Normothermic Perfusion in Organ Preservation and Evaluation. Promising Future in Kidney Transplantation.

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Abstract

Kidney transplantation remains the best treatment for endstage renal disease. Normothermic Machine Perfusion (NMP) could be a valuable tool that could solve the problems that arise from the high demand for organs and their quality, providing a suitable platform for investigating treatments that would not be possible *in vivo*.

Introduction

Due to advanced kidney replacement therapies, improved care, and increased survival of renal transplant patients, the number of people on the waiting list for a deceased donor kidney is stably high or growing, currently standing at ~10.500 patients in UE in 2024 [1]. To meet growing organ demands, using of grafts donated after circulatory death (DCD), or retrieved from extended criteria donors (ECD), is unavoidable. Unfortunately, DCD and ECD grafts are more immunogenic, which leads to difficulties in immune regulation after implantation and a higher risk of acute and chronic rejection. Therefore, the development of organ preservation techniques has the potential to overcome these challenges [2].

Normothermic Machine Perfusion (NMP) is an emerging preservation modality for kidneys prior to transplantation that maintains the organ at body temperature (35-37°C), in contrast to traditional cold storage methods. NMP circulates a warm perfusion solution through the renal vasculature, delivering oxygen and nutrients to maintain metabolic activity [3]. This restoration of function during preservation represents a significant advancement in transplantation science, as it allows clinicians to assess organ quality before transplantation and provides a platform for delivering pre-transplant therapies to promote recovery [4].

Advantage of NMP over other methods

For more than 50 years, the traditional method of pre-transplant kidney preservation has been static cold storage (SCS) in ice. This involves flushing the kidney to remove donor blood, cooling with a preservation solution at 4 °C and storage on ice while arrangements are made for transplantation. Moreover, this leads to the production of the reactive oxygen species that underlie an ischemia reperfusion injury [5].

Compared to conventional static cold storage, NMP maintains and even upregulates oxidative phosphorylation (OXPHOS) genes, preserving the kidney's metabolic functions [6]. The metabolic activity maintained during NMP creates additional opportunities for organ optimization that are impossible with cold storage. By circulating warm, oxygenated perfusion solution through the kidney, NMP enables the removal of toxic metabolites that accumulate during ischemia, thereby reducing organ inflammation. Furthermore, NMP provides a platform for real-time assessment of organ viability before transplantation, potentially reducing discard rates of viable organs [7].

Another significant advantage of NMP is the extended preservation time it provides compared to cold storage. This prolonged ex-situ preservation window enables a more thorough assessment of organ viability and function before transplantation decision-making [3].

In this way, NMP appears to be a perfect platform for the administration of various biological and pharmacological agents directly into the renal graft, avoiding the toxicity caused by systemic treatment of the recipient and the logistical difficulties of donor therapy.

Therapeutic Interventions during NMP

As discussed above, NMP has emerged as an effective platform for testing and performing therapies that would not be possible *in vivo*. Some of the most promising therapies performed on this platform are those aimed at improving transplantation conditions and increasing access to organs that would *a priori* be discarded. Among the most innovative and emerging therapies we highlight cell therapy, nanoparticle therapy and gene therapy. **(Table 1)**.

Our Approach

Our approach, based on the background above context, will be to use the organ *ex vivo* maintenance machine in normothermia ARK KIDNEY® of EBERS Medical Technology SL. to perform genetic modifications in kidneys, to convert them into more immunocompatible transplants. Previous experiments performed on mouse kidneys in subnormothermic machines have been able to modify and downregulate MHC class I and II expression in a prolonged manner, by using RNA interference (RNAi) through lentiviral vectors [8].

This serves as a background to apply these gene editing methodologies in our *ex vivo* model and exploit this new potential in the field of biomedicine. It also opens an opportunity to apply other techniques such as CRISPR-Cas9 technology to implement or eliminate genes of interest directly in the DNA, as in recent research in xenotransplantation [9].

Conclusions

Ex vivo normothermic perfusion emerges as a transformative technology in the field of organ preservation and evaluation, offering a hopeful horizon for kidney transplantation. It has been highlighted, how NMP goes beyond mere preservation, allowing donor kidneys to function and be evaluated in *ex vivo* physiological conditions pretransplantation. This unique ability to resuscitate and optimize organs outside the body not only improves graft viability and reduces the incidence of post-transplant complications such as delayed graft function (DGF), but also opens the door to utilizing kidneys that were previously considered marginal or unfit. By providing a platform for assessing organ function in real time and enabling therapeutic interventions prior to implantation, NMP promises to expand the donor pool and ultimately improve long-term outcomes for patients.

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Tabla 1. Therapeutic interventions during NMP

Reference	Target Mechanism/Pathway	Organ model	Main Findings
[10]	Mesenchymal Stromal Cell (MSC); Bone marrow (BM-MSC) and Adipose tissue derived (A-MSC)	Porcine kidneys	↓LDH (BM-MSC) ↓NGAL (BM-MSC and A-MSC)
[11]	antiCD31-nanoparticles (NP)	Non-transplantable human kidneys	Anti-human CD31 Abs NPs loaded had enhanced vascular retention compared to nontargeted NPs
[12]	FpGalNAc deacetylase and FpGalactosaminidase through *Flavonifractor plautii* to enzymatically convert blood group A to Group O	Non-transplantable human kidneys	Blood group A antigen loss of approximately 80% in renal vasculature as little as 2 h NMP

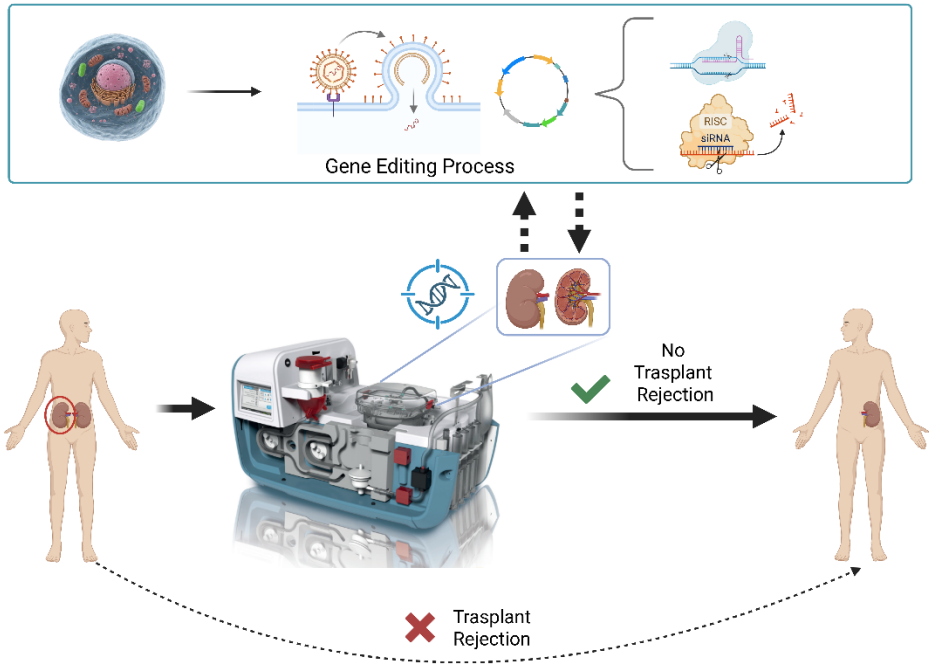

Figure 1. Graphical representation of the immunocompatibility process by the use of NMP (ARK KIDNEY ®)