Establishing a protocol for the isolation of pig vascular endothelial cells

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1. Abstract

Endothelial cells (EC) build the inner layer of all vessels of the human body. They form a selective permeable barrier between blood and tissue and are involved in molecule transport and anti-/coagulation and inflammation processes. The aim of this thesis was to establish a protocol for the isolation of pig vascular endothelial cells for use in research projects aimed at studying the endothelium. For this purpose, different pig vessels and tissues were used to isolate both macro- and microvascular endothelial cells. Once isolated, the cells were expanded, characterized for endothelial cell specific markers like CD31 and von-Willebrand-factor (vWF) by immunofluorescence staining and subsequently frozen for later projects. Unfortunately, isolation of microvascular endothelial cells was not successful. No signal for CD31 or vWF was observed in cell cultures isolated from kidney tissue. This makes further investigation of isolations from this tissue necessary. On the other hand, isolation of endothelial cells from larger vessels was successful and expressed the same markers as endothelial cells in situ. Interestingly, cells isolated from arteries grew faster than those isolated from veins. However, endothelial cells from veins were less contaminated by fibroblasts and more CD31 positive cells expressed vWF. Further studies are needed to explain these observed differences.

2. Introduction

The endothelium, a monolayer of endothelial cells, constitutes the inner cellular lining of blood vessels (arteries, veins and capillaries) and the lymphatic system, and is in direct contact with the blood/lymph and the circulating cells [1]. The blood vessel wall forms a selective barrier for the transport of molecules between blood and tissues. The endothelial lining presents a large surface area for this exchange of materials between blood and tissues. The total area of this interface in human has been estimated to be about 350 m^2 [2].

The intimal surface of healthy endothelium is both anticoagulant and antithrombotic: endothelial cells secrete a variety of molecules important for the regulation of blood coagulation and platelet function. Through numerous anticoagulant pathways, endothelial cells maintain blood fluidity and regulate blood haemostasis. During pathological conditions, vessel damage and proinflammatory cytokines can lead to a procoagulant/prothrombotic phenotype of the endothelial cells [2].

One of the main areas of research is to analyse endothelial cell activation and complement deposition during ischemia/reperfusion injury and in xenotransplantation. For this purpose, isolation of endothelial cells from different anatomical compartments and vessels is of fundamental importance. For all the studies presented in this thesis, pig tissues and vessels were used, and EC were isolated. This because the majority of the experiments in of the cluster for cardiovascular research of the University of Bern are performed using pigs as a large animal model.

3. Aims / Leading Question

- Can the isolation protocol for the pig aortic EC be transferred to other vessel types?
- Can an isolation of EC from pig kidney be improved to create a standardised protocol?

4. Material and Methods

For the cell isolation two different techniques were used. For the isolation of EC from the vessels (aorta, aortic arch, A. abdominalis, A. renalis, A. carotis, V. cava, V. portae, V. renalis, V. jugularis, V. Iliaca, V. femoralis) a sterile cotton-swab was gently rolled over the endothelium to collect cells and put them in culture.

The EC from the kidney were isolated by separating the glomeruli from the rest of the tissue and putting them in culture. In a later step a separation of the EC with CD-31 coated magnetic beads was performed.

After culturing the cells, an indirect immunofluorescence staining was used to check that the isolated cells were indeed EC, by staining the for EC specific markers CD31, VE-Cadherin and vWF. DAPI was used as nucleus staining and α-SMA (alpha-smooth muscle actin) to visualise filaments of fibroblasts, the major contaminating cell fraction in EC isolation. The stained cells were evaluated using a Leica DMI4000 epifluorescence microscope.

5. Results

From the vessels aorta, aortic arch, A. abdominalis, A. renalis, A. carotis, V. cava, V. portae and V. femoralis were successfully EC isolated, while from V. renalis, V. jugularis, V. Iliaca no EC could be isolated. Further could the cells from the V. femoralis not been characterized because of the extremely slow growing speed and the lockdown due to the COVID-19 Pandemic.

Cells were stained as in the following example and a ratio of vWF expression and fibroblast contamination calculated.

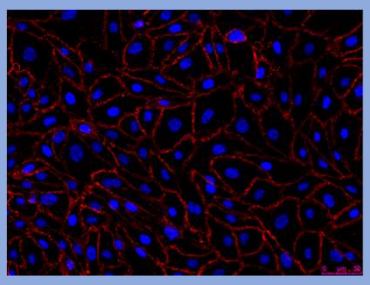

Fig. 5. 1: Characterization of EC cultures isolated from aortic arch stained with anti-CD-31 (red), anti-α-SMA (green), and DAPI (blue).

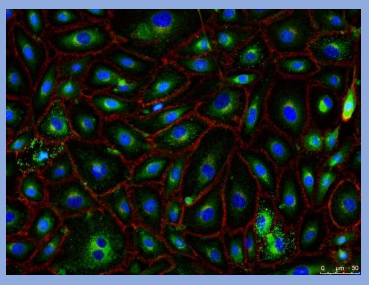

Fig. 5. 2: Characterization of EC cultures isolated from aortic arch stained with anti-VE-Cadherin (red), anti-vWF (green) and DAPI (blue).

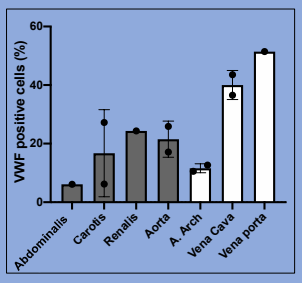

Fig. 5. 3: Graphical presentation of vWF positive cells

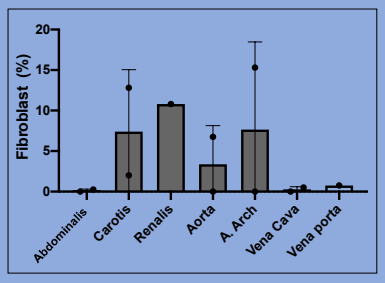

Fig. 5. 4: Graphical presentation of Fibroblast contamination

The staining of the isolated cells from the kidney did not show any signal for CD31, VE-Cadherin or vWF what showed that no EC were present.

6. Discussion

The protocol for the isolation of EC from a pig aorta was successfully performed on other vessels, namely the aortic arch, A. abdominalis, A. carotis, A. renalis, Vena cava, V. portae and V. femoralis. Through immunofluorescence was it possible to characterize the isolated cells and determine the degree of fibroblast contamination. Interestingly, EC cultures from arteries were more affected than those from veins. The different structures and functions of arteries and veins could potentially lead to such differences. For example, is the pressure under flow in arteries significantly higher than in veins, what is a reason for the different vascular composition of these two and this could lead to a higher amount or activity of fibroblasts in arteries compared to veins.

Two other main differences between EC isolated from veins compared to EC isolated from arteries were noticed. So did EC from veins grow much slower. It took 10 to 13 days for arterial EC to grow confluent in a 6 well-plate, while venal EC needed 13 to 14 days. Cells from V. femoralis were left in culture for 43 days without reaching confluency. For all cells the same culture medium was used, what could have influenced the ratio of cell growth of the cells isolated form different vessels.

The second major difference was that more veinal EC express vWF compared to EC isolated from arteries. Here one can only speculated on the reason behind such observations. Again, the cultivation method could influence vWF expression or the cause could be physiological.

From the kidney no EC were successfully isolated. The reason for this is currently unknown and further research needed.

References

[1] Félétou, M. (2011) *The Endothelium: Part 1: Multiple Functions of the Endothelial Cells—Focus on Endothelium-Derived Vasoactive Mediators*. San Rafael (CA): Morgan & Claypool Life Sciences. http://www.ncbi.nlm.nih.gov/books/NBK57149/

[2] Michiels, C. (2003) 'Endothelial Cell Functions'. *Journal of Cellular Physiology* 196(3):430–43. https://onlinelibrary.wiley.com/doi/abs/10.1002/jcp.10333

Figures

Fig. 5.1: Sorvillo, N., Kunz, C. (2020) *Characterization of EC cultures isolated from aortic arch stained with anti-CD-31 (red), anti-α-SMA (green), and DAPI (blue)*. Bern: own figure

Fig. 5.2: Sorvillo, N., Kunz, C. (2020) *Characterization of EC cultures isolated from aortic arch stained with anti-VE-Cadherin (red), anti-vWF (green) and DAPI (blue)*. Bern: own figure

Fig. 5.3: Sorvillo, N. (2020) *Graphical presentation of vWF positive cells*. Bern: own figure

Fig. 5.4: Sorvillo, N. (2020) *Graphical presentation of Fibroblast contamination*. Bern: own figure