European Cells and Materials Vol. 31. Suppl. 1, 2016 (page P283) ISSN 1473-2262

Double-compartment culture system for conditioning vascular grafts with different trans-wall oxygen levels

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INTRODUCTION: The aim of this work is to develop a new *ex-vivo* model for conditioning small vessel structures with inner-outer oxygen gradients, featuring separated intra-luminal and extra-adventitial compartments, where oxygen levels are differentially controlled.

METHODS: The culture system consists of a double-compartment chamber, a vessel housing and a purpose-developed de-oxygenator module [1], the latter consisting of oxygen-permeable silicone tubing hosted in a reservoir (Fig. 1A-B). Silicone tubing length, and recirculating flow rates were pre-dimensioned through mathematical modelling. The performances of the de-oxygenator was tested single-pass and in a recirculated condition imposing different levels of oxygen. Oxygen concentrations were measured via PreSens Microx 4 meter with PSt7-10 spots (Fig 1A). Experiments with human SV (hSV) segments were performed for a conditioning period of 7 days (Fig.1C). Briefly, hSVs were mounted within the culture system, and arterial oxygen level conditions (21% intraluminal, 5% extra-adventitial) were compared with standard oxygen conditions (21% in both compartments). In all experiments, culture medium was recirculated at 5 ml/min with a luminal pressure of 5 mmHg. At the end of the conditioning period, hSV central portion was processed for the histological analyses.

RESULTS: The results of bench tests demonstrated that controlled trans-wall oxygen gradients were generated, exploiting the oxygen saturation of the gaseous mixture as a control parameter (Fig.1D). Regarding the conditioning experiments with hSVs, the main result is that the arterial oxygen condition displayed a significant increase in the density of adventitial small-and-large caliber *vasa vasorum* (Fig.1E, upper panel), as well as a higher proliferation of cells within and around these vessels (Fig.1E, lower panel).

DISCUSSION & CONCLUSIONS: From the technical point of view, the results proved the device to be a versatile, easy-to-use and functional system. Moreover, it is compatible with the best standards for good laboratory practice and a valuable alternative to bubbling a gas mixture in the culture medium. Regarding the hSV experiments, these results suggest that vein adventitial hypoxia promotes neovascularization and growth of *vasa vasorum*, which is known to predispose the arterialized vein to restenosis, increasing the risk of graft failure.

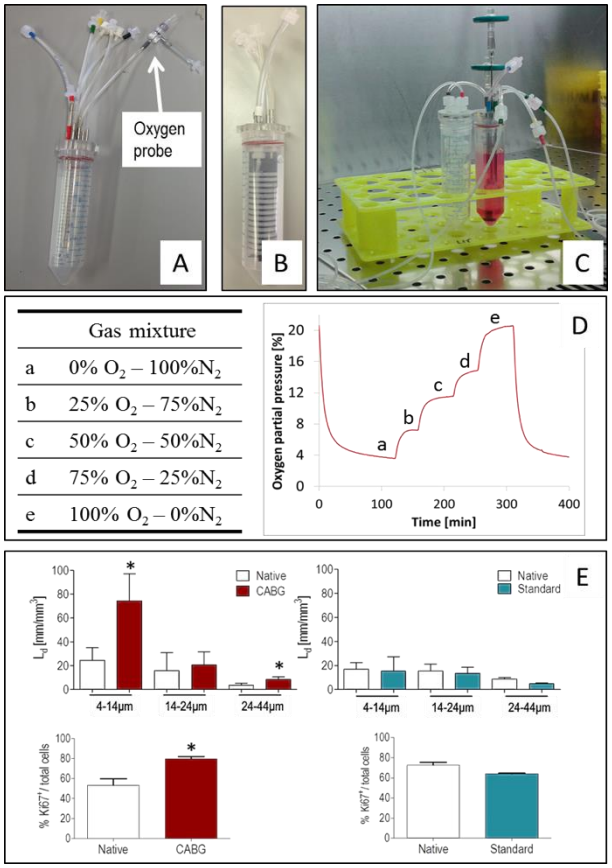

Fig. 1: Pictures of the culture chamber (A), the de-oxygenator module (B) and the assembled system during experiments with hSVs(C). Results of the experiments carried out to verify the system's performances (D), and results of the hSV oxygen conditioning experiments (E).

REFERENCES: [1]Piola et al., (2015) *Ann Biomed Eng* **44**(5):1449-61.

ACKNOWLEDGEMENTS: This work is supported by the Italian Ministry of Health (RF-2011-02346867).