Fabrication and design of channels with laser to mimic blood vessels

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Abstract

In this work we present a laser based technique to fabricate a preclinical device conformed by channels with different geometries to mimic blood vessels in order to study and simulate vascular and tumoral pathologies in-vitro.
The fabrication method is composed by a two-step technique. First, a master of the channel is fabricated over soda-lime glass using a laser back-writing technique and a nanosecond pulsed laser Nd:YVO_4 [1]. Then, the inverse structure is replicated with silicone and the initial master channel is recovered using a soft-lithography method with polidimethylsiloxane (PDMS) [2]. The device is closed with a PDMS cover using a technique described by Unger et al [3]. By using laser to design the master, customized structures can be fabricated and also, the roughness of the channel can be modified by applying a post-thermal treatment to the soda-lime substrate.
Human umbilical vein endothelial cells (HUVEC) are cultured in the channels walls in order to obtain a totally covered channel by cells, achieving the most possible similar device to a human blood vessel. The adherence of the cells to the PDMS is enhanced with a controlled initial roughness of the channel. The cell culture is performed in two steps: first we seed the cells over the bottom of the channel and then on the upper surface; second and once the device is covered with HUVEC, experiments incorporating flow through the channel are performed in order to study cell behaviour on these conditions.

Fabrication method

Master fabrication by laser techniques » Replica procedure by soft lithography methods » Closure and connections

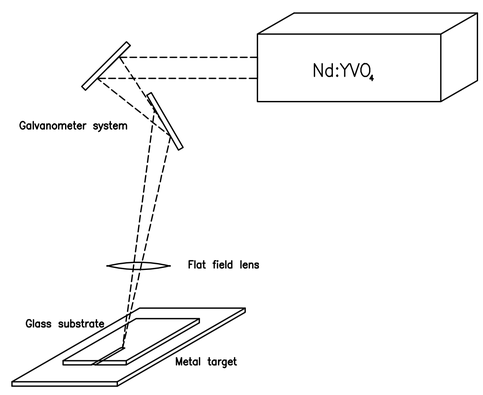

Fig. 1: Experimental setup for the fabrication of the master using laser direct writing techniques

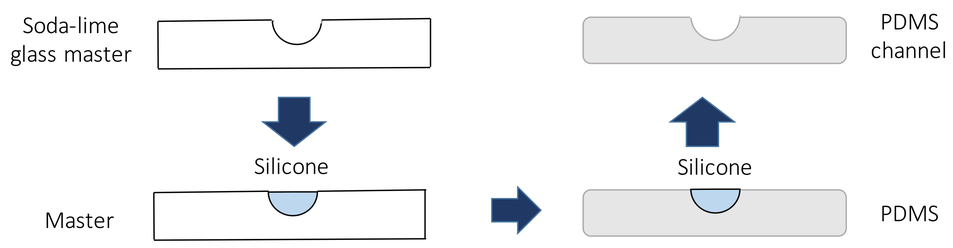

Fig. 2: The master is replicated in PDMS employing soft lithography methods

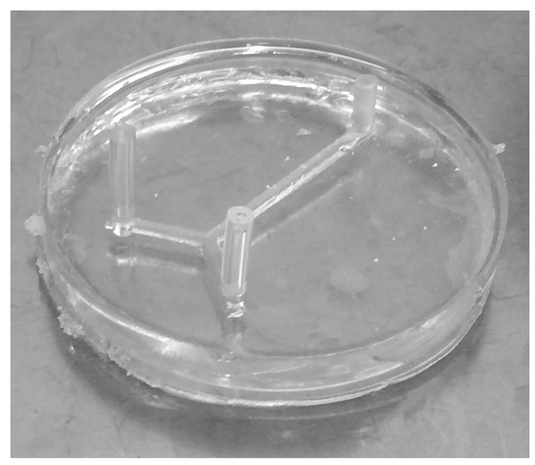

Fig. 3: Picture of the final device fabricated following the Unger et al. protocol

Roughness evaluation and endothelial covering

A post-thermal treatment is applied to the master in order to reduce the roughness of the soda-lime glass and to determine the optimal roughness value for cell attachment. Different thermal treatments are applied at 670^{0}C, 630^{0}C, and 590^{0}C.

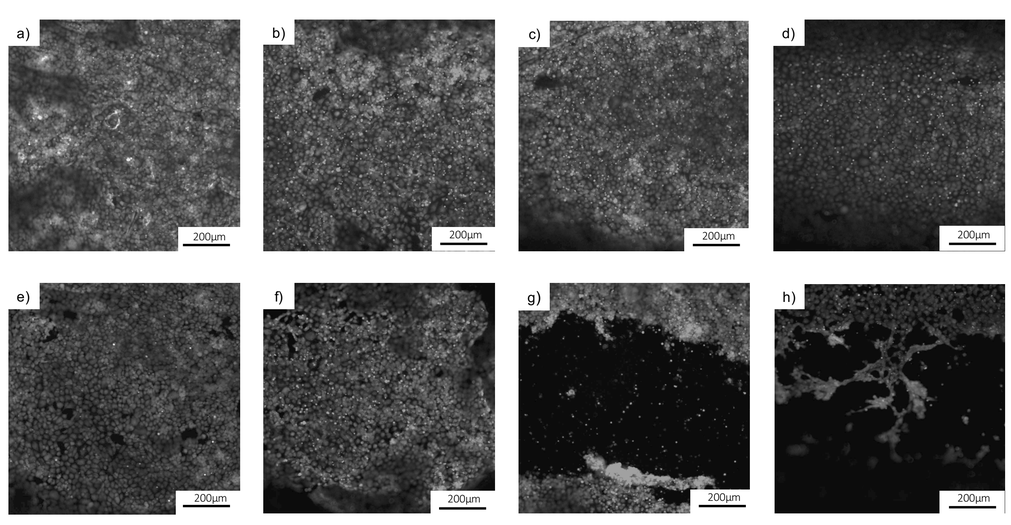

Fig. 4: Fluorescence microscopy images of the endothelial cells over the PDMS device. a) to d): before flux implementation when the master has no thermal treatment and with 590^{0}C, 630^{0}C and 670^{0}C thermal treatment; respectively. e) to h): after the flux implementation, for the same parameters than a) to d)

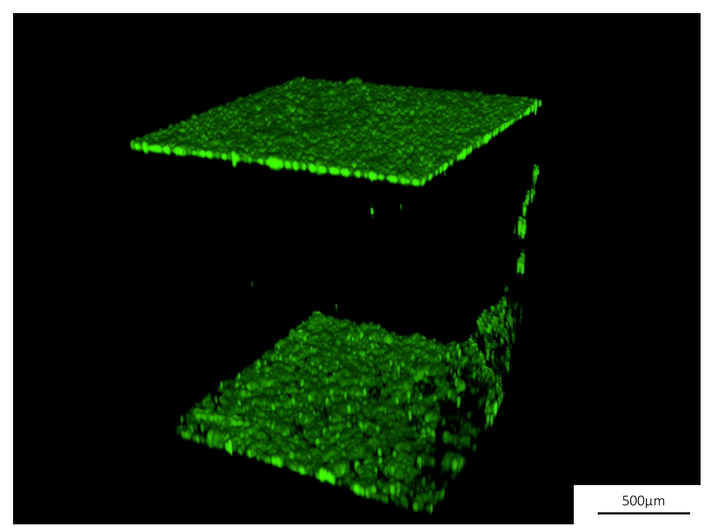

Fig. 5: Confocal image of the endothelial cells attach to both sides of the PDMS device

Conclusions

Preclinical devices that try to mimic blood vessels to perform bioassays were fabricated. The master was manufactured with a laser direct writing technique combined with a thermal treatment to modify the channel roughness and it was replicated in PDMS. The dependence of the endothelial cell attachment on the channel surface roughness was studied. An enhancement of the optical quality of the channel as well as of the cellular adherence was found when a 590 ^{0}C thermal treatment was applied. For this case a channel roughness of 1.53±0.10µm has been measured

References

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Acknowledgements

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