Characterisation of genetically modified pig endothelial cells from aortic and renal blood vessels in xenotransplantation settings

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1. Abstract

Xenotransplantation offers a potential solution to organ shortage, but immune rejection, especially through antibody binding and complement activation, remains a major barrier. Endothelial cells (ECs) are critical as they form the first contact between graft and recipient immune system.

In this study, porcine aortic and renal ECs (wild-type vs. transgenic: α-Gal KO + human CD46 + human Thrombomobulin (TBM)) were analyzed in a 2D microfluidic system under shear stress. Immunofluorescence was used to assess endothelial markers (CD31, VE-Cadherin, von Willebrand Factor (vWF) and complement activation (C3c, IgG).

Results: Transgenic ECs showed reduced complement and IgG deposition while maintaining structural integrity. These findings underline their potential to improve graft survival in future xenotransplantation

2. Introduction

Xenotransplantation, the transfer of organs or cells between species, is a promising strategy to address the global organ shortage. Patients with kidney, liver, or heart failure are most affected. The main barrier remains the immune response of the recipient, especially antibody (IgG) binding and complement activation, which can be measured by C3c deposition. [1]

Pigs are considered the most suitable donors due to their anatomical and physiological similarity to humans, rapid reproduction, and the possibility of genetic modifications to reduce rejection. [1]

Endothelial cells (ECs) line blood vessels and form the first point of contact between graft and recipient blood. [2] They regulate vascular tone, hemostasis, and barrier function (markers: CD31, VE-Cadherin, vWF). [3] The aorta (largest artery) ensures continuous flow via the Windkessel effect, [4] while the renal arteries supply the kidneys under high pressure. [5]

Shear stress influences EC alignment and stability. Physiological flow supports vascular homeostasis, while disturbed flow promotes dysfunction and graft failure. [6]

To overcome immunological barriers, αGal knockout and the introduction of human genes such as hCD46 and hTBM aim to reduce IgG binding, limit complement activation, and protect the endothelium in xenotransplantation. [1&6]

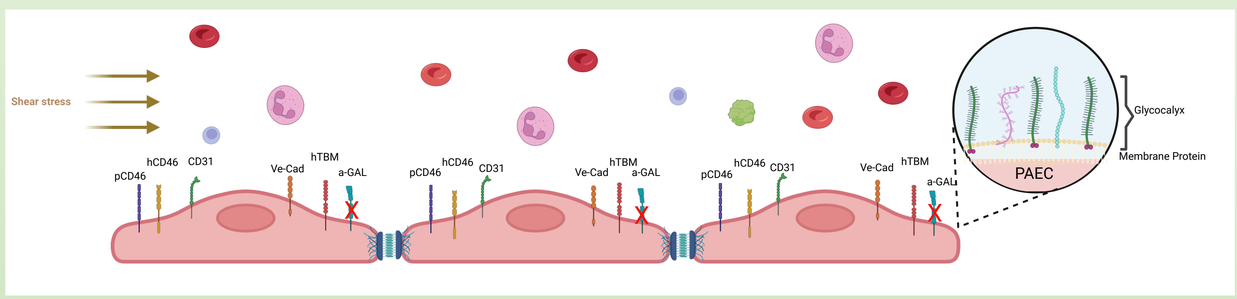

Figure. 1: Schematic illustration of endothelial cells under shear stress showing CD31, VE-Cadherin, hCD46, hTBM, α-Gal knockout, and the glycocalyx structure. (Teixeira, 2025; created with BioRender)

3. Aims and leading questions

Aim: The effect of genetically modified cells on the complement system of different types of endothelial cells in xenotransplantation.

Lead question: How efficient is the complement protection of transgenic modifications in renal endothelial cells in com- parison to the wild-type cells in xenotransplantation?

4. Material and Methods

Porcine aortic and renal endothelial cells, wild-type and genetically modified (α-Gal KO, hCD46, hTBM), were expanded in DMEM with serum and antibiotics. Cells were seeded on fibronectin-coated chamber slides or in a custom 2D microfluidic system to mimic shear stress and perfused with human or porcine serum for 48 h.

After fixation, immunofluorescence staining was performed for CD31, VE-Cadherin, F-actin, IgG, and C3c. Imaging was done with a Zeiss LSM980 confocal microscope, and analysis with Fiji and Prism software.

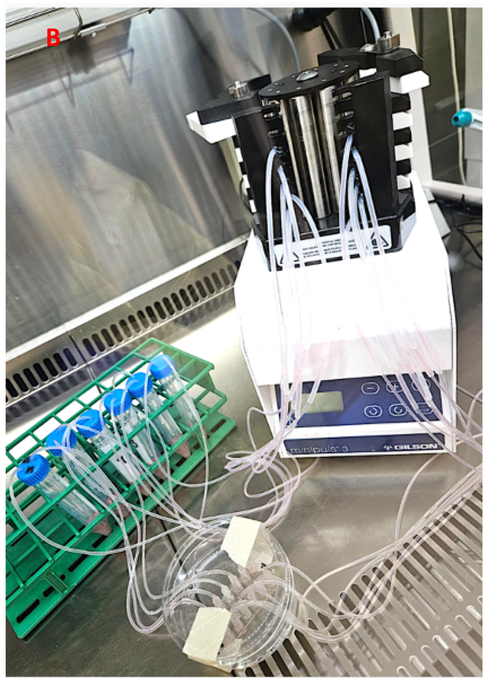

Figure 2: Microfluidic perfusion system with pump, tubing, and culture chamber for simulating physiological shear stress on endothelial cells. (Teixeira, 2025 Own image)

5. Results

Wild-type renal endothelial cells showed strong C3c deposition and IgG binding, especially under human serum, indicating robust immune activation. In contrast, transgenic cells displayed reduced signals with statistical significance (IgG: $p < 0.03$; C3c: $p < 0.05$), while maintaining intact cytoskeleton and nuclei. These findings highlight the protective effect of genetic modifications in reducing immune activation.

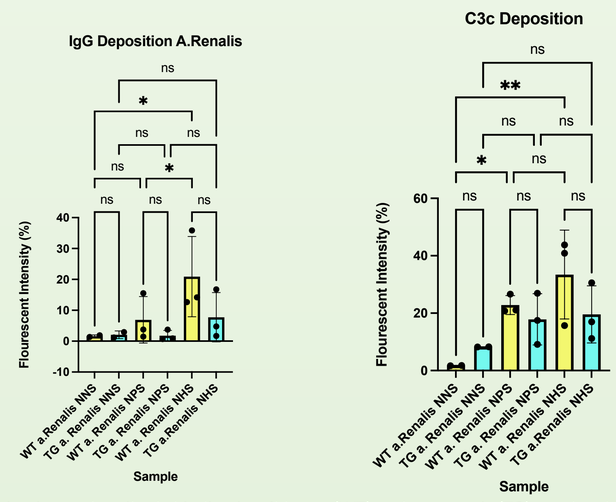

*Figure 3: Quantification of complement activation and antibody binding in wild-type (WT) and transgenic (TG) renal endothelial cells under different serum conditions. **A) C3c deposition:** WT cells showed strong complement activation under human serum, while TG cells displayed significantly reduced deposition ($p = 0.05$). **B) IgG binding:** WT cells had higher IgG signals under human serum compared to TG cells, which showed significantly reduced binding ($p = 0.03$). These findings demonstrate the protective effect of genetic modifications (αGal KO, hCD46, hTBM) in reducing immune activation and improving compatibility in xenotransplantation. (Teixeira, 2025, own image)*

6. Discussion and conclusion

Wild-type endothelial cells showed strong immune activation under human serum with high C3c deposition and IgG binding. In contrast, transgenic cells (αGal KO, hCD46, hTBM) displayed significantly reduced signals (*C3c p = 0.05; IgG p = 0.03*) while maintaining structural integrity. These results highlight the protective effect of genetic modifications in reducing complement activation and antibody recognition.

Genetic modifications in porcine endothelial cells significantly reduce immune activation and preserve cell stability, representing a promising strategy to improve xenograft survival.

References:

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Figure:

Fig. 1: Own image, Teixeira, 2025 : *Schematic illustration of endothelial cells under shear stress showing CD31, VE-Cadherin, hCD46, hTBM, α-Gal knockout, and the glycocalyx structure*

Fig. 2: *own Image, Teixeira, 2025Microfluidic perfusion system with pump, tubing, and culture chamber for simulating physiological shear stress on endothelial cells.*

Fig. 3: own image, Teixeira, 2025 Quantification of complement activation and antibody binding in wild-type (WT) and transgenic (TG) renal endothelial cells under different serum conditions. A) C3c deposition: WT cells showed strong complement activation under human serum, while TG cells displayed significantly reduced deposition ($p = 0.05$). B) IgG binding: WT cells had higher IgG signals under human serum compared to TG cells, which showed significantly reduced binding ($p = 0.03$). These findings demonstrate the protective effect of genetic modifications (αGal KO, hCD46, hTBM) in reducing immune activation and improving compatibility in xenotransplantation.