Co-culture of ECs and SMCs Promotes Vascular Tissue Remodeling

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Introduction:

Scaffold-based vascular tissue engineering aims to regenerate vascular tissue for the replacement of diseased small-calibre blood vessels (diameter < 6mm). Collagen gel is a commonly used scaffold because of its biological properties with a high potential for supporting and guiding the regeneration process by vascular cells [1]. The approach we privileged consists in first reproducing the media, which provide the high elasticity properties of the vessel wall, thus making it an essential and effective component for blood transportation. In previous works, an original method for processing scaffolds already containing smooth muscle cells (SMCs) was reported [2]. In this work, the overall goal is to seed endothelial cells (ECs) on gels composed by collagen and smooth muscle cells. This is expected to provide in the functionality of engineered tissue approaching its overall mechanical and hemocompatible properties close to those of blood vessel walls.

Materials and Methods:

Collagen extraction: Type I collagen was extracted from rat-tail tendons and solubilized in acetic acid solution (0.02 N) at a concentration of 4 g/L according to a protocol previously describe [3]. The collagen solution (2 g/L) was mixed with DMEM (1.1X), NaOH (15 mM), and Hepes (20 mM) in deionized water. This mixture was then poured in a specific mold and then let jellify for 30 min at room temperature.

Cells isolation: ECs were isolated by trypsin treatment of human umbilical cords vein (HUVEC) and expanded in HyClone Media M199/EBSS (Fisher). SMCs were isolated from human umbilical cord artery (HUASMC). Initially, the Wharton's jelly that surrounds the arteries was carefully removed by cutting with scissors. Afterwards, the arteries were chopped to rectangle pieces using scissors and finally placed in a Petri dish with M199 medium. After two weeks, rectangle pieces of the artery were removed and the cells were expanded.

Cells viability: To examine HUVEC viability and adherence, the cells were incubated on collagen gel containing SMCs for 48h at different concentration. HUVECs incubated on Petri dishes without collagen were used as the normal control for the study. At the end of the 2-day incubation period, collagen gel were rinsed with a saline solution (PBS) in order to remove non adherent and died cells. Viability was measured by adding 18 µL of the 3-[4,5-dimethylthiazol-2-yl]-5-[3-carboxymethoxyphenyl]-2-[4-sulfophenyl]-2*H*-tetrazolium] (MTS) reagent to each of the wells. The optical density was measured at two wavelengths, 490 and 600 nm.

Immunofluorescence staining: Cells were seeded at 1×10^5 cells per well in six-well plates that contained 22-mm glass coverslips and incubated for 24 h at 37 °C. Afterward, the cells were washed twice with PBS and then fixed with 3.7% formaldehyde in PBS for 20 min. The cells were then permeabilized with 0.1% saponin in PBS during 10 min at room temperature. The cells were then additionally incubated with a first antibody: vWF (Abcam), CD31 (Dako), α-actine (Sigma) or calponin (Abcam) and with the secondary antibody: Alexa 546, Alexa 488 (Invitrogen). The cellular distribution of the fluorescent was assessed using an Olympus BX51 microscope. Images were captured with a Q imaging RETIGA EXI digital camera driven by Image pro express software.

Results

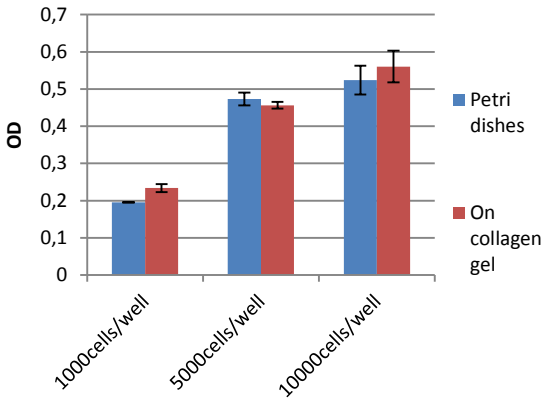

Figure 1: HUVEC viability and adherence using MTS assay. HUVEC incubated on Petri dishes without collagen were used as the normal control (blue). HUVEC incubated on collagen gel are in red.

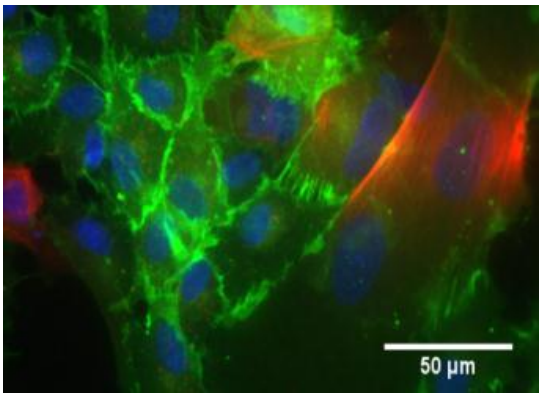

Figure 2: Co-culture of HUVEC and HUASMC. HUVEC are characterised by CD31 in green and HUASMC are characterized by calponin in red. Nuclei are stain in blue (DAPI).

No significant difference was observed between number of cells on collagen surface and cells on tissue culture polystyrene control after 48 hours. They can also growth and proliferate as they are in a normal culture flaks condition regardless the initial concentration of cells (Figure 1). The immunofluorescence staining shows us that endothelial and smooth muscle cells can be cultivated at the same time using a common media (figure 2).

Discussion

Co-culture of SMC and endothelial cells were achieved on collagen scaffold without losing endothelial cells as compared to tissue culture polystyrene control. Furthermore, the non-circular morphology of both cells shown by immunofluorescence staining suggests that cells are happy and are searching to make focal attachments. This ongoing experiment shows that it is possible to do vascular co-culture using collagen gel scaffold. The interaction between cells will enhance the matrix remodeling and the properties of the arterial construct. This presentation will show the selection of the common medium to cultivate two cells type, and the determination of HUVEC seeding parameters on collagen gel seeded with smooth muscle cells. Characterization of cells localization in the collagen matrix will be also shown. The addition of mechanical stimulation should be performed to further understand the mechanisms of vascular remodeling.

Acknowledgement: CL was awarded of a PhD Doctoral Scholarship from NSERC CREATE Program in Regenerative Medicine (www.ncprm.ulaval.ca).

References

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