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Intra-Organ Delivery of Nanotherapeutics for Organ Transplantation

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Abstract

Targeted delivery of therapeutics through the use of nanoparticles (NPs) has emerged as a promising method that increases their efficacy and reduces their side effects. NPs can be tailored to localize to selective tissues through conjugation to ligands that bind cell-specific receptors. Although the vast majority of nanodelivery platforms have focused on cancer therapy, efforts have begun to introduce nanotherapeutics to the fields of immunology as well as transplantation. In this article, we provide an overview from a clinician's perspective of current nanotherapeutic strategies to treat solid organ transplants with NPs during the time interval between organ harvest from the donor and placement into the recipient, an innovative technology that can provide major benefits to transplant patients. The use of *ex vivo* normothermic machine perfusion (NMP), which is associated with preserving the function of the organ following transplantation, also provides an ideal opportunity for a localized, sustained, and controlled delivery of nanotherapeutics to the organ during this critical time period. Here, we summarize previous endeavors to improve transplantation outcomes by treating the organ with NPs prior to placement in the recipient. Investigations in this burgeoning field of research are promising, but more extensive studies are needed to overcome the physiological challenges to achieving effective nanotherapeutic delivery to transplanted organs discussed in this review.

Graphical Abstract

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Author Contributions

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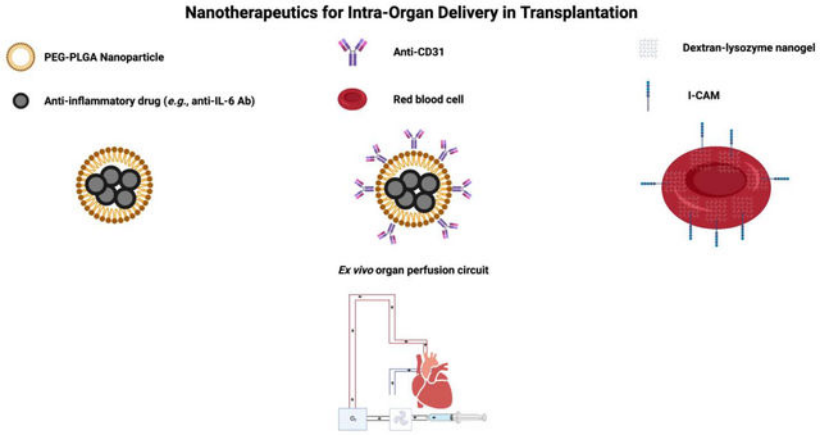

Keywords

nanotherapeutics; nanoparticles; transplantation; endothelial cells; immunology; machine perfusion; ischemia; ischemia-reperfusion injury; dendritic cells; transplant rejection

Adverse effects that accompany the delivery of systemically administered immunosuppressive agents (ISAs) constitute major obstacles to their use and also hinder long-term success in transplantation.[1–4] The emerging method of nanodelivery holds the potential to surmount this barrier and produce a transformative impact on the administration of ISAs by facilitating their transport to specific organs and tissues in a targeted fashion. This targeted method of delivery for ISAs can limit their interactions with unintended sites and the subsequent off-target toxicity.[5–7] Other advantages of nanotherapy over conventional systemic medical therapy include a reduction in the required dose of ISAs and a capacity for customization, such as surface modification.[8–10]

Nanoparticles (NPs) are enclosed bodies of matter with a typical diameter of around 100 nm that can encapsulate a variety of therapeutic agents. The composition of NPs can vary from organic materials, including polymers, liposomes, and proteins, to inorganic materials, such as gold, iron oxide, and quantum dots.[8, 11–13] NPs have widespread potential medical applications, due to the principle of active targeting. Active targeting refers to a method in which conjugation to the NP of ligands to receptors that are present in cells of the destination tissue confers the potential capacity of specific delivery of the NP to that tissue.[14–18] Successful targeting by NPs relies on physical contact of the NP with the intended target cell and subsequent ligand-mediated retention at the site.[14]

In clinical practice, successful delivery of NPs to their target faces significant obstacles created by potential interactions with cells in other parts of the body, clearance by the kidney[19–21] or mononuclear phagocytic system (MPS) in the liver[22–24] and spleen,[25–27] as well as trapping and sequestration in the lung capillary bed.[28–31]

Solid organ transplantation is an ideal clinical scenario in which therapeutic agents can be delivered directly to the organ, during the time period between removal from the donor and placement inside the recipient.[32–34] This opportunity for direct access to the organ is

a rarity in medicine, a setting that enhances the potential utility of nanotargeting through simplification of the kinetics of administration.[35–38]

Pertinent physical properties of NPs for intra-organ delivery

A major potential benefit to the use of NPs is optimization of the pharmacokinetics of a drug with a narrow therapeutic index or low bioavailability. These pharmacokinetic properties can be tuned by altering the chemical and physical characteristics of the NP, including its size, surface charge, shape, and surface composition.[39–41]

Size:

NP size is an important parameter that can be modified to direct the delivery of a drug to a particular organ.[42] A significant body of work exists on the importance of NP size variations for applications in cancer.[42, 43] Historically, alterations in the size of NPs have been undertaken to increase their systemic circulation. Following intravenous injection, those NPs smaller than 5 nm in diameter are cleared very rapidly by kidneys, while NPs larger than 200 nm are cleared rapidly by the MPS in the liver and spleen.[21, 43, 44] The endothelium of the liver is non-continuous with fenestrations of 50–100 nm in diameter, so NPs in this size range will accumulate preferentially in the liver.[45] Due to the size of the gap junctions (GJs) between endothelial cells in the spleen, NPs in the 200–500 nm range will accumulate there.[46] A diameter between 100 to 150 nm is often considered an ideal NP size in the field of cancer therapeutics, as this range exceeds the size threshold for clearance by the kidneys but also falls short of the size that results in internalization by the MPS.[43, 47, 48] Increasing the circulation time of the NP raises the chance that the NP will accumulate in the tumor.[43] Nonetheless, the rise in awareness of the importance of lymphoid tissues to mounting an effective anti-cancer immune response may signal a need to revise this size guideline, as the internalization of some NPs that incorporate ISAs by the MPS in the spleen may boost anti-cancer immunity.[48]

NPs can be injected directly into the arterial blood supply of the organ prior to transplantation, but future experiments are required to determine the proper size for maximizing the intra-organ penetrance of NPs following intraarterial injection.[47] As the organ could be perfused by a pump in this scenario, additional studies are required to assess the clearance kinetics of NPs in this setting. Nonetheless, the first layer of cells with which these NPs interact are endothelial cells (ECs). Some NPs traverse passively through the endothelium *via* intercellular connections called GJs, which are formed between adjacent ECs.[49–52] GJs are vital to intercellular transfer of ions, small molecules, nutrients, and secondary messengers.[53, 54] GJs also play an important role in vascular inflammation and the stiffness of ECs.[50] On the other hand, the surface of NPs could also be coated with various antibodies to produce an active interaction with the ECs (*e.g.*, anti-CD31 antibody).

Cold ischemia time refers to the storage period of transplanted organs in cold solution, immediately following their removal from the donor and prior to the time that they are warmed through restoration of blood supply.[32, 55] Ischemia results in the closure of GJs and opening of hemichannels,[56] which are comprised of six subunits of a protein called connexin and form one half of the GJ. In the heart, cardiac ischemia can result in

closure of GJs between myocytes, decreasing intercellular conductance[57–61] and thus also the passive entrance of NPs into the heart tissue. Cold ischemia is a specific cause of hypoxic uncoupling, which results in endothelial GJ damage, a major factor responsible for transplant rejection.[62] In fact, addition of the Cx43 mimetic peptide ACT-1 to University of Wisconsin (UW) preservation medium stabilized the endothelial GJs of mouse cardiac tissue in cold storage and notably reduced ischemia-reperfusion injury (IRI).[63] Therefore, this tool could be used to increase the targeting efficacy of NPs that rely on GJs to enter transplanted organs.

IRI of the kidney has been associated with disruption of tight junctions, areas of close contact between adjacent epithelial cells in the kidney, through which solutes greater than 1.8 nm typically cannot pass.[64, 65] These losses in the integrity of tight junctions suggest that ischemic organs may permit the entrance of NPs of greater size and quantity. However, future studies are required to test this hypothesis.

Surface charge:

NP surface charge, measured as zeta potential (ξ), can also be manipulated to prolong the circulation time and effect targeted delivery.[43] NPs with neutral and negative surface charge have decreased serum protein adsorption, thereby extending circulation time.[66] However, positively charged NPs undergo more robust non-specific uptake by organs.[42, 67] The positive charge can interact specifically with the glycocalyx, a negatively charged layer of polysaccharides that covers the cell membrane of some endothelial cells.[44] Thurston *et al.* showed that cationic liposomes are internalized robustly by tumor-associated endothelial cells.[67] Positive surface charge also promotes endosomal release of payloads and limits intracellular drug degradation.[68] Thus, NPs with neutral or negative charge have prolonged circulation following intravenous (iv) administration, but a positive surface charge facilitates efficient uptake by some target cells and successful release of the payload inside these cells.[42, 69]

The kidney, the most commonly transplanted organ, presents particular challenges to effective targeted drug delivery on the basis of charge considerations.[70–72] The glomerular basement membrane, a major component of the blood filtration apparatus of the kidney, has a strongly negative charge and thereby functions as a major barrier to the filtration of negatively charged molecules.[73] Prior studies have demonstrated that among similarly sized molecules, those that are positively charged cross the filtration barrier faster than neutral molecules, which in turn cross faster than negatively charged molecules.[74] Therefore, localization of the NPs to the tubular compartment *via* passage through the glomerular filtration apparatus in the kidney likely requires the synthesis of neutral or positively charged NPs.[75]

Shape:

Another important parameter to consider is the shape of NPs, which is also a crucial determinant of their half-life in the circulation.[42] Gentile *et al.* showed that discoidal particles tend to marginate and adhere to endothelium more readily than spherical particles, due to specific tumbling and margination dynamics.[76] Geng *et al.* demonstrated that filo-

micelles (filamentous polymer micelles) align naturally with blood flow, resulting in longer circulation time (>1 week) than spherical NPs (2–3 days).[77] The shape of NPs also affects cellular internalization, as the initial contact angle of the NP upon macrophage contact determines its rate of phagocytosis.[43] Parallel alignment of the short axis of the NP with the cell membrane facilitates more rapid internalization than alignment along the long axis.[43, 78] For rod-shaped NPs, internalization is faster when they are perpendicular to the cell axis, *i.e.* $\theta = 90°$.[79] For spherical NPs, NPs with a length of normalized curvature, denoted Ω, of ≤45° undergo faster internalization than particles with a $\Omega \geq 45°$.[42, 80] Park *et al.* showed that tumors internalized paclitaxel filo-micelles more robustly in comparison to spherical NPs upon IV administration.[81]

Surface composition:

Many different materials can be harnessed to form NPs used for intra-organ drug delivery, of which the most salient will be highlighted here. Poly(lactic-co-glycolic acid) (PLGA) is a biodegradable organic polymer that is used commonly to form NPs. Drug encapsulation into PLGA NPs leads to stabilization, prolongation of circulation time, and guided release of the drug.[82]

The surface of PLGA NPs can also be altered through the attachment of different molecules, such as sialic acid or glycolipids. Polyethylene glycol (PEG) provides the PLGA NP with the capacity for evasion of uptake by the mononuclear phagocytic system. For example, cyclosporine, an ISA used commonly for suppression of transplant rejection, can be encapsulated into PEG-PLGA NPs for controlled release, thereby stabilizing the inherent variability in its pharmacokinetic profile as it maximizes its therapeutic efficacy and minimizes its toxicity.[83, 84] Additionally, envelopment of the ISAs tacrolimus and sirolimus into liposomes has also yielded similar success.[85–88] Active targeting can be achieved through modification of the PEG molecule with monoclonal antibodies or ligands.[89–93]

High-density lipoproteins (HDLs) can also be used to construct small dynamic NPs that modify the activity of the immune response through their internalization by macrophages.[94] NPs formed by HDL target myeloid cells with high specificity, delivering immune therapeutics to APCs readily *in vivo*.[95] Binding by HDL NPs to ATP-binding cassette receptor A1 and scavenger receptor type B-1 on the surface of myeloid cells mediates this interaction.[96] Therefore, HDL-NPs represent another efficacious approach to improving ISA therapy in transplant recipients.[97]

Finally, intracellular adhesion molecule-1 (ICAM-1) antibody-coated nanogels composed of a mixture of dextran and lysozyme have been found to provide efficient delivery of drugs *in vitro* and *in vivo* to endothelial cells.[98]

NP stability:

NP stability refers to the preservation of a specific NP parameter in a given condition.[99] One major property that correlates to the integrity of NPs upon their collision with each other is aggregation.[99–101] Aggregation must be mitigated to maintain stability of NPs, due to their tendency to cluster following these collisions.[102] The creation of this protection can be achieved through adjustment of pH, temperature, and the use of stabilizing agents,

such as poly(vinyl pyrrolidone).[99, 103] Stability of the chemical composition of the NP is another major factor that must be considered. Oxidation from reactive components in the medium can result in chemical instability.[104] Approaches using inert metals,[105, 106] graphene oxide,[107] or a complex surfactant[108] have proven to preserve the chemical composition of NPs.[99] Successful strategies to stabilize NP shape correlate to retention of its original structure and radius of curvature.[99, 100, 109] Surfactants, such as oleylamine, can be utilized to maintain the shape of NPs by reducing adsorption.[110] Stability of NP size refers to conservation of the dimensionality of the NP throughout the course of an experiment or in storage solution.[99, 111] Low-density stabilizing agents assist in preserving the stability of large NPs; alteration of surface composition through the use of PEG or EDTA can serve to accomplish this goal.[112–114] Finally, stabilization of surface chemistry refers to maintenance of the original surface potential, structure, and composition of the NP.[99, 100, 105] Altering the pH of the solution can assist in retention of surface chemistry; for example, silica NPs are stable in a solution with a pH of 5.5 to 6.5 for up to 50 hours.[115]

Endocytosis of NPs

Equally as important as the forces that govern the extracellular localization of the NP is the intracellular distribution of the NP at its target, as this determines the efficacy and bioavailability of the drug that it carries.[116] NPs are internalized into cells *via* an active transport mechanism called endocytosis.[117] During this process, the cell membrane invaginates and engulfs the NP from the extracellular environment, forming an intracellular membrane-bound vesicle called an endosome.[117, 118] Then, the endosome fuses with a lysosome, another membrane-bound vesicle that contains hydrolytic enzymes, resulting in degradation of the encapsulated NP and release of the drug.[119, 120] Endocytosis can be classified into several different processes, such as phagocytosis, pinocytosis, clathrin-mediated endocytosis, and caveolae-mediated endocytosis.[121, 122] Phagocytosis and pinocytosis can be differentiated on the basis of endosome size; large particles are engulfed by large endosomes in the former, whereas small endosomes capture small volumes of fluid in the latter.[117, 123, 124]

Clathrin-mediated endocytosis is a receptor-mediated pathway, in which a ligand binds to a receptor on the cell membrane in a clathrin-rich area, where the ligand-receptor complex is engulfed subsequently in clathrin-coated vesicles.[125–127] These vesicles fuse with endosomes and are degraded *via* the endosomal-lysosomal pathway.[117, 128–131] Caveolae-mediated endocytosis requires caveolae, which are flask-shaped invaginations of the cell membrane.[132, 133] Following detachment from the cell membrane, caveolae fuse with caveosomes, thereby avoiding lysosomes.[134, 135] Therefore, this internalization pathway does not involve degradation and could be most favorable for the transport of NPs.[117, 136, 137]

Organ transplantation as a notable medical application for nanotherapy

As discussed earlier, when drug-loaded NPs are administered systemically, many of the NPs accumulate at off-target sites, despite the specificity created by surface conjugation and the principle of active targeting. The dilemma posed by competition with off-target binding

sites and interference by the mononuclear phagocytic system during *in vivo* administration of therapeutics can be circumvented *via* solid organ transplantation, a clinical scenario in which the organ is temporarily accessible for direct *ex vivo* treatment. A key feature of organ transplantation is the emergence of *ex vivo* normothermic machine perfusion (NMP).[138]

NMP can expand the pool of available organs to include those of marginal initial quality, by enhancing graft assessment, preservation, and resuscitation, through constant supply of oxygen and nutrients as well as clearance of toxic metabolites. Currently, *ex vivo* kidney perfusion is the most highly developed extracorporeal perfusion technique, although liver, heart, intestine, and lung perfusion have also been introduced into clinical practice.[139–143] In addition, machine perfusion of vascularized composite allografts, such as limbs, has created possibilities for transplantation or autologous reimplantation.[144]

NMP is an ideal process by which therapeutics can be delivered directly to the organ, prior to its implantation in the recipient, In a localized, sustained, and controlled fashion[87] (Figure 1). Therefore, this modality can be explored to overcome the limitations of *in vivo* administration, as discussed earlier. This technology has been associated with a reduction in delayed graft function (DGF) by reducing the cold ischemia time of the organ.[145] DGF is an important and common complication of transplantation, affecting around 31% of kidney transplants in the United States.[146] DGF is also a significant risk factor for acute rejection and reduced long-term survival of the organ.[147–149]

ISAs can also be delivered *via* NMP to reduce the total exposure to systemic immunosuppressive medications post-transplantation.[8] In addition, machine perfusion also permits *ex vivo* assessment of isolated kidneys through the analysis of secreted factors in the perfusion fluids,[150, 151] which can indicate the quality of the organ prior to its transplantation.[152]

Application of NPs for Organ Transplantation

Research into the application of NPs for organ transplantation remains in its infancy. However, several recent publications have demonstrated important breakthroughs in this field. ECs, the single layer of cells that line the inside of a blood vessel and regulate the exchange of molecules between blood and tissue, represent the first point of contact for NPs with the organ in both *in vivo* and *ex vivo* administration settings. They are also the primary site of damage from both IRI and preformed anti-donor antibodies. Therefore, limiting the perioperative injury to ECs through targeted drug delivery can mitigate the severity of the alloimmune response in the recipient and thereby yield long-term benefits.[153]

ECs as the first points of contact for NPs position them well for drug targeting. However, several factors must be considered to ensure proper efficacy of the therapeutic platform. First, some target molecules may be shed by ECs under pathological conditions, such as acute inflammation and ischemia. An example is thrombomodulin, which escapes from the lung following acute lung injury, thereby reducing by half the localization of anti-thrombomodulin antibodies to the lung.[44, 154, 155] The exact location of the ligand within the EC membrane is important as well. Molecules that are obscured by glycocalyx or located

within intercellular junctions will be more difficult to target.[156] In addition, proteins that are situated inside membrane invaginations (such as caveolae) will be more challenging to access, as the mouths of the caveolae are typically no greater than 50 nm in diameter.[157, 158] Conversely, some pathological conditions that are associated with loss of the glycocalyx may uncover some ligands, such as intracellular adhesion molecule-1 (ICAM-1), and boost their interaction with targeting moieties.[159]

The authors of an innovative investigation examined the efficacy of a practice that they termed “red blood cell hitchhiking,” in which nanocarriers described above, comprising of a dextran and lysozyme mixture,[98] were adsorbed onto red blood cells and injected *ex vivo* into subsegmental branches of pulmonary arteries of human lungs.[160] These red blood cell-adsorbed nanocarriers, which were found earlier to internalize readily to ECs *in vitro*,[98] accumulated in the lungs at 3.7-fold higher density than free nanocarriers.[160] However, whether the ECs of the human kidney, the most commonly transplanted organ in clinical practice, could be targeted successfully by NPs was unclear. To answer this question, an important study conducted by Tietjen *et al.* evaluated whether surface conjugation to polymeric NPs of an Ab reactive with an EC surface molecule could enhance NP targeting to vascular ECs during *ex vivo* NMP of human kidneys. They adapted an established approach for conjugating a mouse monoclonal Ab to fluorescent dye-loaded poly(lactic acid)–PEG (PLA-PEG) NPs of a consistent diameter and initially used these NPs to analyze attachment to cultured human umbilical vein ECs (HUVECs) under static conditions or in a microfluidic flow chamber under conditions of shear stress that more closely resemble those created during *ex vivo* NMP. In these initial experiments, the investigators compared anti-CD31-conjugated NPs (CD31-NPs) to NPs conjugated with the relevant isotype control (Control-NP), and they quantified the effects of NP concentration as well as duration of treatment by fluorescence microscopy and flow cytometry on transplant rejection. Then, they assessed the localization of NPs to ECs in 8 human kidneys that had been declined for transplant during *ex vivo* NMP, under the same conditions as would be applied to organs used for transplantation.[153] This study revealed that the attachment of monoclonal anti-CD31 Ab to the surface of PLGA-PEG NPs loaded with a fluorescent dye and administration of these CD31-NPs to isolated human kidneys during *ex vivo* NMP can lead to enhanced vascular retention, as compared to unconjugated NPs. Using two-color quantitative microscopy on cryosectioned biopsies, the authors observed that CD31-NPs accumulated at a 5- to 10-fold higher rate in the renal vasculature, as compared to the Control-NPs. This approach showed that attachment to NPs of Abs targeted to ECs can augment their accumulation in the ECs of both the glomerular and peritubular capillaries in the kidney during *ex vivo* NMP.[153]

Cell-mediated transplant rejection occurs through the recognition of the human leukocyte antigen (HLA) expressed by cells of the donor graft as foreign by the T cells of the recipient. The most accessible HLA molecules in the donor graft to the T cells are located on the surface of the ECs. HLAs are member proteins of the major histocompatibility complex (MHC) that are found in humans. Cui *et al.* developed small interfering RNA-releasing poly(amine-co-ester) nanoparticles (siRNA-NPs), containing a high content of a hydrophobic lactone. They showed that a single transfection of siRNA-NPs targeting class II transactivator attenuated MHC class II (MHC-II) expression on ECs for at least 4 to 6 weeks

after transplantation into immunodeficient mouse hosts. Furthermore, silencing of MHC-II reduced allogeneic T-cell responses *in vitro* and *in vivo*. These data suggest that siRNA administered during *ex vivo* NMP of human organs could be used to modify ECs with a sustained tolerogenic effect following transplantation.[161]

In one study, a micelle of approximately 10 nm in size that encapsulated the ISA sirolimus was developed, consisting of PEG-PE-amine and N-palmitoyl homocysteine (PHC) modified with a targeting peptide (cRGD) for ECs. These targeted rapamycin (sirolimus) micelles (TRaMs) were internalized successfully by HUVECs *in vitro*. In addition, treatment with these TRaMs resulted in inhibition of production and release of the pro-inflammatory cytokines Il-6 and IL-8 by HUVECs and mouse cardiac endothelial cells, including in hypoxic conditions, such as those encountered during IRI. The study also demonstrated a dose-dependent uptake of TRaMs by aortic ECs *ex vivo*.[162, 163] In a model of skin transplantation, a visual light-crosslinkable biomaterial composed of gelatin methacryloyl that eluted an antibody against IL-6 receptor (anti-IL-6R) was created and placed between skin allografts and areas of excised skin of mouse recipients.[164] This biomaterial doubled the length of survival of the skin allografts by reducing the infiltration of alloreactive T cells and macrophages.[164]

A key advantage of intra-organ delivery of NPs is to suppress the activation of Toll-like receptors (TLRs) in the antigen presenting cells (APCs). Dendritic cells (DCs) can project cellular extensions into the lumina of blood vessels that can capture NPs from the circulation.[165, 166] Thus, coating the surface of NPs with antibodies against the macrophage antigen CD11b and dendritic cell antigen CD11c may increase their uptake into organs through direct interaction with these cells. IRI results in the opening of connexin hemichannels,[56] so a greater number of NPs may cross the endothelium between adjacent cells and undergo internalization by APCs of ischemic organs.

One study demonstrated that *ex vivo* NMP of heart allografts with PLGA nanoparticles encapsulating anti-IL-6 Ab resulted in lower chronic rejection rates of heart allografts in mice, as compared to systemic administration of IL-6. This beneficial effect, which minimized the amount of anti-IL-6 Ab delivered to the animals, was mediated though mitigating the sequelae of IRI-induced autophagy in heart allograft-resident dendritic cells.[167] An additional investigation sought to determine whether *ex vivo* NMP of heart allografts with NPs loaded with the ISA mycophenolate mofetil (MMF) prior to transplantation reduces the rate of transplant rejection in comparison to systemic administration of MMF post-transplant.[35] *Ex vivo* treatment with MMF-loaded NPs prevented the onset of rejection of the heart transplant through the inhibition of pro-inflammatory cytokines and chemokines in the heart graft. The NPs were internalized mainly by $CD11b^+$ macrophages in the organ. Together, these studies show that treatment of transplant organs *ex vivo* with ISAs prior to transplantation can become a clinically feasible method of reducing the rate of graft rejection post-transplantation through inhibition of APC activity.

Clinical pitfalls and obstacles

Vascular thrombosis:

Aggregation of NPs within the microvasculature of organs can add to the risk of microthrombi, especially in an ischemic organ that might be more predisposed towards microthrombi formation.[168, 169] NMP and cold storage can lead to endothelial dysfunction, arterial thrombosis, post-reperfusion syndrome (intraoperative hypotension following reperfusion of liver grafts),[170–172] and potential post-transplantation arterial thrombosis.[173] Injection of NPs containing heparin could reduce this potential complication of microthrombosis.[174] Amongst the various materials used to create NPs, PLGA may represent a safe choice, due to its safety profile with respect to potential endothelial toxicity.[175] On the other hand, attachment to the NP surface of antibodies that cross-react with integrins expressed on ECs may constitute additional risk, as these antibodies could potentiate EC activation and vascular thrombosis.

Effect of temperature on NP stability:

The instability of NPs in *ex vivo* perfusate also presents a major limitation to their widespread clinical use, even though considerable advances have been made to solve this problem, as previously explained. NPs are more stable in a colder environment than a warmer environment.[176] After donor death, both hypothermic machine perfusion (HMP) and static cold storage (SCS) are used to maintain the viability of the organ, and several studies have compared the efficacy of these methods in maximizing function. Jochmans *et al.* advised the use of HMP by demonstrating that it is associated with reduced risk of delayed renal graft rejection and better early post-transplant graft function.[177] Jia *et al.* demonstrated that HMP is superior to SCS for liver allograft preservation, as it improved short-term outcomes and protected against early allograft dysfunction and biliary complications.[178] These techniques can also assist in preserving the stability of NPs that are administered during this time period. Future studies are required to compare the stability and efficacy of nanotherapeutics for intra-organ delivery between HMP and NMP.

Temperature affects many properties of NPs, including size, structure, magnetism, aggregation, and stability. Intrinsic temperature-sensitive characteristics of the NP determine how temperature alters its properties. For example, increasing the reaction temperature in the synthesis of cobalt ferrite NPs ($CoFe_2O_4$-NPs), augments their size, increases their saturation magnetization, and results in the formation of an equiaxial-shaped, single-phase cubic spinel structure.[179] On the other hand, increasing the reaction temperature decreases the size of maghemite NPs (γ-Fe_2O_3-NPs), lowers their magnetization, and improves their stability.[180] Increasing the reaction temperature boosts the aggregation of gold NPs (Au-NPs).[181] Finally, intermediate reaction temperatures have been demonstrated to produce smaller silver NPs (Ag-NPs) with narrow size distribution.[182] Extensive investigation is required to expand the understanding of the effect of temperature on the characteristics of NPs and its application to machine perfusion of transplanted organs, a process that can occur in normothermic or hypothermic conditions.

Toxicity of payload:

ISAs packaged inside NPs have side effects that must still be considered in their administration. For example, calcineurin inhibitors like cyclosporine and tacrolimus cause endothelial dysfunction due to vasoconstriction, hypertension, and enhanced formation of superoxide.[183] Other ISAs like sirolimus and mycophenolate mofetil may be more favorable due to their vascular safety profile. In addition, nanosized drug delivery devices can be developed to control the release of a drug at a concentration within a specific therapeutic range, thereby circumventing the threat of toxicity and overdose, while ensuring consistent efficacy.[184, 185]

Finally, alternative molecules that dampen the immune response by shutting down the inflammasome like MCC950,[186] 3,4-Methylenedioxy-β-nitrostyrene (MNS),[187] tranilast,[188] or oridonin,[189, 190] or inhibit nuclear factor-kappa B (NF-κB) like emetine, fluorosalan, sunitinib malate, bithionol, narasin, tribromsalan, or lestaurtinib[191] can be placed inside the NPs.

Targeting recipient lymphoid tissue *via* intra-organ delivery of NPs:

An interesting concept that remains unexplored is targeting the lymphoid tissue with NPs through intra-organ delivery. As systemically administered NPs pass through blood vessels and arrive in the interstitium of the organ, some enter the lymphatic capillaries through solvent drag and arrive to draining lymph nodes (DLNs)—the quintessential sites for the mounting of adaptive immunity--*via* afferent lymphatic ducts.[192, 193] Whether NPs that enter the lymphatic capillaries during intra-organ delivery subsequently home to the LNs following anastomosis in the recipient is unknown.

NPs of higher molecular weight (1000–16,000 kDa) drain through lymphatic channels instead of blood vasculature[194, 195] However, NPs of higher size do not diffuse as easily through the interstitium, resulting in a slower drainage rate into lymphatics.[196] Ischemia and increased interstitial pressure may enhance the trafficking of these larger NPs from the interstitium to the lymphatics. These NPs could then transport potent immunomodulatory molecules directly to the DLNs, important sites for immune activation.

Conclusion

Nanotherapeutics offer a promising approach to the targeted delivery of ISAs for prevention of solid organ transplant rejection. NPs assist in optimizing pharmacokinetic properties to maximize therapeutic bioavailability, specificity, and efficacy, while minimizing toxicity. However, *in vivo* application of nanotherapeutics still faces significant physiologic barriers, such as the accumulation of NPs at off-target sites and uptake by mononuclear phagocytes for elimination. *Ex vivo* NMP permits direct administration of NPs containing ISAs to the solid organ prior to transplantation, circumventing the limitations of *in vivo* application. An inadequate supply of transplant organs has led to extended wait times, resulting in increased waitlist mortality for chronic organ disease patients. From this perspective, NMP has proven to be a promising advancement, offering opportunities to improve graft preservation and viability at the time of transplantation, as well as to prevent rejection post-transplantation.

A small set of studies have tested the feasibility and efficacy of administering NPs containing immunosuppressive agents during *ex vivo* NMP to prevent transplant rejection (Table 1), and the data from these preliminary studies have showed promise in prolongation of bioavailability as well as reduction of rejection. Therefore, the use of nanomedicine during preclinical studies of *ex vivo* NMP has provided a route to potentially groundbreaking progress in the clinical management of solid organ transplant recipients, but extensive investigation remains to overcome the barriers to effective drug delivery imposed by ECs and to translate these promising preliminary findings to significant advances in the prevention of allograft rejection, which remains the largest obstacle to the long-term survival of the transplant.

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VOCABULARY

Antibodies are molecules in the blood that are produced by B cells in host organisms in response to proteins (antigens) that the immune system of the host recognizes as foreign. Potential sources of these antigens include bacteria, viruses, toxic materials, or any other foreign substance, and the antibodies assist the immune system of the host in eliminating these potentially pathogenic sources.[197]

Poly(lactic-co-glycolic acid) (PLGA) is a synthetic biodegradable and biocompatible polymer, which is established as the "gold standard" polymer in controlled release systems for prolonged release of drugs.[198] It is eliminated *via* hydrolysis in the body by degradation into lactic and glycolic acid.[199, 200]

Liposomes are lipid vesicles that can be used as carriers to transport biologically active molecules to their intended sites of action.

High-Density Lipoproteins (HDLs) are heterogenous lipoproteins that are responsible for cholesterol and lipid transport in the body.[201] They are involved in reverse cholesterol transport (RCT), which leads to removal of excess cholesterol from blood vessels and uptake by the liver for elimination.[202, 203] HDLs also have anti-atherogenic, anti-oxidative, and anti-inflammatory roles in the body.[203–206]

Antigen-presenting cells (APCs), comprised of macrophages and dendritic cells, are major participants in the innate immune response. Their primary role is to internalize foreign antigens and process them for presentation to T cells, which are the chief arbiters of adaptive immunity. This function positions APCs at the nexus of innate and adaptive immunity.

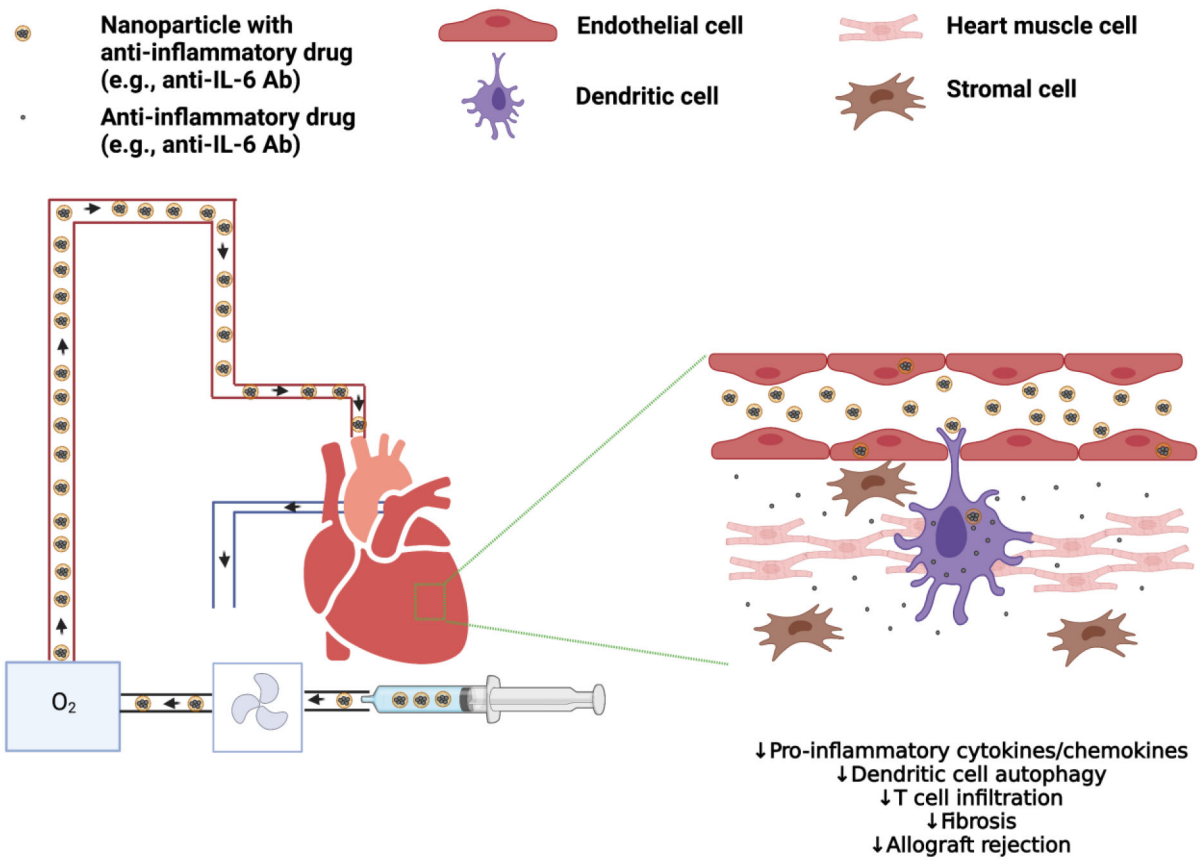

Figure 1. Administration of NPs containing anti-inflammatory drugs during *ex vivo* NMP of heart.

Ex vivo NMP provides an ideal scenario for direct administration of NPs containing anti-inflammatory drugs for intra-organ delivery prior to transplantation of the heart and other solid organs. Anti-IL-6 Ab: antibody against IL-6. Created with BioRender.com.

Table 1.

Summary of preclinical *ex vivo* nanovehicle administration studies in transplantation.

Nano-vehicle	Method of Delivery	Therapeutic	Target Organ and Cell population	Results	Reference
ICAM-1-conjugated dextran-lysozyme nanogel adsorbed onto RBCs	Vascular perfusion	None	Human lung ECs	3.7-fold higher localization to lung ECs than free nanocarriers	160
Anti-CD31-conjugated PLA-PEG NP	NMP; whole organ/tissue immersion	None	Human kidney ECs	5–10-fold higher localization to kidney ECs than isotype control Ab-conjugated NPs	153
High-lactone poly(amine-co-ester) NP	NMP; whole organ/tissue immersion	siRNA targeting MHC class II transactivator	Human blood vessel ECs	Lowered MHC class II expression by ECs for 4–6 weeks; suppressed allogenic T cell responses	161
Light-crosslinkable gelatin methacryloyl biomaterial	Whole organ/ tissue immersion	Anti-IL-6R Ab	Mouse skin macrophages, T cells	Decreased alloreactive T cell and macrophage infiltration; doubled survival length of skin allografts	164
PEG-PLGA NP	NMP; whole organ/tissue immersion	Anti-IL-6 Ab	Mouse heart macrophages, T cells	Decreased T cell and macrophage infiltration; inhibited chronic rejection in comparison to ischemic control	167
PEG-PLGA NP	NMP; whole organ/tissue immersion	MMF	Mouse heart macrophages, T cells	Decreased expression of pro-inflammatory cytokine and chemokines; decreased T cell and macrophage infiltration; inhibited fibrosis and chronic rejection in comparison to free MMF	35

ICAM-1: intercellular adhesion molecule-1; RBC: red blood cell; PLA: poly(lactic acid); NP: nanoparticle; PEG: polyethylene glycol; PLGA poly(lactic-co-glycolic) acid; siRNA: small inhibitory RNA; anti-IL-6R Ab: antibody against IL-6 receptor; anti-IL-6 Ab: antibody against IL-6; MMF: mycophenolate mofetil; EC: endothelial cell; MHC: major histocompatibility complex