Improving Kidney Transplantation: Evaluating the Efficacy of Polysulphide Nanoparticles in the LIVING-KIDNEY System

Introduction

Kidney transplantation is the primary therapeutic intervention for patients suffering from end-stage renal disease, offering a significant improvement in both life expectancy and quality of life compared to long-term dialysis.

However, ischemia-reperfusion injury (IRI) significantly impairs graft function and affects long-term patient outcomes. IRI is a complex pathophysiological phenomenon that occurs when blood supply to the organ is temporarily cut off and then restored, leading to a series of deleterious cellular and molecular events.

This white paper delves into a study conducted using Pebble's LIVING-KIDNEY system, evaluating treatment of the donor graft with polysulphide nanoparticles (PPS-NPs) to mitigate IRI. The study highlighted the LIVING-KIDNEY systems capability to closely mimic human physiological responses in transplantation, providing a robust platform for testing novel therapeutic interventions.

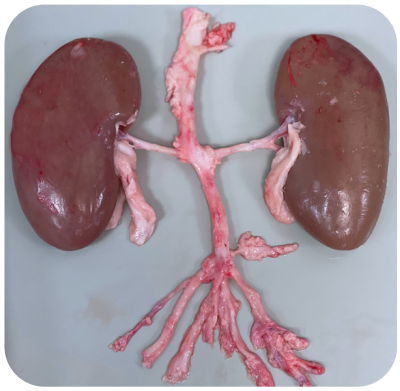


Figure 1- Bilateral kidneys with corresponding renal arteries arising from the aorta, its primary source of arterial blood supply.

Pebble's LIVING-KIDNEY System

Pebble's LIVING-KIDNEY system is a state-of-the-art platform in preclinical transplantation research, designed to closely emulate the human transplantation process.

Pebble use pig kidneys, chosen for their physiological and anatomical parallels to human kidneys. This system models static cold storage (SCS), normothermic machine perfusion (NMP), and allogeneic reperfusion - essential stages in the kidney transplant process.

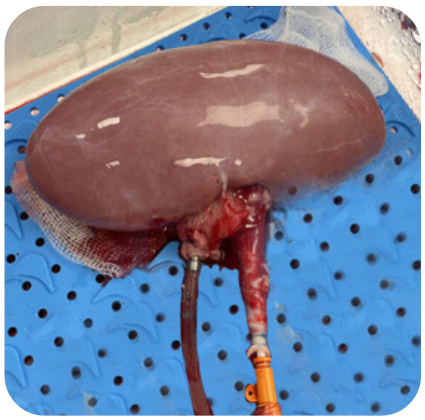


Figure 2- A kidney being perfused on Pebble's LIVING-KIDNEY System.

Pebble support high welfare farming practices, using organs that are surplus to the food industry in their systems, aligning with contemporary ethical standards in biomedical research.

Study Design

Pebble's study employed a randomized, two-stage preclinical trial design with paired pig kidneys. The primary objective was to assess the effectiveness of PPS-NP treatment in ameliorating IRI during donor kidney preservation.

The trial was divided into two key experiments: the first focused on the impact of nanoparticles on renal haemodynamics and IRI during normothermic machine perfusion (NMP), while the second assessed the outcomes of post-transplant reperfusion using allogeneic blood, mimicking clinical transplantation.

In each experiment, kidneys were procured from pigs and randomly assigned to either a standard preservation flush or a flush supplemented with PPS-NPs. This setup allowed for a direct comparison of the effects of the nanoparticle treatment on renal function and IRI, under conditions closely resembling those experienced during human kidney transplantation.

Methodology

Following procurement, kidneys underwent a standardised period of SCS, followed by NMP, allowing for the assessment of both preservation techniques. Key parameters measured included renal blood flow, intra-renal resistance, and biochemical and functional parameters, providing comprehensive data on the kidney's physiological state.

Advanced analytical techniques were employed to assess total protein and albumin content in urine, DNA damage, TNF-α expression levels in plasma, and complement activation. The study incorporated infra-red imaging to assess perfusion homogeneity, while needle-core biopsies were taken at various time points for histological analysis. These techniques ensured a thorough evaluation of the effects of PPS-NP treatment on the kidneys.

Statistical analysis of the data was conducted to ensure the validity and reproducibility of the findings. The approach to data collection and analysis underlines the scientific rigor of the study and its potential to contribute significantly to the field of transplantation medicine.

Results and Discussion

Treatment with PPS-NPs during SCS and NMP resulted in improved post-transplant outcomes, evidenced by better renal hemodynamics, reduced reactive oxygen species (ROS), and decreased IRI (Figure 3A and 3B)

Figure 3 - Renal haemodynamics during perfusion and reperfusion.

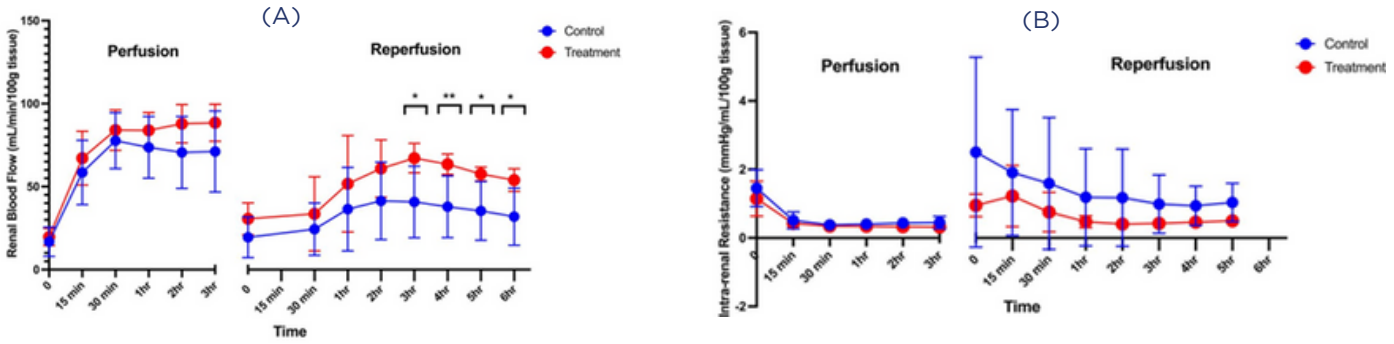
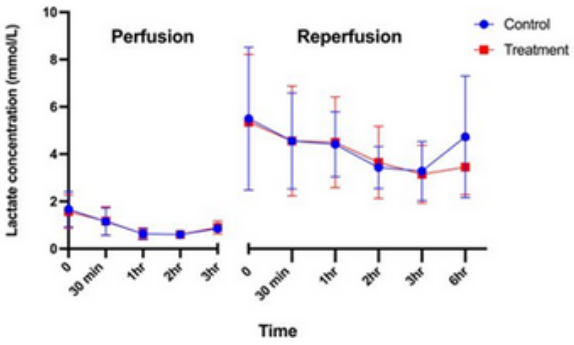


Figure 4 - Lactate concentration during perfusion and reperfusion.



Biochemical analysis revealed that PPS-NP treatment led to lower lactate levels (Figure 4), a surrogate marker for anaerobic metabolism and tissue perfusion, suggesting benefit to vascular integrity. Furthermore, treated kidneys displayed a reduction in oxidised DNA in the plasma perfusate, indicating reduced cell and tissue damage, as well as lower levels of oxidative stress.

Histologically, PPS-NP treated kidneys exhibited better preservation of tissue architecture and reduced acute tubular necrosis (Figure 5), pointing to reduced oxidative stress and cellular damage. Immunostaining revealed that PPS-NPs infiltrated renal tissue and were associated with areas where VCAM-1 was not expressed (Figure 6), suggesting a role in adhesion molecule downregulation.

Figure 5 - Areas of cellular necrosis in a kidney from the control group (A). Limited cellular necrosis present in a kidney from the treated group (B). Endothelial denudation in a kidney from a control group (C). Improved endothelial preservation in a kidney from a treated group (D).

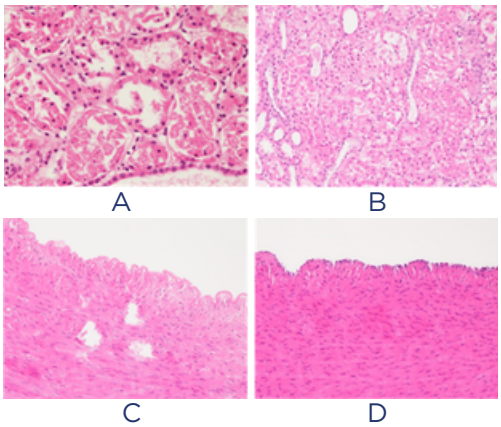
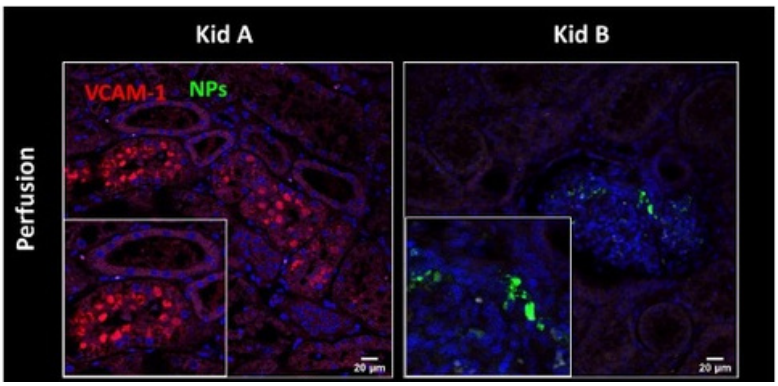
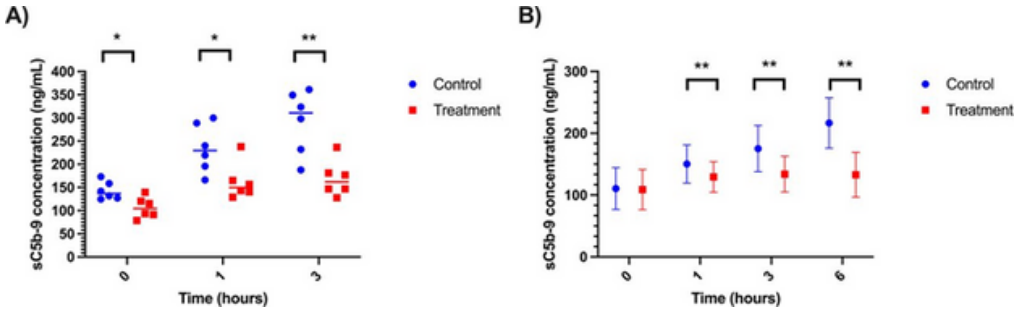


Figure 6 - VCAM-1 and nanoparticle expression in control and treated groups after perfusion



The reduction in the soluble form of the terminal cytolytic membrane attack complex (MAC), C5-9b, in the perfusate of kidneys treated with PPS-NPs is particularly noteworthy (Figure 7). This finding suggests that PPS-NP treatment may have beneficial effects via ROS neutralisation and indirect complement inhibition, both of which are intricately involved in IRI.

Figure 7 - Complement activation during perfusion and reperfusion

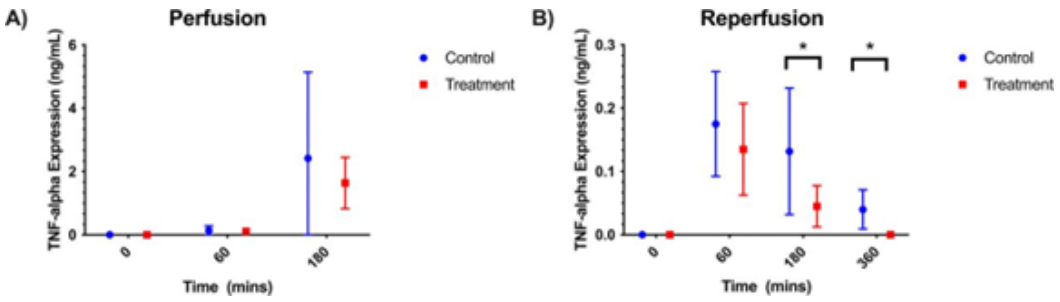


Additionally, PPS-NP treated kidneys presented with improved tissue integrity and reduced urine albumin concentration during reperfusion, potentially in response to reduced oxidative stress, cell damage, complement activation, and MAC formation.

The release of ROS and subsequent complement activation contributes to endothelial cell injury and death, causing the upregulation of leukocyte adhesion molecules and leading to vascular wall remodeling and reduced blood flow. The observed reduction in VCAM-1 in the presence of NPs supports this notion.

Furthermore, TNF-α perfusate levels were diminished in NP treated kidneys (Figure 8). TNF-α is a therapeutic target in transplant settings due to its roles in rejection, and the study's findings indicate that PPS-NP treatment might mitigate TNF-α-mediated effects.

Figure 8 - TNF-α concentration in perfusate



These results have direct implications for clinical transplantation, suggesting a novel approach to organ preservation and the potential to reduce delayed graft function. The translational relevance of these findings is significant, with the potential to enhance graft survival and patient outcomes in clinical settings.

Conclusion

The study conducted using Pebble's LIVING-KIDNEY system offers compelling scientific evidence for the effectiveness of PPS-NP treatment in improving kidney transplantation outcomes. The research highlights the system's ability to generate clinically relevant data, bridging the gap between preclinical and clinical transplantation research. Pebble's commitment to scientific excellence and innovation is evident, with potential wide-reaching impacts on the field of transplantation medicine.