Assessing the anticoagulant properties of transgenic porcine endothelial cells using a 3D microfluidic assay in xenotransplantation research settings

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1. Abstract

Xenotransplantation is a promising solution to organ shortage in which hearts from pigs, are transplanted into humans. However, immunological barriers need to be overcome. Endothelial cells (EC) maintain an anticoagulant and anti-inflammatory phenotype. Furthermore, they function as first point of contact between recipient's blood and xenograft. Therefore, it is crucial to find out if the currently used genetic modification sets, in transgenic (TG) donor pigs with gene knock-out (KO), can elicit better protection from coagulation.

A 3D microfluidic system is used for 72 hours perfusion, afterwards the cells get activated either with TNFα or human serum (hSerum) or both simultaneously. The 1xKO 2xTG EC show prolonged clotting time after TNFα and simultaneous activation, suggesting that human thrombomodulin (hTBM) play an important role. Nevertheless, the 2xKO 5xTG EC show prolonged clotting time after hSerum activation probably due to increased human genes implementation.

2. Introduction

As of 2023, in Switzerland alone, 2’225 people are on the waiting list for an organ transplant. However, only 675 transplants have taken place during the year [1]. Using pigs for xenotransplantation is an alternative solution to the organ shortage and pigs recognized as the best choice of animals [2].

A functional endothelium is essential for the regulation of vascular homeostasis and the maintenance of an anticoagulant and anti-inflammatory phenotype (fig. 1). The endothelium consists of EC that line the lumen of the blood vessel. The EC function as first point of contact between recipient's blood and xenograft [3].

After xenotransplantation, the immune system plays the main role in the rejection of the xenograft. This happens, among other things, due to the difference of the molecules [4].

To improve the acceptance of the xenograft gene editing is used for porcine KO and implementation of human genes. Important porcine KO are αGal and CMAH [5]. Human produce natural antibodies among others against αGal causing an hyperacute rejection (HAR) [4]. The implement of human genes, for example hTBM, lead to an improved anticoagulant properties[2]. There are genes for complement regulation (hCD46, hCD55, hCD59) and maintaining an anti-inflammatory and anti-apoptotic effect (hA20, hHO1) [5].

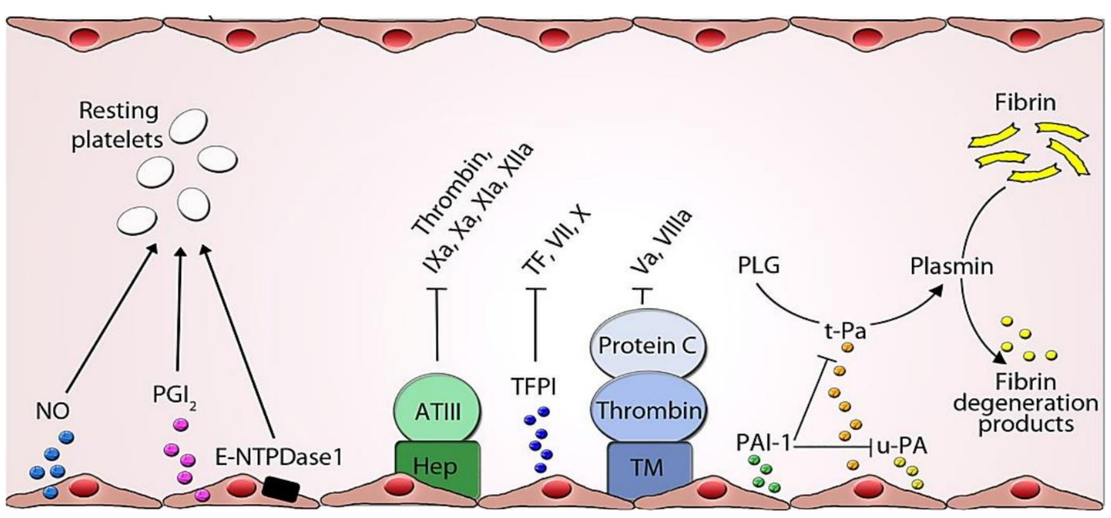


Fig. 1: Anticoagulant properties of the endothelium

3. Aims and leading questions

Aim 1-3
Examine anticoagulant response of two different transgenic aortic endothelial cells, using a 3D microfluidic assay:

- under inflammatory condition
- under xenogeneic activation
- simultaneous exposure of inflammatory condition and xenogeneic activation

4. Material and Methods

1xKO 2xTG EC (αGal KO and hCD46 & hTBM implemented), 2xKO 5xTG EC (αGal & CMAH KO and h-CD46, -CD55, -CD59, -A20 & -HO1 implemented) and wild type (WT) EC are used, with two donors each.

The EC get aligned in a microchannel, which get connected to a 3D microfluidic system allowing a pulsating movement through the microchannel, similar to a blood vessel [6]. After 72 hours of perfusion and 2 hours with hSerum or 4 hours with TNFα or both in succession, the clotting assay with recalcified human citrated plasma get performed under the confocal microscope. Clot formation is recorded and defined as time to occlude the channel and the formation of the saturated fluorescently labeled human fibrinogen is visible.

5. Results

The 1xKO 2xTG EC have a significant longer clotting time when activated with only inflammatory cytokine TNFα (fig. 2A) and simultaneous with TNFα and human serum (fig. 2C) compared to the WT EC. The 2xKO 5xTG EC show prolonged coagulation time after xenogeneic activation with human serum compared to the WT EC and 1xKO 2xTG EC (fig. 2B).

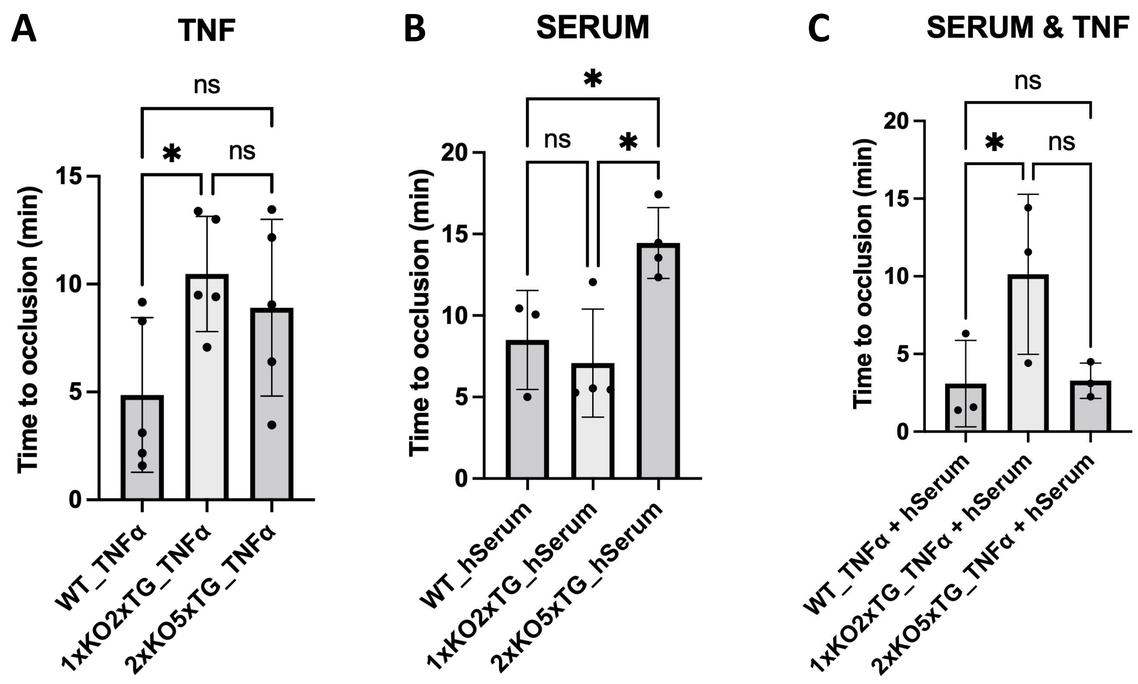


Fig. 2: A) Comparison clotting time TNFα activated cells. Statistical significance evaluated using Kruskal-Wallis test. ns = not significant, *p-value <0.05. B) Comparison clotting time hSerum activated cells. Statistical significance evaluated using Kruskal-Wallis test. ns = not significant, *p-value <0.05. C) Comparison clotting time simultaneously activated cells. Statistical significance evaluated using one-way ANOVA test. ns = not significant, *p-value <0.05

6. Discussion and Conclusion

The 1xKO 2xTG EC express hTBM, which is the only thing missing in the 2xKO 5xTG EC gene set. From this result, it can be concluded that hTBM likely has a major influence on the anticoagulant response during inflammation and, therefore, should be included into the genetic modification sets for xenotransplantation.

The higher number of implemented human genes, as well as the additional KO from CMAH appear to have a positive influence in the experimental setting, simulating xenogeneic circumstances. This underlines the importance of the implementation of human regulatory genes in the field of hemostasis.

As this thesis demonstrates, a significant improvement of the anticoagulant phenotype during inflammation and xenogeneic environment is possible with the corresponding genetic modifications.

Referenzen

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Figure

Fig. 1: Neubauer, K., & Zieger, B. (2022). Endothelial cells and coagulation. Cell and Tissue Research, 387(3), 391–398. https://doi.org/10.1007/s00441-021-03471-2

Fig. 2: Thomi, N.A. (2024). A) Comparison clotting time TNFα activated cells. Statistical significance evaluated using Kruskal-Wallis test. ns = not significant, *p-value <0.05. B) Comparison clotting time hSerum activated cells. Statistical significance evaluated using Kruskal-Wallis test. ns = not significant, *p-value <0.05. C) Comparison clotting time simultaneously activated cells. Statistical significance evaluated using one-way ANOVA test. ns = not significant, *p-value <0.05. Medi.