# Characterization and use of primary porcine vascular cells in a xenotransplantation model

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## 1. Summary

With the help of xenotransplantation the aim is to reduce the dependency on the limited number of human organ donors and use pigs as potential donors. Since the human immune system has preformed antibodies against certain porcine antigens, the porcine graft needs to be genetically modified. Therefore a new type of pig was established that contained four knock out genes. The aim of this project was to characterize these modified porcine aortic endothelial cells (PAECs) with the help of immunofluorescence staining with specific endothelial cell markers and staining for the four knocked out antigens to confirm their absence.

Unfortunately, after taking the 4x knock out (4xko) cells in culture, no typical endothelial cell growth could be seen. It was suspected that the cells were in fact not all endothelial cells but contaminated with fibroblasts. Therefore I tried to purify these contaminated cells with the help of FACS and the endothelial cell marker CD31 to get a pure cell culture.

## 2. Introduction

It has previously been proposed as a possible solution to the worldwide shortage of human organs for patients with terminal organ failure to use xenotransplantation. Humans are closer matched to primates than pigs, however, it is remarkable how similar these two species have remained, both physiologically and immunologically. [1]

Humans have preformed antibodies against porcine proteins expressed on the endothelial surface. With CRISPR/Cas9, a large variety of genetically modified pigs have been generated to reduce cell activation, complement activation and organ rejection in a xenografic setup. [2]

To characterize the 4xko PAECs, the endothelial cell specific markers CD31, Ve-Cadherin and von Willebrand Factor (vWF) were being used to confirm contamination of the isolated primary cells with fibroblasts. To confirm the knocked out genes, cells were stained for the four antigens. As a control, wild-type (wt) and transgenic (tg) PAECs with two knocked in human genes that act anticoagulant and decelerade the complement cascade (human Thrombomodulin (hTM) and CD46) were used as shown in Table 1.

| | 4x ko cells | tg PAECs | wt PAECs |
|---|---|---|---|
| αGal | X | X | ✓ |
| Sd(a) | X | ✓ | ✓ |
| Neu5Gc | X | ✓ | ✓ |
| SLA-I | X | ✓ | ✓ |
| hTM | X | ✓ | X |
| CD46 | X | ✓ | X |

Tab. 1: Overview of the genes present (Indergand, 2021).

## 3. Aims and leading questions

Aim 1: Characterization of 4x ko cells in comparison to tg PAECs and wt PAECs.
*Question 1: Is there a difference in the antigen expression of the genes present between 4x ko cells in comparison to wt PAECs and tg PAECs?*

Aim 2: Purification of 4x ko cells with Fluorescence-activated Cell Sorting (FACS).
*Question 2: Is it possible to purify the 4x ko cells that are contaminated with fibroblasts with FACS?*

## 4. Material and methods

Wild type , transgenic and 4x knock out primary cells between passage 3 and 4 were kept in culture at 37°C, 5 % $CO_2$ until they reached confluency.

For the characterisation staining, the cells were seeded in a 8-well chamber slide and incubated for 24 hours. Afterwards, the cells were fixed and stained with immunofluorescence labelled antibodies as shown in Figure 1.

For the purification, cells in culture were trypsinised, stained with CD31, sorted with FACS and put back in culture.

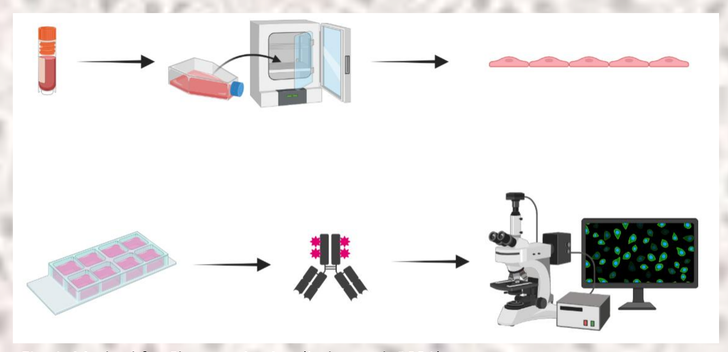

Fig. 1: Method for Characterization (Indergand, 2021).

## 5. Results

The immunoflurescence staining showed a negative result for the four knocked out genes of the 4xKO cells as shown in Figure 2.

The purification of the contaminated 4xKO cells showed 46.6 % CD31+ cells and were taken back in culture for cell expansion.

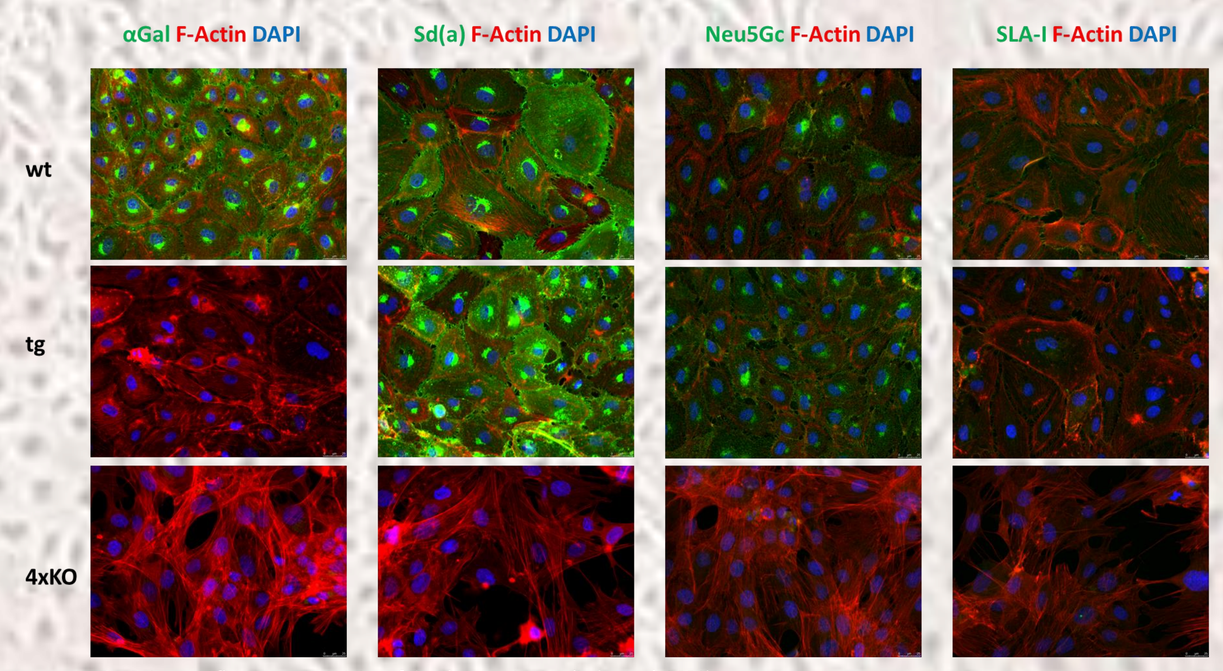


Fig. 2: Immunofluorescence staining of the characterization (Indergand, 2021).

## 6. Discussion

The characterization of the 4xKO cells confirmed the absence of the four knocked out genes. Unfortunately, staining for endothelial cell markers confirmed that the cells were contaminated wih fibroblasts and were not a pure culture of endothelial cells.

The purification with FACS was successfull and after taking the sorted CD31+ cells back in culture, a pure endothelial cell culture with the typical monolayer grwoth could be observed.

### References

[1] Sachs, D. H. (1994). The pig as a potential xenograft donor. Veterinary Immunology and Immunopathology, 43(1–3), 185–191. https://doi.org/https://doi.org/10.1016/0165-2427(94)90135-X

[2] Sykes, M., & Sachs, D. H. (2019). Transplanting organs from pigs to humans (Vol. 4). http://optn.transplant.hrsa.gov

### Figures

Fig. 1: Indergand, S. (2021). *Method for Characterization*. Bern: own figure
Fig. 2: Indergand, S. (2021). Immunofluorescence staining of the characterization. Bern: own figure

### Tables

Abb. 1: Indergand, S. (2021). *Overview of the genes present*. Bern: own table