Exploring endothelial cell protection in Auckland Island pigs: A gateway to advancing xenotransplantation compatibility

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1. Abstract

Xenotransplantation offers a promising approach to overcome organ shortage, but strong immune reactions against vascular endothelial cells (ECs) remain a major obstacle. ECs regulate coagulation and inflammation, and act as the first barrier between recipient blood and xenograft tissue. Auckland Island (AI) pigs, a genetically homogeneous and pathogen-free strain from New Zealand, present an attractive donor source. In this project, we evaluated genetically modified transgenic (TG) AI porcine endothelial cells (PECs) with α-Gal knockout and human CD46 expression for their ability to improve graft compatibility.

PECs from wild-type (WT) and TG AI pigs were cultured in a 2D microfluidic flow system for 48 hours. Endothelial identity was confirmed by marker expression, and complement activation was assessed after perfusion with human serum. TG PECs showed markedly reduced antibody binding and complement deposition compared to WT PECs, while endothelial integrity was preserved under flow conditions.

These results demonstrate that TG AI PECs display reduced immunogenicity and support the use of microfluidic culture as a robust platform for xenotransplantation research.

2. Introduction

Xenotransplantation has emerged as a promising strategy to address the global shortage of donor organs. Pigs are considered the most suitable donor species because they share key physiological similarities with humans, have short breeding cycles, and can be genetically modified with relative ease [1]. A milestone was reached in 2022 with the first pig-to-human heart transplant, which demonstrated the feasibility of this approach, although graft survival was limited to 49 days [2].

One of the greatest obstacles to long-term success is graft rejection. This response is largely driven by human antibodies binding to porcine endothelial cells (ECs), which in turn activate the complement system and coagulation cascades. Hyperacute rejection, for example, can occur within minutes and is primarily caused by natural antibodies targeting the porcine antigen αGal. To counteract this, genetic modifications such as αGal knockout (KO) and the introduction of human complement regulatory proteins like hCD46 have been developed, significantly reducing graft immunogenicity in preclinical models [3].

To model these mechanisms under conditions that mimic human physiology, advanced 2D microfluidic systems have been established by the research group of Prof. Robert Rieben (Bern) in collaboration with partners in Munich. Within this framework, my thesis investigates endothelial cells derived from Auckland Island (AI) pigs bred in Münich - a pathogen-free, genetically homogeneous donor strain originally from New Zealand with favorable organ size - under flow conditions, to assess their potential role in advancing xenotransplantation.

Fig. 1: Structural overview of a built 2D microfluidic system. (Walther, 2025)

3. Aims and Leading Questions

Establishment of AI PECs (WT and transgenic αGal knockout and hCD46 expression) in the microfluidic system.

- Are the genetic modifications successfully implemented?
- How do genetic modifications regulate complement activation when exposed to human serum during xenogeneic activation?

4. Materials and Methods

Porcine endothelial cells (PECs) were isolated from thoracic aorta and vena cava of Auckland Island (AI) pigs. Two groups were used: wild-type (WT) PECs and transgenic (TG) PECs carrying a GTα1 knockout (αGal KO) combined with human CD46 (hCD46) expression. Endothelial identity was confirmed by immunofluorescence staining of CD31, VE-Cadherin, and vWF.

Cells were seeded into 2D microfluidic slides (200,000 cells/channel, fibronectin-coated) and cultured under dynamic flow conditions using a peristaltic pump, generating physiological shear stress (10 dyn/cm²) for 48 hours. This model replicates aspects of the vascular microenvironment, including alignment of endothelial cells in the direction of flow.

After flow conditioning, channels were perfused with normal human serum (NHS) for 2 hours, while normal pig serum (NPS) served as baseline control. Following perfusion, cells were fixed with 4% paraformaldehyde and stained for complement activation (C3c) and human IgG deposition.

Microscopy was performed using a confocal microscope. Fluorescence intensity was quantified with Fiji and statistical analysis was carried out in GraphPad Prism.

5. Results

Under flow with human serum (NHS), TG AI PECs showed significantly reduced C3c deposition ($p < 0.05$) and IgG binding ($p < 0.01$) compared to WT PECs. In contrast, with pig serum (NPS) no significant differences were observed, confirming the specificity of the human immune response.

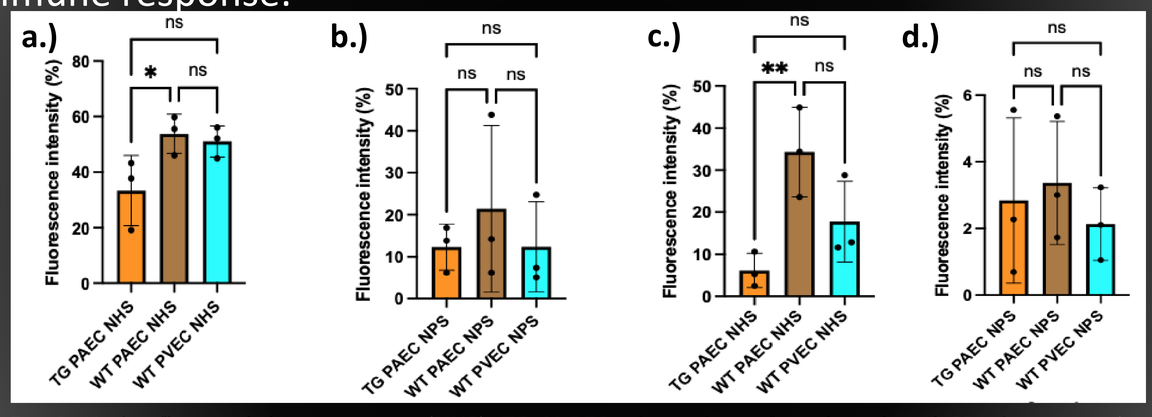

Fig. 2: Under flow with human serum (NHS), TG AI PECs showed significantly reduced C3c deposition ($p < 0.05$) compared to WT PAECs, while no difference was observed between WT PAECs and WT PVECs (Fig. 2a). With pig serum (NPS), no significant changes in C3c were detected, confirming that complement activation is specific to human serum (Fig. 2b). Similarly, IgG binding was markedly lower in TG AI PECs compared to WT PECs ($p < 0.01$) under NHS, indicating that αGal KO and hCD46 expression reduce antibody recognition (Fig. 2c). In contrast, IgG binding remained low across all groups under NPS (Fig. 2d). Statistical analysis: one-way ANOVA, ns = not significant. (Walther, 2025)

6. Discussion and Conclusion

This study demonstrates that endothelial cells (ECs) from Auckland Island (AI) pigs can be successfully maintained in 2D microfluidic slides under physiological flow, providing a controlled model to study xenogeneic immune responses. When perfused with human serum, wild-type ECs showed strong IgG binding and C3c deposition, reflecting hyperacute rejection mechanisms. In contrast, transgenic ECs with αGal knockout and human CD46 expression displayed markedly reduced IgG binding and complement activation, confirming the protective effect of these genetic modifications.

These findings are consistent with previous reports that αGal is the main target of natural antibodies and that hCD46 regulates complement deposition. The microfluidic approach further strengthens these results by replicating shear stress conditions, which are not captured in static culture.

While limited to a small donor pool and focused on IgG and C3c readouts, this study supports the strategy of combining genetic modifications in pigs with advanced in vitro models to advance xenotransplantation research.

References

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Figures

Fig. 1: Walther, B. (2025). *Overview of the structure of a built 2D microfluidic system.* Bern: own figure.

Fig. 2: Walther, B. (2025). *Complement (C3c) and IgG deposition under human and pig serum.* Bern: own figure.