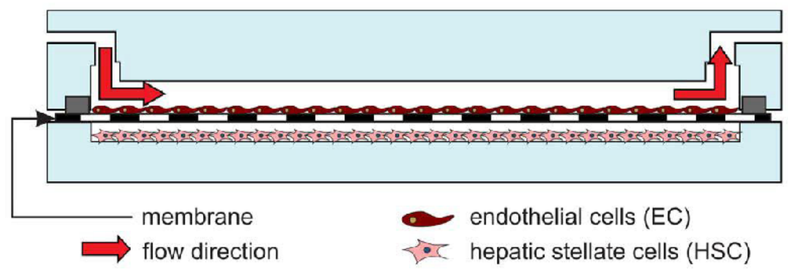
METHODS II

1– *FUNCTIONAL VALIDATION*

Endothelial cells stimulated with shear stress (5dyn/cm^2), HSC, and hepatocytes were cultured for 24h in the chamber. Morphology, viability and endothelial nitric oxide production was analyzed.

2–*TRANSLATIONAL VALIDATION*

The applicability of the chamber in the field of cirrhosis was assessed co-culturing activated HSC and capillarized LSEC under shear stress, or in static conditions. Furthermore, the effects of adding the vasoprotective agent simvastatin were analyzed and compared with traditional culture methods.

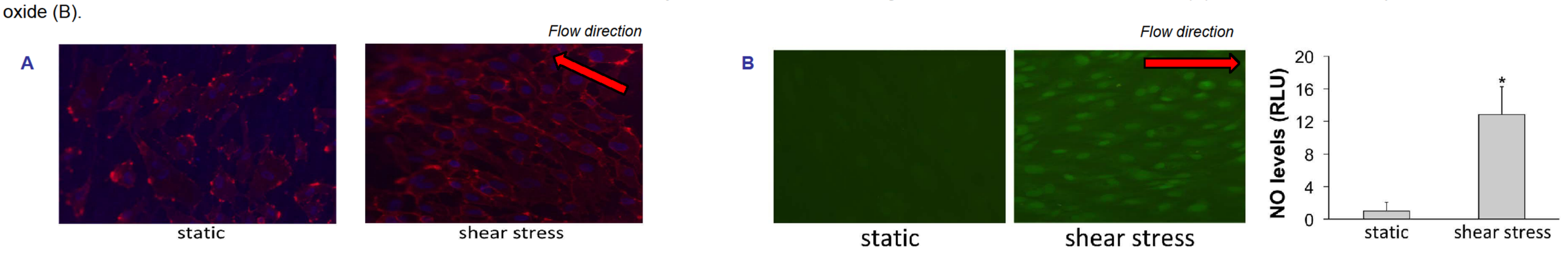


Cross-section schematic of the mounted bioreactor with the endothelial cells culture on top of the home-modified membrane and the HSC culture on top of the lower plate.

RESULTS

1- Functional validation:

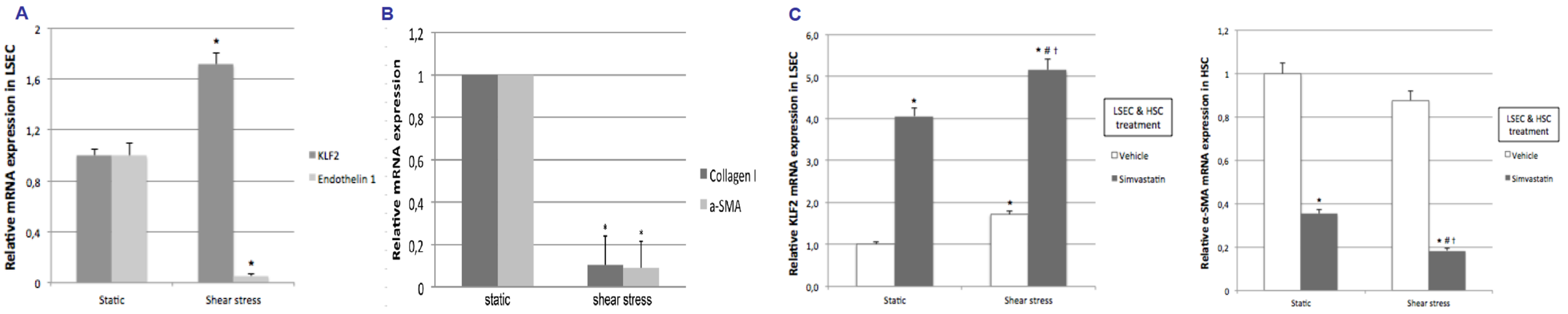
Endothelial cells cultured under shear stress stimulation showed excellent viability. Endothelial cells were aligned in the direction of shear stress (A), and increased their production of nitric oxide (B).



Cells grown in the chamber present an improvement in their phenotype maintining their morphology and markedly increasing the production of NO in comparison to those cells cultured in static conditions.

2- Translational Validation:

Dysfunctional LSEC cultured in the device showed a marked improvement in their phenotype (activation of the KLF2 pathway and decrease in endothelin-1) (A). The shear stress-derived improvement in LSEC led to a beneficial paracrine effect on HSC (reduced collagen I and alpha-SMA) (B). Simvastatin addition produced a strong protective effect on both cell types, which was significantly higher than that obtained using traditional "transwell" methods (C).



*p<0.05 versus vehicle-static.

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*p<0.05 versus vehicle-static. # p<0.05 versus simvastatin-static. †p<0.05 versus vehicle-shear stress.

The bioreactor here presented allows paracrine interactions between co-cultured cells.This is a significant improvement over the previous method of using common cell culture inserts under static conditions since our bioreactor simulates the *in vivo* situation of the hepatic vasculature.

CONCLUSIONS

We herein describe a novel, versatile, easy to operate and highly reproducible device that can be applied in different fields of vascular biomedical research, including hepatology.

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