A mechanobiology approach aimed at understanding the role of pulsatile pressure on human saphenous vein after coronary artery bypass grafting

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***Abstract*—Saphenous vein graft disease represents an unresolved problem in coronary artery bypass grafting. After surgery, progressive structural modifications in the vein wall occur with gradual occlusion of graft lumen. This is due to a complex process, called intima hyperplasia, involving activation of vein-resident smooth muscle cells. We have recently devised a novel *ex vivo* vein culture system, able to reproduce the wall strain associated to coronary-like pressure. This system was used to expose human saphenous vein segments to pulsatile luminal pressure or venous perfusion conditions, for a period of 7 days. After stimulation, saphenous vein segments were examined by histology and immunofluorescence techniques. Our findings revealed a 'mechano-biology' mechanism involved in the pro-pathologic remodelling of the vein grafts.**

***Keywords*—ex vivo vessel culture system, coronary artery by-pass grafting, saphenous vein remodelling, pulsatile pressure stimulation.**

I. Introduction

CORONARY Artery Bypass Grafting (CABG) using autologous Saphenous Vein (SV) is a standard surgical procedure to recover myocardial perfusion in patients with coronary artery disease [1]. After CABG, a progressive remodeling of the SV wall occurs, possibly leading to the lumen occlusion. This process is termed intima hyperplasia (IH). The beginning of the pathology occurs at much earlier stages after grafting (7 days), with the activation of biomechanical- and inflammatory-driven cascades which prime vessel remodeling [2] [3]. Immediately after grafting, SV segments are exposed to a variety of hemodynamic stimuli activating several cellular pathways and responses in the venous vessel wall. In particular, after transposition into the coronary circulation, the vein is subjected to a high pulsatile pressure, oscillating between 80 and 120 mmHg, and a pulsatile flow (with mean flow rate of 250 ml/min), which results in an elevated shear stress (1 - 7 Pa) [4], [5]. In these conditions, smooth muscle cells respond with apoptosis, modified proliferation, as well as enhanced/reduced migratory activity, losing their typical contractile phenotype and starting to proliferate and to invade the intima layer, thus reducing the vascular lumen.

The use of bioengineering approaches is an option to study Saphenous vein graft disease (VGD). Several organ culture systems [6] were designed to recapitulate arterialization in cultured human veins for studying the physiopathology of VGD consequent to SV exposure to coronary artery-mimicking flow and pressure. Here we investigated the impact of arterial pressure (thus, wall strain and wall stress) on early human SV remodelling within a novel *ex vivo* vessel culture system. Using this system, segments of human SV were exposed to CABG-pressure stimulation or venous perfusion for a period of 7 days.

II. Materials and Methods

A. The Ex Vivo Vessel Culture System

We used an *ex vivo* vessel culture platform (EVCS), developed in our Lab [7] (Fig.1), able to apply the CABG-like pressure (CABG-PS) stimulation or a venous-like perfusion (VP) conditioning to human SVs, and to maintain the vessels viability. Briefly, the CABG-PS consists in:

I. a loading step, in which the culture medium is delivered through the vessel until the intraluminal pressure reaches the lower pressure80 mmHg;
II. a pulsatile stimulation step, during which the vessel is inflated and deflated under 80-120 mmHg cyclic pressure regimen;
III. an unloading step, in which the intraluminal pressure within the vessel is lowered again to zero;
IV. a recirculation step, characterized by a constant flow rate allowing a metabolic supply to the vessel for a predefined recirculation period.

During the pulsatile stimulation, a circumferential wall stress is applied to the SV wall. Thus, the cells covering the lumen and those embedded in the medial layer are subjected to circumferential stress and strain typical of the arterial circulation, but in the absence of the elevated shear stress characterizing the coronary flow. VP conditioning consists, instead, in perfusion with culture medium with a constant low pressure (5 mmHg).

B. Mechanical conditioning of human SV within the EVCS

The use of human SV segments was authorized by the local Ethical Committee with the approval of an informed consent. Human SV samples were harvested using a 'no touch' technique avoiding venous spasm and dilatation and

ensuring endothelial, medial and adventitial integrity. The distal end of each SV segment was cannulated in the operating room, thus allowing the identification of the valve direction, side branches were ligated and the SVs were immediately stored at 4 °C in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 1% L-glutamine and 1% penicillin–streptomycin. All the SV segments were maintained at 4 °C for up to 48 h before use.

Twenty-four segments of human SV were cultured for a period of 7 days under: i) venous perfusion condition (5 mmHg, 3 ml/min; n=12), or CABG-like pressure stimulation condition (80-120 mmHg, f = 0.5 Hz for 10 min; recirculating flow rate equals to 1ml/min for 2 min; n=12). Native SV samples serve as control.

C. Morphological and morphometric assessment of the conditioned human SV segments

Cultured and control SV samples were fixed with 4% formaldehyde overnight, paraffin embedded, and cut in 5-µm-thick sections using a microtome. Sections were stained with hematoxilin and eosin (H&E) and Masson's trichrome staining (Bio-Optica Milano SpA, Italy), according to manufacturer's protocol. To better evaluate the organization and arrangement of cellular components of the cultured SVs, immunofluorescence analysis for smooth muscle cells (SMC) marker αSMA, endothelial cells (EC) markers (CD31 and vWF), were performed. The number of proliferating cells in the tunica media was analyzed by Ki67/Hematoxylin immunohistological staining in native, VP, and CABG-PS samples.

Morphometric analyses were performed on Masson's trichrome stained sections in order to measure wall thickness and luminal perimeter. Thickness measurements were manually processed on digital images taken with AxioVision Bio Software (Carl Zeiss®, Germany) at 10X magnification. At least 40 measurements per sample were made. The inner perimeter was calculated using Image-J software (Version 1.47f-sofware for Java, National Institutes of Health, USA).

To measure the vasa vasorum length density [8], H&E stained sections of native and cultured samples were analyzed. Digital images were acquired using a light microscope at a magnification of 40x. Major and minor axes of each vasa vasorum were measured in a range comprised between 4µm and 44µm (three subsets were identified: 4-14 µm, 14-24 µm, and 24-44 µm). The classification of the vasa vasorum was performed based on the minor axis assumed as representative of the diameter. The adventitial area (*Ad*) was calculated on digital micrographs (2.5x) after manual contour identification using GIMP (Version 2.6.12) and Image-J software (Version 1.47f-sofware for Java, National Institutes of Health, USA).

The number of proliferating cells in the tunica media was analyzed on digital images, acquired using a light microscope at a magnification of 20X. For each samples at least 5 randomly chosen fields were acquired. A manual counting protocol of $Ki67^+$ cells was performed by using Image-J software. The quantitative analysis of positive cells for Ki67 was accomplished by only one observer and in a blinded fashion. The number of proliferating cells was normalized to the total cell number.

III. Results

Results showed a relatively good integrity of the vessel structure with a good preservation of SMCs and the adventitia layers, and without signs of tissue degeneration and swelling (Fig.2). The observation of the vessel structure by immunofluorescence showed that vessels cultured under CABG-PS conditions had relatively thinner wall and larger lumen compared to VP or native vessels (Fig.2). Remodeling of the SMCs layers in the media (Fig.2, white arrows) in CABG-PS groups was evident. As expected, computer-assisted morphometry indicated a significant reduction of the wall thickness (327.3±40.5µm, vs. 575.5±60.8µm, $P<0.05$, t test) and an increase in the lumen perimeter (12.10±1.14mm vs. 8.77±0.49mm, $P<0.05$, t test) in CABG vs. native SVs. Evident ruptures of the ECs layer, and significant increase in cellular proliferation (46.33±2.9 vs. 29.29±2.14, $P<0.001$, t test) were also observed. Finally, exposure of SV segments to CABG-PS induced changes especially in adventitial and vasa vasorum (VV) cells. This correlated with an increase in VV thickness and length density (Fig.3), and their structural disarrangement.

IV. Conclusion

An arterialization campaign was extensively conducted using the EVCS. Our results showed morphological changes in the wall of CABG-PS cultured SVs, demonstrating a remodelling activity, suggesting that the CABG-PS has an important role in the remodeling of the SV wall. Studies are ongoing in our Lab to correlate each of these changes to the establishment of cellular and molecular pro-pathologic pathways involved in the SV graft disease. The discovery of molecular targets regulated by biomechanical stimuli will produce a unique opportunity for devising novel translational protocols to reduce the SV intima hyperplasia.

In this scenario, the EVCS will be a novel laboratory-oriented tool useful for elucidating the phenomena responsible for *in vivo* early arterialization of SV after CABG surgery.

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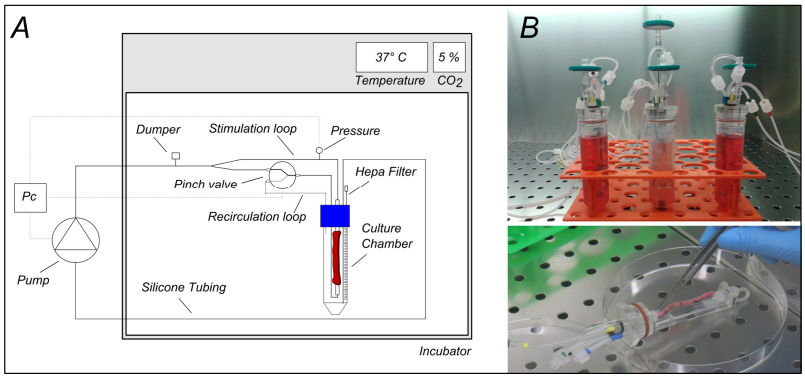

Fig. 1. The compact and automated ex-vivo vessel culture system able to mimick the effects of the pulsatile arterial pressure-related cyclic wall distention. A) Schematic representation of the system's layout, and B) prototype of the EVCS during the assembling phase under laminar flow hood.

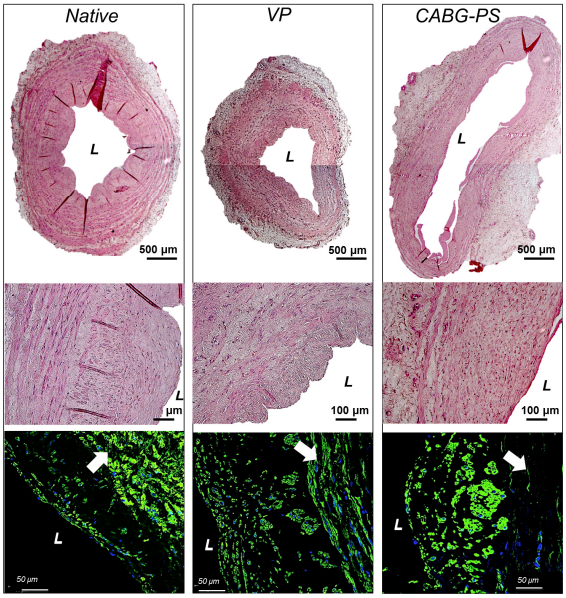

Fig. 2. Representative SV tissue sections stained with H&E (up) and α-SMA/DAPI (bottom) after 7-days of culture. L indicates the lumen.

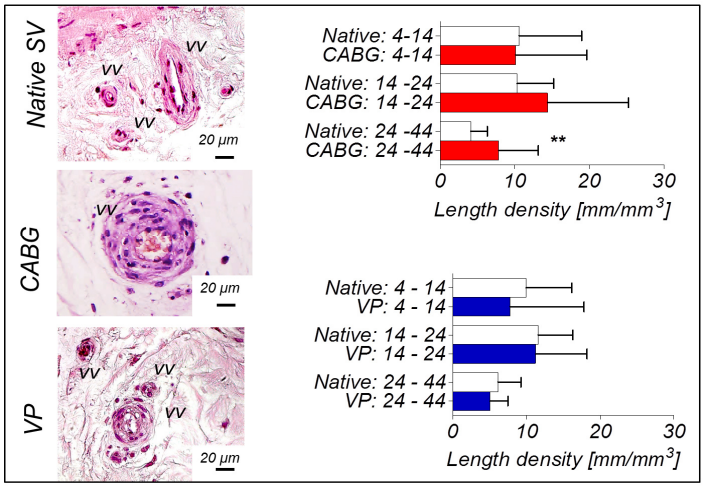

Fig. 3. Representative H&E stain section of vasa vasorum of native, VP, and CABG-PS samples. Vasa vasorum length density analysis were performed on SV sections stained with H&E staining.