MINIREVIEW

Nanotherapeutics in transplantation: How do we get to clinical implementation?

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Patients undergoing organ transplantation transition from one life-altering issue (organ dysfunction) to a lifelong commitment—immunosuppression. Regimens of immunosuppressive agents (ISAs) come with significant side effects and comorbidities. Recently, the use of nanoparticles (NPs) as a solution to the problems associated with the long-term and systemic use of ISAs in transplantation has emerged. This minireview describes the role of NPs in organ transplantation and discusses obstacles to clinical implementation and pathways to clinical translation.

KEYWORDS
basic (laboratory) research/science, bioengineering, graft survival, immunosuppressant, immunosuppression/immune modulation, organ perfusion and preservation, organ transplantation in general, solid organ transplantation, translational research/science

1 | INTRODUCTION

Nanoscale materials, that is, nanoparticles (NPs) were first used for targeted drug therapy in the 1960s and have been a subject of great interest and research in many fields for their ability to be used as both a therapeutic and/or diagnostic tool.[1] NPs are engineered materials with particle diameters generally under 100 nm. They can be synthesized using a broad range of materials from polymers to lipids and can specifically be engineered to form a variety of structures like micelles, solid particles, and core/shell (Figure 1). NP synthesis is a reproducible and scalable process that generally includes either a top-down or bottom-up approach, reviewed by Baig et al.[2] NPs offer

Liposomes

Payload	Allograft model	Administration
Prednisolone	Mouse kidney (31)	*In vivo* post-transplantation
Cyclosporine	Rat liver (32)	*In vivo* post-transplantation

Solid Lipid Nanoparticle

Payload	Allograft model	Administration
Mycophenolate mofetil	Not tested (34)	Not tested

Payload	Allograft model	Administration
anti-IL6	Mouse heart (14)	*In vivo* post-transplantation
anti-CD3; coated with MECA 79 antibody to enhance lymph node accumulation	Mouse skin and heart (8)	*In vivo* post-transplantation
Class II transactivator (CTIIA) siRNA	Human artery (33)	*Ex vivo* normothermic pre-treatment

Polymeric Micelle

Micelle

Payload	Allograft model	Administration
Tacrolimus and rapamycin	Mouse skin (9)	*In vivo* post-transplantation
Rapamycin; coated with cRGD peptide to target endothelial cells	Mouse aorta and trachea (12)	*Ex vivo* hypothermic pre-treatment

Polymersome

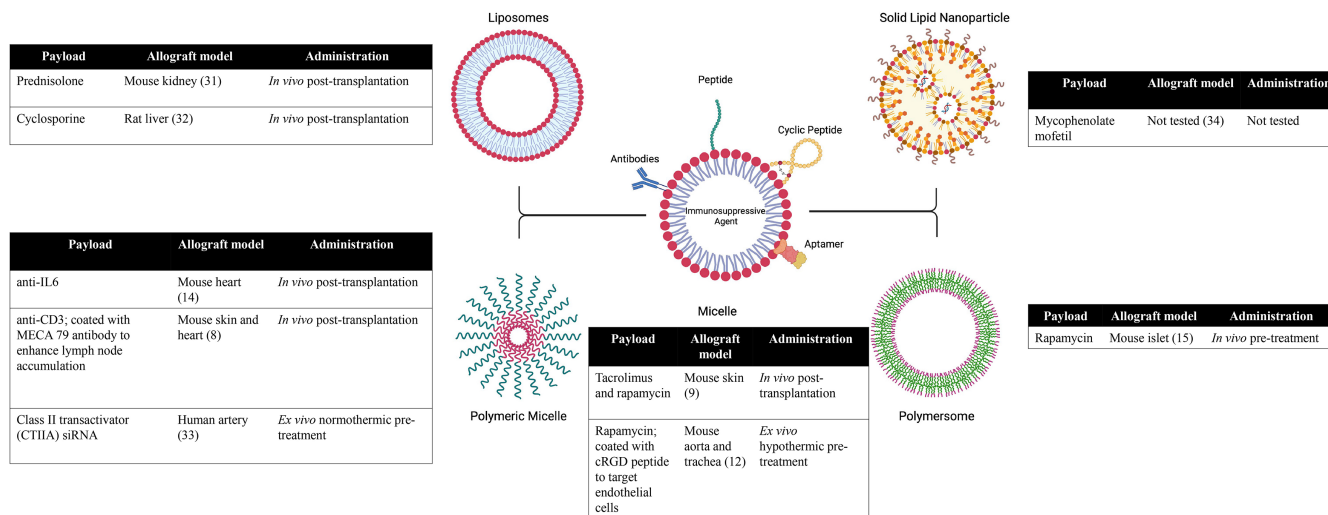| Payload	Allograft model	Administration
Rapamycin	Mouse islet (15)	*In vivo* pre-treatment

FIGURE 1 Various formulations and structures like micelles, liposomes, polymeric micelles, solid lipid nanoparticle (NP), and polymersomes have successfully been used in pre-, peri-, and posttransplant studies. Typically, an immunosuppressive agent is encapsulated inside the NP, and cell or tissue targeted delivery is achieved by conjugating antibodies or aptamers or peptides or cyclic peptides on the surface of the NPs. Some examples of each NP developed and/or tested for transplantation are shown[8,9,12,14,15,31-34]

several advantages including targeted payload delivery, sustained and controlled drug release, improved stability and solubility of payload, and improved efficacy with reduced toxicity of payload therapeutics.[3] This can be accomplished through the modification of the particle itself, such as including moieties on the surface of NPs that facilitate cell-specific targeting or triggered drug release based on pH or other factors. A recent example includes the use of lipid NPs in vaccine development toward SARS-CoV-2 in the delivery of mRNA.[4]

Although the use of NPs as drug delivery vehicles is not new, their use in transplantation is growing rapidly. Here, we describe the role of NPs in transplantation and identify barriers and pathways to clinical implementation.

1.1 | Role of nanoparticles in transplantation

Significant progress has been made in improving early outcomes in transplantation but improvements in long-term survival have been elusive. Poor long-term outcomes and deleterious effects of current immunosuppressive agents (ISA) predispose recipients to a wide range of complications ranging from infection and metabolic derangements to cancers, further exacerbated by the current cost and financial burden associated with these medications.[5,6] The use of NPs could overcome the current limitations in transplantation including systemic immunosuppressive drug delivery, lack of organ therapeutic pretreatment strategies, cell-specific drug targeting, personalized immunosuppressive regimens, and use of marginal organs. Ongoing investigations in transplant research are centered around manufacturing NPs using mechanisms that can blunt early allograft insults and/or minimize ISA risk, which is important to improve transplant outcomes.

Due to the unique nature of transplant surgery, there are multiple time points in which the use of a nanotherapeutic can be investigated, with current research primarily focused around either in vivo systemic delivery posttransplant or ex vivo pretreatment of the organ prior to transplantation.[3] NPs administered posttransplant provide an opportunity for controlled and localized targeting of the antigen-presenting cells (APCs) involved in systemic immune responses. The goal of targeting APCs is to potentially induce immune tolerance to prevent graft rejection. NPs administered to donor organs prior to transplantation (pretreatment) provide a unique opportunity to target specific organs, cell types, and harmful signaling mechanisms that are inevitably triggered as a consequence of ischemia-reperfusion injury (IRI).[7] In addition to targeting, pretreating donor organs prevent the risk of systemically exposing the therapeutic to the recipient patient. Moreover, targeting the signaling pathways to condition organs ex vivo or mitigate early injuries in the graft could potentially allow for the inclusion of marginal organs and increase the donor pool. A list of various NPs used specifically in transplantation is enumerated in a comprehensive review by Yao and Martins.[3] Below, we discuss specific NP-based therapeutic strategies administered to the recipients or the donors.

1.2 | Recipient-specific treatment strategies

Recent advances in NP bioengineering have explored the conjugation of targeting moieties to NPs as a means to specifically deliver NPs to predetermined sites to further reduce off-target effects. NP targeting moiety alterations have allowed for targeting of diverse cell populations, including resident lymphocytes in lymph nodes (LNs) and the allograft's vascular endothelium.[8-12] The goal is to lower the systemic exposure to these potentially toxic, but necessary, immunotherapeutics. As described above, many alterations are being created to work towards either inducing long-term tolerance in ideal organs or mitigating the negative impacts of IRI in marginal organs.

NP targeting moiety alterations represent a method for enhanced NP retention in tissues expressing the desired target proteins, thus increasing the bioavailability of the drug at the desired location.

Bahmani et al. reported on a novel intravascular delivery platform for LN-targeted administration of drugs to lymphatics that bypasses the current limitation, which relies on the administration of drugs to lymphatic vessels through the skin.[8] They synthesized a MECA79 monoclonal antibody (mAb)-coated NP that carries an anti-CD3 payload (MECA79-anti-CD3-NP). MECA-79-anti-CD3-NPs have a reduced rate of clearance by the mononuclear phagocyte system, allowing it to have a prolonged circulatory time with minimal physical entrapment in the lung. Their data demonstrated that targeted delivery of anti-CD3 to LNs dramatically increased efficacy by inducing long-term allograft survival in a murine heart transplant model. The use of these NPs as an induction strategy could greatly improve the safety profile of induction immunosuppression by enabling significantly lower systemic dosing without sacrificing efficacy.

These studies enabled lower systemic dosing, raising the question of whether NPs could induce long-term tolerance and eliminate the need for ongoing immunosuppression. Braza et al. identified a macrophage activation pathway through which dectin-1 and toll-like receptor 4 activation drive immunity-associated cytokine production contributing to allograft rejection.[13] In this study, a myeloid-specific nanoimmunotherapy based on high-density lipoprotein (HDL) nanobiologics was developed and characterized to synergistically target mTOR (mTORi-HDL) and CD40-TRAF6 (TRAF6-HDL) as a means to induce long-term organ transplant acceptance in a murine cardiac transplant model. Treatment with mTORi-HDL alone resulted in significantly lower TNF-α and IL-6 protein expression and lactate production by graft-infiltrating macrophages after ex vivo lipopolysaccharide stimulation. When TRAF6i-HDL NPs were added to the treatment, the mTORi-HDL/TRAF6i-HDL treatment synergistically promoted organ transplant acceptance, with >70% allograft survival at 100 days posttransplant. They concluded that short-term treatment with mTORi-HDL/TRAF6i-HDL combination therapy was able to induce long-term allograft survival with the potential to facilitate successful organ transplantation without needing continuous immunosuppression.

Solhjou et al. observed an early increase in intragraft IL-6 levels in ischemia in a murine heterotopic intra-abdominal cardiac transplantation model which prompted an evaluation of the role of allograft-derived dendritic cells as a potential source of IL-6 which promotes CD4^{+} alloreactive T cell activity.[14] They went on to develop the first controlled-release formulation of an anti-IL-6 nanomedicine and intragraft NP delivery platform using poly(lactic-co-glycolic acid) (PLGA, 50:50 lactic acid:glycolic acid) NPs with anti-IL6 antibody. In their murine heart transplant model, they treated recipients with systemic anti-IL-6 or single-dose local intragraft treatment. Systemic anti-IL-6 treated grafts had significantly less macrophage infiltration and vascular injury compared with controls. Moreover, it protected the grafts from the development of chronic rejection as demonstrated by a reduction in inflammatory cell infiltrates, macrophage

infiltration, and vascular injury at a dose that was nine times lower than systemic dosing.

Burke et al. designed poly(ethylene glycol)-b-poly(propylene sulfide) (PEG-b-PPS) polymersome (PS) nanocarriers for subcutaneous delivery of rapamycin (rPS).[15] Rapamycin is a potent immunosuppressive drug and, yet, is not readily used in the perioperative period due to a host of adverse side effects when administered systemically.[16] By encapsulating rapamycin in PS nanocarriers, rPS achieved antigen-specific tolerance for transplanted pancreatic islets in a major histocompatibility complex (MHC)-mismatched, allogeneic, intraportal (liver) transplantation model during the treatment of type I diabetes. This was achieved due to the ability of NPs to selectively concentrate rapamycin within APCs, while avoiding direct drug delivery to T cells, effectively limiting side effects, avoiding systemic immunosuppression, and selectively tolerizing donor antigen.

1.3 | Organ-specific treatment strategies

Transplant is unique in that the donor organ is isolated prior to implantation into the recipient. A logical first step to the application of NPs could be applied to the donor organ prior to transplantation. Incorporation of NPs into preservation solutions or as part of pulsatile organ perfusion systems could allow for direct, as well as targeted delivery of the drugs to the donor organs. In addition, it will enable detailed biodistribution and pharmacokinetic studies to provide the means to optimally deliver NPs to the donor organ.

There is growing appreciation that injuries like IRI occurring early posttransplant set in motion a cascade of events that contribute to long-term graft dysfunction. During IRI, endothelial cells (ECs) lining the graft vasculature are subjected to oxidative stress and mitochondrial dysfunction.[17-20] Consequently, they are activated and behave as semi-professional APCs to elicit pro-inflammatory responses[21] and contribute to the development of downstream vasculopathy, the hallmark pathology of chronic transplant dysfunction.[22] Modulation of the EC-mediated early immune responses in the graft could, therefore, be key to improving transplant outcomes. Our group has developed pH-sensitive micelle NPs loaded with rapamycin (TRaM) and decorated with cyclic arginine-glycine-aspartate moieties to target integrin alpha-v beta-3 on the EC.[10] The targeting moiety significantly improved TRaM uptake and downregulated production and release of IL-6 and IL-8 in mouse cardiac and human umbilical vein ECs in vitro. In an in vitro injury model using cold-storage hypoxia followed by reperfusion, ECs treated with TRaM NPs significantly downregulated memory T cell responses. To further validate TRaM NPs in vivo, mouse trachea, and aorta transplantation models were used in which TRaMs were delivered as a constituent of the organ preservation solution.[12] When comparing donor organ TRaM augmented University of Wisconsin solution (UW) pretreatment with standard-of-care UW alone and free rapamycin augmented UW, TRaM pretreatment was associated with significant protection against the development of chronic rejection in both allograft

models, as compared to all other groups. In the trachea model, TRaM-treated tracheas had minimal evidence of disease pathology and no fatal tracheal dehiscence, which was seen in half of the free rapamycin-treated recipients. In the aortic interposition model, a single dose of 100 ng/ml TRaM (1/10th the dose of free rapamycin) significantly decreased vasculopathy that was similar to isogeneic controls.

Tietjen et al. used NPs targeting the endothelium in studies of normothermic machine perfused human kidneys.[11] Normothermic machine perfusion (NMP) creates an opportunity for the ex vivo delivery of the therapeutic agents to the isolated organ and may also provide a therapeutic benefit itself by reducing the severity of IRI to graft vasculature.[23] They conjugated anti-human CD31 antibodies to poly(lactic acid)-poly(ethylene) glycol (PLA-PEG) NP loaded with a fluorescent dye and administered them to isolated human kidneys during ex vivo NMP. Conjugating the anti-CD31 to the PLA-PEG NP led to enhanced vascular retention compared with nontargeted NPs. Renal vascular ECs are highly susceptible to both IRI and damage by pre-existing donor-specific antibodies, making them an ideal target for NPs. In a follow-up study, they evaluated the efficacy of the addition of plasminogen at a concentration of 10 μg/ml and tissue plasminogen activator (tPA) dosed at 100 μg/kg of graft to organ preservation solution in NMP of marginal human organs as a method of lysing microvascular obstructions that impaired microvascular blood flow leading to nonspecific accumulation of the targeted NP.[24] Treatment with both plasminogen and tPA during NMP not only lysed the fibrinogen plugs but also resulted in enhanced retention of the NPs in the glomeruli and microvessels. This improved delivery/retention led to improved urine production and vascular resistance and reduction in kidney injury markers such as NGAL, ICAM-1, and IL-6.

These studies demonstrate that, even in an isolated setting, the delivery of NPs is not straightforward. The results of these studies outline the progress being made in the delivery of NPs directly to a vulnerable cell population and the mitigation of the negative impacts of IRI and improvement in transplant outcomes; however, they are only initial steps in uncovering the role that NPs can play in impacting organ transplantation.

1.4 | Barriers to clinical implementation of nanoparticles

NPs offer several advantages in transplantation, demonstrated through the preclinical studies discussed above. Yet, barriers exist that prevent the translational use of NPs for humans. Differences between humans and animal models can influence the distribution and efficacy of NPs.[25,26] Identifying the differences and finding solutions can improve their clinical implementation. NP properties like shape, size, and charge are also a barrier, as these properties dictate its stability, interaction with cells including immune cells, distribution in the tissues, and release and bioavailability.[27] These are important attributes to consider when designing NP systems. Further

strategies to maximize the efficacy of NP targeting are under development and are heavily dependent on the type of organ undergoing transplantation. The goal of the NP in development will also play a role—is the primary objective of the NP to improve marginal organs through minimizing the negative impacts of IRI, induce long-term tolerance or both? These all represent areas undergoing further research, development, and testing.

Barriers to implementation from a regulatory standpoint exist. There are significant costs and time associated with FDA approval of a "new drug," which some NPs are deemed. For those nanotechnology-based platforms that use an existing FDA-approved therapeutic, it is expected to take between 3 and 4 years, costing between $20 and 50 million, to be able to use the therapeutic commercially.[28] It is difficult to ascertain the full impact that preclinical research in non-transplanted human organs would have on the timeline and cost of the introduction of new NP-based therapeutics, but one can speculate that it would expedite the process while being less expensive than other options. Use in transplantation could facilitate rapid demonstration of safety and efficacy, which would open the door to other indications. In fact, the FDA's designation of "orphan status" to therapeutics intended for use in transplantation, can help expedite the demonstration of safety and efficacy in many of the aforementioned nanoformulations due to clinical trial tax credits and market exclusivity. However, translation of some "new" delivery systems, including NP-based products containing genetic material, biomimetic proteins, or artificial APCs, would be more difficult as those technologies will likely be deemed new drug(s). Their FDA approval process would likely cost over $500 million and take 10+ years before being able to bring the new nanotherapeutic to market.[28]

The introduction and implementation of any new technology or therapeutic faces challenges. Some of these will directly impact the development and adoption of NPs as part of the standard of care, including things like controllable and reproducible synthesis, evaluation and screening, and scalable manufacturing.[29] Addressing these challenges is an area of ongoing research and process improvement. Challenges still exist in the evaluation and screening of NPs both in vitro and in vivo. Additionally, preclinical and early phase clinical trials represent a necessary but timely and costly step in clinical translation. We anticipate that the initial introduction of NPs into transplantation will be focused on the delivery of existing drugs, and once NPs have gained acceptance, further work exploring the introduction of "new drugs" will be undertaken.

1.5 | Future directions of nanotechnology in transplantation

The FDA is a member agency in the National Nanotechnology Initiative, a federal research and development program that was established to coordinate the multi-agency efforts in nanoscale science, engineering, and technology. They have recognized the importance and promise of nanotechnology and have taken steps to accelerate progress for those making developments in the

field, including publishing formal guidance for the industry regarding the types of information that should be included in drug applications.[30]

A wide range of FDA-approved ISAs exist and are readily captured into various NPs. Renal allografts are frequently placed into pulsatile flow perfusion machines, making the addition of NPs to the preservation solution a simple modification that could readily be adapted. NP delivery during perfusion allows for assessment of the efficacy of NP binding dynamics and retention. Assuming minimal off-target effects and systemic absorption, NP pretreatment during perfusion provides benefit from a regulatory perspective of eliminating the need to collect data on the systemic pharmacokinetics and pharmacodynamics of the NPs within the recipient. A key determinant to the ease of obtaining FDA approval for this approach hinges on if the NP would be considered a part of the perfusion device or a separate therapeutic as this could greatly accelerate the FDA review process and applications clinically.

Whether immunosuppressive NPs are delivered ex vivo or in vivo, determining off-target effects and identifying ways to minimize them is imperative and requires an enhanced understanding of the biodistribution and trafficking of various delivery methods to allow for delineation of the off-target effects and kinetic patterns for delivery modes and organs transplanted. A growing body of evidence supports the role of pretreatment strategies abrogating some pathways that contribute to long-term graft dysfunction and failure. Ex vivo perfusion of organs is intended to mitigate ischemic injury that occurs during organ preservation. This "therapeutic window" has been used experimentally to effectively immunosuppress or deliver anti-inflammatory agents to grafts prior to, or during, preservation; to focus delivery of the therapeutic to grafts; to control the release of payload cargo; and to protect grafts from injury immediately posttransplant, potentially facilitating tolerance induction by modifying the allogenicity of the grafts. Thus, ex vivo delivery may represent a strategy that would facilitate the introduction and adoption of nanotherapeutics into transplantation, potentially paving the way for their adoption and use in other fields of medicine.

2 | CONCLUDING REMARKS

Although barriers to the implantation of NPs in transplantation exist, they are not insurmountable. NP-based therapeutics can minimize recipients' ongoing needs to ISAs through systemic recipient therapy or organ pretreatment. NPs hold promise to deliver drugs within a specific therapeutic range, improving the efficacy of treatments while reducing necessary drug dosage and associated toxicities.

DATA AVAILABILITY STATEMENT
Data sharing is not applicable to this article as no new data were created or analyzed in this study/minireview. Data sharing is not applicable to this article/minireview as no data sets were generated or analyzed during the current study.

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