Towards the development of a pathological *ex vivo* blood vessel model

Noemi Vanerio[1,2], Alberto Leopaldi[1], Marco Stijnen[1], Bas de Mol[1,2] and Linda Kock[1]

[1]LifeTec Group BV, Eindhoven, The Netherlands
[2]Dept. of Cardiothoracic Surgery, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

Introduction

In the cardiovascular field, the evaluation of medical devices, like drug-eluting stents and bioresorbable vascular scaffolds is mainly based on animal models or *in vitro* platforms. These have several limitations, such as complexity and lack of biological response respectively. New approaches are involving the assessment of the devices in *ex vivo* blood vessel platforms with the advantage of having both controlled hemodynamic parameters like pressure and flow rate, and the presence of native tissue with a reduction of animal experimentation [1]. One drawback of this approach is that healthy blood vessels are being used to test devices that will actually be implanted in diseased vessels, which could lead to biased results. Therefore, the aim of this study is to develop a pathological *ex vivo* blood vessel model for long-term culture in a controlled way, which can then be used to test therapies and devices.

Methods

Fresh porcine carotid arteries were harvested in a local slaughterhouse, rinsed and prepared. The carotids were incubated inside and outside with a solution of 1% Rose Bengal (RB) for 3 minutes. RB is a photo- and sono-sensitive compound used to induce collagen crosslinking [2] and photochemical tissue bonding [3]. In this study, we hypothesize that RB will induce collagen crosslinking in the vessel wall after activation by sonication of the carotid artery at 3.5 MHz for 2 minutes (Aloka SSD-2000 ultrasound machine). Next, the arteries were mounted in an in-house developed *ex vivo* platform (VABIO – VAscular BIOreactor [4]), longitudinally pre-stretched to 150% and cultured under physiological conditions (T = 38°C, 5% CO_2, 100% humidity), physiological pressure values (120/80 mmHg) and physiological shear stress for 7 to 10 days. During culture, ultrasound imaging was obtained daily to measure the diameter of the carotid and to assess changes in intima-media thickness, shape and vessel wall structure. Pressure and flow rate values were recorded and monitored hourly during the entire experiment. At the end of each culture, samples of the carotid arteries were snap-frozen and histological analyses were performed. Untreated carotid arteries, kept in culture under the same conditions, were used as control.

Results

Pressure and flow rate values were in the physiological range and were stable during the entire culture period for both treated and untreated vessels. Ultrasound imaging of treated vessels showed a clear increase in the inner and outer diameter of the artery and general and local thickening of the vessel wall. Interestingly, the internal structure, the organization of the layers and the intima-media thickness underwent significant modifications, such as bulge formation and increase of the media thickness, that were visible at ultrasound images. Histological analyses show significant changes in structure of intima and media layers.

Conclusions

Results show that the treated vessels underwent pathological changes compared to the untreated vessels, kept under the same physiological conditions. These findings suggest that this method can be used to create an *ex vivo* pathological model that can be used in the *ex vivo* vascular platform to evaluate medical devices in a realistic but controlled environment, overcoming the main drawbacks of *in vitro*, *in vivo* and healthy *ex vivo* models. Future studies will focus on obtaining more insight in the mechanism of pathology induction and on testing of innovative technologies, treatment methods and therapies.

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