Application of PLGA nano/microparticle delivery systems for immunomodulation and prevention of allotransplant rejection

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Running title: PLGA delivery systems against allotransplant rejection

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Abstract

Introduction: Allograft transplantation is an effective end-point therapy to replace the function of an impaired organ. The main problem associated with allotransplantation is the induction of immune responses that results in acute and chronic graft rejection. To modulate the response of the immune system, transplant recipients generally take high dose immunosuppressant drugs for life. These drugs are associated with serious side effects such as infection with opportunistic pathogens and the development of neoplasia.

Areas covered: We reviewed the obstacles to successful transplantation and PLGA-based strategies to reduce immune-mediated allograft rejection.

Expert opinion: Biomaterial-based approaches using micro- and nanoparticles such as poly (lactic-co-glycolic acid) (PLGA) can be used to achieve controlled release of drugs. This approach decreases the required effective dose of drugs and enables local delivery of these agents to specific tissues and cells, whilst decreasing systemic effects.

Keywords: PLGA; Transplantation; Allograft rejection; Immunosuppression

Article highlights

- One of the main concerns of allotransplantation is overcoming and modulating immune responses.
- Common immunosuppressant drugs cause adverse effects because of their general and non-specific impacts.
- The development of nano/microparticles such as PLGA, which allows controlled release and targeted delivery of these drugs presents a promising therapeutic approach.
- The lower toxicity of drug-encapsulated PLGA nanoparticles compared with the soluble form and the potential for combination therapy with additional induction therapies are the main advantages of nano/microparticle delivery systems.

Abbreviation

aAPCs: Artificial antigen-presenting cells (APCs)
CsA: Cyclosporine A
CMV: Cytomegalovirus
DAMPs: Danger associated molecular patterns
ECDI: 1-ethyl-3-(30-dimethyl aminopropyl)-carbodiimide
HGMB1: Heat-shock proteins
IRI: Ischemia-reperfusion injury
IL: Interleukin
IMPDH2: Inosine 88 monophosphate dehydrogenase-2
IBD: Inflammatory bowel disease
LIF: Leukemia Inhibitory Factor
MNPs: Micro and nanoparticles
MMF: Mycophenolic acid
MHC: Major histocompatibility complex
PLGA: Poly (lactic-co-glycolic acid
PRRs: Pattern recognition receptors
PD-L1: Programmed death ligand-1
RA: Retinoic acid
SD: Sprague Dawley

1. History of Transplantation

Most common organs and tissues (including transplanted kidneys, liver, heart, corneal and musculoskeletal grafts) have been successfully transplanted. Long-term acceptance of allografts is the main goal of clinical transplantation. The obstacles to successful transplantation are immunologically mediated damage (termed allograft rejection), and adverse effects of immunosuppressant drugs [1]. Improvement in surgical techniques occurred in parallel with developments in the understanding of the immune mechanisms mediating allograft rejection and these developments enabled the first kidney transplant in 1963 [2-5]. The promise of improved immunosuppression and increased survival opened the door to the transplantation of other organs, including the heart, liver, pancreas, lung and small bowel. [6-10].

During the past two decades, several immunosuppressive drugs have minimized the risk of allograft rejection, but all current immunosuppressive drugs lack specificity, resulting in severe side effects [11]. The frequency of all organ graft rejection is approximately 15 % during the first six months after transplantation. For these reasons, physicians and immunologists are making every effort to develop new approaches to decrease the side effects of immunosuppressive drugs in organ transplants or to eliminate the use of these drugs by inducing allograft-specific tolerance mechanisms [12].

2. Mechanism of allograft rejection

Despite advances in techniques to limit transplant rejection, allograft rejection mechanisms have not yet been completely explained.. [13]. Clinical rejection is classified according to the length of time after the transplant at which rejection happens, as follows:

2.1. Hyper-acute rejection

Hyper-acute rejection is uncommon and occurs in within minutes to hours after transplantation in vascularized grafts such the kidney and heart. Rejection occurs due to alloantibodies preformed by the recipient which can be classified into two subclasses: 1) preformed IgG antibodies against MHC class I molecules, these are most common in sensitized individuals especially after transplantation, repeated transfusions, or multiple pregnancies. 2) Pre-existing "natural" IgM alloantibodies against non-self carbohydrate determinants, such as alloantibodies against ABO blood group antigens [14,15].

2.2. Acute rejection

Acute rejection, occurs days to months after transplantation, and is mediated by both humoral (alloantibody) and cell (CD4+ T cells and CD8+ T cells)-mediated immune reactions, mainly in response to MHC antigens that are present on vascular endothelial and parenchymal cells. [16].

2.3. Chronic rejection

Chronic rejection [17] is the main cause of allograft failure, and occurs months or years following transplantation. The exact pathogenesis of chronic rejection is unknown. HLA incompatibility, ischemic damage, and the number and the severity of acute rejection events and infections are the main risk factors for the development of chronic rejection [18,19]. Current immunosuppressant

drugs cannot entirely prevent chronic rejection, so the new generation of agents could help to improve survival of graft by vasculoprotective effects, preventing neointima formation [20,21] and enabling the clinical induction of tolerance [22].

3. The mechanisms of immune-mediated rejection

3.1. Ischemia-Reperfusion Injury

After removal of the allograft from the donor, the graft becomes ischemic. This situation would ultimately result in necrosis of the tissue if it was not implanted into a recipient, and the blood supply restored (reperfusion). Ischemia-reperfusion injury (IRI) encompasses the damage that occurs to the tissue whilst it is ischemic, and damage which occurs at reperfusion.

3.2. Alloreactive- T cell activation mechanisms

In this field, three mechanisms have been described that result in allograft rejection, including direct, semi-direct and indirect presentation.

3.2.1. Direct presentation:

Donor APCs, mainly resident DCs in the allograft which have allogenic MHC molecules, migrate to the draining lymph nodes where they present alloantigens to alloreactive recipient T cells. This results in the development of helper- and cytotoxic- T cells which play a central role in the rejection process [23].

3.2.2. Indirect presentation:

Recipient DCs process alloantigens (donor MHC molecules) as exogenous antigens in a self-MHC context and present various alloantigens from the graft to alloreactive recipient T cells. This pathway is responsible for chronic rejection and hence is the main reason for organ failure, which cannot be prevented or treated at present. [24-26] (Figure 1).

3.2.3. Semi-direct presentation

Donor membrane fragments which carry MHC class I molecules can transfer to recipient APCs [24,27]. The semidirect presentation involves cell-to-cell interaction through the release and uptake of MHC-I-containing vesicles [28]

4. Mechanisms of clinical immunosuppression drugs and their side effects

As explained above, immunosuppressant drugs are essential for transplant recipients, and play a vital role in maintaining the integrity of grafts. Nevertheless, they have some serious side effects, described below.

4.1. Mycophenolic acid (MMF)

MMF has an anti-proliferative immunosuppressant effect through the inhibition of inosine 88 monophosphate dehydrogenase-2 (IMPDH2), which is the rate-limiting enzyme in the de novo synthesis of guanine. Gastritis, leukopenia, esophagitis, and opportunistic CMV infection are a complication of MMF therapy.

4.2. Calcineurin inhibitors—FK506 and Cyclosporine A

CsA is a calcineurin inhibitor which decreases the expression of pro-inflammatory cytokines, resulting in the suppression of the effector function of T cells.CsA has some severe side effects such as diabetes, end-organ toxicity, neurotoxicity and hypertension [29]. FK506 or tacrolimus is a next-generation calcineurin inhibitor with a potency 10–200 times greater than that of CsA [30]. Unfortunately, FK506 treatment is associated with a range of side effects, including nephrotoxicity and neurotoxicity, opportunistic infection and diabetes [31-33].

4.3. Rapamycin

Rapamycin or sirolimus inhibits the mammalian rapamycin target (mTOR), thereby regulating cell proliferation by suppressing cell cycle transition from the G1 to the S phase and by preventing protein synthesis [34]. In addition to suppression of effector T cells, rapamycin can shift DCs toward a more tolerogenic phenotype [35-37] and drive the naïve T cells toward a regulatory T cell (Treg) phenotype [38-42]. Side-effects include non-specific effects on immune cells, altered fibroblast activity resulting in impaired wound healing, proteinuria and in some cases, diabetes and life-threatening pneumonitis [34,43].

5. Limitations of currently used immunosuppressive drugs

As discussed in the previous sections, the current immunosuppressive agents used in transplantation are associated with notable toxicity and serious side effects. Additionally, systemic administration (oral or intravenous) of these drugs is associated with unpredictable blood concentrations of the drug, resulting in toxic or non-therapeutic concentrations.
[44-46].

Taken together, these limitations highlight the importance of developing new therapeutic technologies to improve graft outcomes in the setting of allotransplantation. Of particular interest, is the development of delivery systems to target therapeutic drugs to the tissues/organs of interest. This approach limits systemic toxicity and allows for the sustained release of agents, reducing the frequency with which patients are required to take their medicine [47].

Inducing allograft-specific immune tolerance is another field of transplant research for the promotion of graft survival, allowing a reduction in the dose and number of immunosuppressive drugs administered and maintenance of immunosurveillance. The main approach employed to induce transplant tolerance is the delivery of alloantigens to the graft recipient in a “tolerogenic form” to stimulate peripheral tolerance mechanisms to the allograft. The remaining sections of this review focus on nano- and microparticles, particularly PLGA, as novel approaches to targeted and sustained drug delivery [48].

6. Particle drug-delivery systems

Recent research in the context of material and biomaterial science, such as micro-nano particles has resulted in an expansion in the availability of drug delivery systems that overcome the current shortcomings with conventional immunosuppression, through improved outcomes by specific targeting, sustained, and controlled release of drugs [49]. Particles used for drug delivery reduce

the systemic toxicity of immunosuppressant drugs by specific targeting to the relevant tissues where they maintain a therapeutic concentration of the drug. Additionally, the most recent advances in this field, include biocompatible and biodegradable delivery systems that can target several tissue types and which can degrade in response to specific environmental conditions [50,51].

Polymeric microspheres can be loaded with drugs or proteins if they possess certain properties. These include biocompatibility, biodegradability and non-toxicity, protein friendly (they do not induce aggregation or other structural modifications to the protein which result in immunogenicity), they maintain the biological activity of encapsulated-protein, exhibit high loading efficiency and high loading capacity of the protein, and release the drug or protein during contact with the release medium [50-54].

Various natural and synthetic biodegradable micro/nanopolymers have been employed for the development of drug/protein delivery systems. Characteristics of particles, such as the particle size may affect the tissue and cellular distribution of the micro/nanopolymers systems. In particular, large microparticles (around 10 μm) have a depot effect because phagocytic clearance is avoided, and because of their inability to cross most biological barriers. Smaller microparticles (around 2–3 μm) can be taken up by antigen-presenting cells (APCs) but are too large for non-specific pinocytotic cellular uptake [51,55].

Therefore, current research is focused on optimizing and promoting common immunotherapies by integrating them into micro/nanopolymer systems. Micro/nanopolymer delivery systems have been studied in many fields, such as vaccine development, cancer therapy, and infectious diseases [49,51,56].

Various natural and synthetic biodegradable polymers have been employed for the development of drug delivery systems. Poly (lactide-co-glycolide acid) (PLGA) is a widely studied polymer used for the improvement of polymeric drug delivery systems [57-59] (Figure 2 and 3). We will discuss the use of PLGA in transplant immunology.

7. Use of PLGA to improve clinical outcomes compared to administration of free drug.

As discussed above, various nanotechnologies have been employed to overcome the many weakness of therapy with common immunosuppressive drugs. Here, we focus on one subset of nanotechnology - PLGA-based nanoparticles, which confer several advantages over conventional approaches to drug delivery.

Drugs-encapsulation by micro- or nano-particles of PLGA, protects the drug from degradation, and thereby improves stability. These particles can target and access many parts of the body, owing to their size, and their ability to penetrate specific tissues via the fenestrations present in the endothelium of inflamed tissue or overexpressed receptors on the surface of target cells. Additionally, drug-PLGA particles can achieve greater therapeutic efficacy compared to the soluble form of drugs, when nanoparticles are decorated with specific targeting agents. This enables drug delivery to be targeted to specific tissues, and permits the use of lower doses than would be needed to achieve a similar effect with soluble drugs. This approach could significantly

decrease the toxicity of some immunosuppressive drugs. PLGA-based nanoparticles can improve pharmacokinetic and pharmacodynamic profiles of drugs via the sustained release of the therapeutic agent, resulting in the improved treatment efficacy [50].

The first investigations in this field were conducted by Sanchez et al., who synthesized, characterized and examined CyA-loaded micro/nanospheres based on poly (DL-lactide-co-glycolide) (PLGA) formulations [49]. The average size of the particles was 0.2-30 μm. Their results showed that the loading capacity of all formulation was high, and was influenced by the size of the microspheres. Assays monitoring the release of CyA from the microspheres showed a biphasic shape. The size of the microspheres and the molecular weight of PLGA, were found to be the main factors that affected the duration and intensity of each phase of release. Consequently, these factors were considered when micro/nanospheres were developed for the controlled release of CyA, and were optimized to provide therapeutic levels of the drug over a prolonged period [56]. The same investigators conducted in-vivo studies to evaluate the biopharmaceutical and clinical benefit of new formulations of CyA-encapsulated PLGA. Tritium-labelled CyA was loaded into the PLGA microparticles (30 and 1 μm) and nanoparticles (0.2 μm) which were administered subcutaneously to mice. The results showed that the micro/nanoparticles act as a depot, which releases the CyA continuously and prolongs the duration over which the drug is present in the blood. Furthermore, they reported that levels of the peptide in tissues (liver, spleen, kidney, and adipose) increased for up to several weeks after intravenous administration of a single dose of microencapsulated CyA. Humoral immunosuppressive responses in the week following the single-dose intraperitoneal administration of the above-mentioned formulations were related to the in vivo kinetic profiles. Nanoparticles and 1-μm microparticles demonstrated a greater response initially (0-24 h), whereas 30-μm microparticle led to more extensive immunosuppression on day 7. These studies demonstrated that PLGA micro/nanoparticles could be used as novel dosage forms, and could modulate the duration and intensity of immunosuppression, compared with free CyA, ultimately resulting in long term immunosuppressant therapy [56,60,61].

Targeted delivery and release of CsA-encapsulated PLGA nanoparticles (average diameter 260 nm) to both cells and lymphatics have been investigated in assays by Yoshikawa et al. They observed that nanoparticles enable a depot release of CsA for 30 days and compared with free CsA, resulted in a greater local concentration of drug in the targeted tissues and cells. Furthermore, they reported that the intramuscular injection of the CsA-encapsulated PLGA-nanoparticles in the femoral site of mice maintained the drug at high concentrations in the inguinal lymph nodes up for up to 1 month. The local concentration was 20 times larger than that achieved when administering soluble systemic CsA, whereas CsA was present at very low concentrations or was undetectable in the plasma [62].

Miyamoto et al. performed a similar study using FK506-encapsulated PLGA micro/nanoparticles in a rat model of liver transplantation. They reported gradual and sustained delivery of FK506 from micro/nanoparticles for ten days, with dose-dependent immunosuppression, after a single subcutaneous administration. The main site of particle uptake was the regional lymph node of the subcutaneously injected site. In addition, in models of liver transplantation, they observed that the

survival rate of a group of rats which received 4.8 mg/kg tacrolimus-loaded into PLGA nanoparticles was 88.6 days, which was more than other groups (2.4 mg/kg tacrolimus-loaded PLGA nanoparticles , 0.16 mg/kg tacrolimus loaded PLGA NPs and 1.0 mg/kg free tacrolimus [63].

Similar studies on mouse models of islet transplantation demonstrated that FK506-encapsulated PLGA particles improved the immunosuppressive effects on islet allograft rejection effectively, with limited effects on insulin secretion. They found that a single subcutaneous injection of 20 mg/kg of tacrolimus-loaded PLGA nanoparticles could significantly prolong the survival time of the allogeneic islet grafts for 30 days, compared with daily tacrolimus treatment at a dose of 2 mg/kg (11.5 days). Furthermore, a series of administrations of tacrolimus-loaded PLGA nanoparticles at a dose of 10 mg/kg at 7-day intervals for 1 month notably prolonged the survival of islet grafts and resulted in 60% allograft acceptance. These results suggest that tacrolimus-loaded PLGA nanoparticles could effectively suppress the immune rejection and prolong allogeneic islet graft survival at lower doses of drug than when free tacrolimus is used [64]. Lamprecht et al. evaluated the oral administration of FK506 -encapsulated PLGA nanoparticles to male Wistar rats with experimentally-induced colitis. FK506-nanoparticles resulted in 3-fold higher concentrations of the drug in inflamed regions, compared with healthy tissue, presumably because the nanoparticles could escape from efflux systems and mucosal metabolism. The higher selective adhesion and increased drug penetration into the inflamed region by drug-loaded nanoparticles compared with free drugs suggests that this drug delivery system could be a promising approach for the treatment of IBD [65].

Other researchers evaluated daily oral or subcutaneous administration of tacrolimus-loaded PLGA or pH-sensitive Eudragit P-4135F nanoparticles in a dextran sulfate mouse model of colitis. Their results showed oral tacrolimus-loaded nanoparticles were less efficient in alleviating the experimental colitis compared to subcutaneous drug solution but were better than oral administration of the drug. Nanoparticles resulted in less toxicity (particularly nephrotoxicity) than free drug solution [66]. Tacrolimus-loaded PLGA nanoparticles entrapped in pH-sensitive microspheres were orally administrated in a rat model of colitis. Investigators observed that tacrolimus-loaded nanoparticles delivered the drug more selectivity to their site of action (colon) than free drug, resulting in significant improvement in the clinical activity index after three days of treatment [67]. Although these nanoparticles delivery systems have been developed to treat murine models of inflammatory bowel disease (IBD), they could be readily applied in the setting of small bowel transplantation.

Rapamycin-encapsulated micro/nanoparticles delivery systems are an interesting field of allograft immunosuppressant therapy, since rapamycin not only inhibits T cell proliferation but also suppresses DC maturation and function and enhances inhibitory FoxP3+ Regulatory T cells [34-37,68]. Several investigators have targeted delivery of rapamycin to DCs using PLGA based Micro/nanoparticles [69]. Jhunjhunwala et al. showed that DCs treated with rapamycin can suppress transplant rejection [37]. They designed rapamycin-encapsulated PLGA microparticles (~ 3.4 μm) and examined the release profile under intra-phagosomal (pH = 5) and extracellular

(pH = 7.4) conditions. Four days after phagocytosis of rapamycin-encapsulated PLGA microparticles, they observed that the ability of DCs to activate T cells was weak compared to DCs treated with soluble rapamycin. As a result, they reported specific intracellular delivery of rapamycin to DC cells through PLGA particles results in better efficacy of the drug, because of its capacity to modulate DC function in comparison with extracellular rapamycin -treated DCs [69].

Other similar studies were performed by Haddad et al. and Saswati et al. They synthesized rapamycin-encapsulated PLGA nanoparticles (average size: 280 nm). Their results showed that PLGA-encapsulated rapamycin reduced the expression of all maturation markers, including MHC class II, CD86, ICAM-I and CD40 in DC to a greater extent than soluble rapamycin. Additionally, PLGA-encapsulated rapamycin suppressed the production of cytokines, and their functional effects on the proliferation of T cells [70,71].

The field of research into improving allograft survival is very extensive, and many drug-delivery systems have been investigated. Research on murine models of skin transplantation has demonstrated that mycophenolic acid (MMF)-PLGA based nanoparticles (at a 1/1000 of the dose of free MMF), prolonged the survival of allografts compared with daily systemic injection of MMF. This occurred via upregulation of the programmed death ligand-1 (PD-L1) and by reduced priming of alloreactive T cells. Furthermore, MMF based nanoparticles showed no toxicity, whereas recipients obtaining systemic free MMF developed iatrogenic cytopenias [72].

Induction of self-tolerance or acquired tolerance could be applied by cytokine pro-regulatory factors loaded into micro/nanoparticles based delivery systems in the setting of allotransplantations, through the immunoregulatory function of Treg. Metcalfe et al. identified that Leukemia Inhibitory Factor (LIF) plays a role in the regulation of transplantation tolerance in vivo [73]. Later, Park et al. demonstrated that LIF-loaded nanoparticles directed towards CD4+ T cells suppressed the IL6-driven Th17 development, developed FOXP3+ CD4+ T cell numbers in a non-human primate model in vitro and prolonged the survival of vascularized heart grafts in mice. Conversely, IL-6 encapsulated nanoparticles directed to CD4+ T cells enhanced Th17 maturation [74,75].

The two main barriers to cell-based therapy of diabetes are alloimmune rejection of donor pancreatic islets, or stem cell-derived insulin-secreting cells and maintainance of autoimmunity against the diabetogenic endogenous target antigen. Nanotherapeutics could be an innovative approach to solving these problems. Dong et al. developed “stealth” islets by PEGylation decorated with LIF-encapsulated nanoparticles (100–200 nm thick) to make islet transplantation viable. They showed that PEGylated nanotherapy prolonged islet viability and functionality in vitro/in vivo and improved long-term normoglycemia. Furthermore, delivery of immune modulators such as LIF improved the viability of this innovative therapeutic strategy in the cell-based therapy of diabetes [76]. Jhunjhunwala et al. reported that the (IL-2, TGF-β plus rapamycin)-encapsulated PLGA nanoparticles induced the FoxP3+ Treg in human cells in vitro by releasing these components over 3–4 weeks [38].

In addition to Treg expansion, current evidence suggests that the recruitment of self-regulatory cells through micro/nanoparticle formulations the so-called “homing” systems could serve as an

effective strategy to enrich cells in a given location in order to overcome aberrant inflammation in the setting of transplant immunology and autoimmunity [68]. In this way, chemokine (chemoattractant cytokines) secretory cells induced the migration of cells which express the relevant receptors. For example, the chemokine CCL22 is expressed by a variety of tumor cells. It is an effective factor for the recruitment of associated CCR4 receptor-expressed Tregs to the site of the malignancy and facilitates tumor-specific immune evasion [77]. Such strategies to recruit Tregs using CCL22 have helped to develop a novel therapy that employs local immunological hyporesponsiveness [78]. Jhunjhunwala et al. designed CCL22-loaded PLGA which released drug within a 3–8-week window and which promoted the site-specific recruitment of endogenous Tregs in vivo. In a mouse model they observed that the adoptively transferred Treg cells migrated to the site of microparticle injection and simultaneously delayed the rejection of transplanted allogeneic cells, which had been implanted at the site of microparticle injection. As a further design parameter, microparticles remain immobilized at the site of injection because their large size prevents their uptake by phagocytic cells [79].

DC cells also have a central role in inducing a tolerogenic condition. Lewis et al. recently designed two classes of PLGA microparticles including phagocytosable microparticles loaded with either rapamycin or retinoic acid (RA) (~2 μm) and non-phagocytosable microparticles loaded with either TGFβ or IL-10(~30 μm). Four separate combinations of microparticles (Rapa/IL-10, Rapa/TGFβ, RA/IL-10, and RA/TGFβ) were cultured with DCs and the results showed that the levels of surface expression of MHC II, CD80 and CD86 decreased in these DC resistant to LPS stimulation in comparison to untreated DCs and soluble proteins controls, but not single-factor microparticles. Furthermore, dual microparticle-treated DC cells inhibited the allogeneic T cell proliferation and shift T cells toward a regulatory phenotype, especially for the RA/IL-10 MPs. The results of this research demonstrate that combined (intracellular and extracellular) delivery of immunomodulatory factors (cytokines and small molecules) can promote immunoregulatory responses for therapeutic application in transplantation and autoimmunity by preventing maturation of DCs.

As mentioned before, DCs play an important role in T-cell activation, by providing co-stimulation and paracrine cytokine signaling. Hence, the improvement of artificial particle-based materials which mimic the function of DCs to target specific T-cells for drug delivery is a topic of ongoing research [80,81]. Steenblock et al. synthesized artificial antigen-presenting cells on a biodegradable PLGA microparticle that was targeted by both recognition and co-stimulatory ligands (anti-CD3 and anti-CD28 ligands) for the T cell receptor and loaded with the cytokine interleukin-2. They reported that this artificial APC system stimulates and develops polyclonal and specific T-cells and improves the stimulatory capacity of these acellular systems through the sustained release of IL-2 in the region of T-cell contact [82].

The development of antigen-specific CTLs for use in clinical therapy is ongoing. Artificial antigen-presenting cells (APCs) have been designed and decorated with a soluble major histocompatibility complex–immunoglobulin fusion protein (MHC-Ig) and anti-CD3 and CD28. Anti-CD3 is a non-specific activator for all T cell receptors, however the presentation of specific

antigen by MHC-Ig provided the target specificity. These APCs induced and developed specific CTLs for cytomegalovirus (CMV) or melanoma which identified endogenous MHC complexes presented on melanoma cells. Therefore, the results of these studies confirm the importance of MHC-Ig–based APCs for the reproducible development of disease-specific CTLs for clinical approaches to adoptive immunotherapy [83].

Later work demonstrated that IL-2-encapsulated PLGA that coupled to anti-CD3 and CD28 as an aAPC could significantly affect the differentiation of CD8+ and CD4+ T cells through the paracrine release of IL-2. After specific contact of aAPC/T cells, paracrine delivery of IL-2 induces substantial accumulation of IL-2 in the synaptic contact region. The sustained release and accumulation of IL-2 enhanced CD25 (the inducible IL-2 Rα chain) on responding T cells and augments proliferation of CD8+ T cells in vitro, to a 1000-fold greater extent than equal amounts of bulk IL-2. This condition could promote activation-induced apoptosis in CD4+populations [84]. Based on the aforementioned studies, future studies will attempt to expand our knowledge of how these therapies could be translated for use in solid organ transplantation.

Because of the complexity of DC/T cells interactions in the immune synapse, more research is necessary to develop a full understanding of these interactions. Two studies have investigated the principle of paracrine factor accumulation among cells and nanoparticles and also the thermodynamic interactions between micro/nanoparticles and cells [85,86]. These studies provide the required knowledge to allow the development of an effective biomimetic artificial cell construct for the next generation immunotherapeutics through collaboration between engineers and immunologists [47].

The numerous FDA-approved Micro/nanoparticle-based drug delivery systems (particle-loaded current FDA-approved drug, genetic material, and biomimetic protein delivery and aAPC) decrease toxicity and improve patient satisfaction by enabling controlled and sustained release of loaded materials. This approach of improving the efficiency of existing drugs is likely to be more cost-effective than the development of new drugs, and may lead to relatively rapid FDA approval [87-89]. Because of unresolved concerns, no micro/nanoparticle delivery system yet exists in the clinic for immunosuppressive drugs in any application.

CsA encapsulated PLGA Nano/microparticles (15, 30, 45 mg/kg) were administrated daily by oral gavage over 28 days to Sprague Dawley (SD) rats to investigate the drug- or carrier-dependent change of biochemical, hematological and histological parameters. CsA-encapsulated PLGA Nano/microparticle did not cause any common adverse effects, but high doses (45 mg/kg) induced notable loss of body weight and resulted in diminished food consumption and lymphocyte numbers in comparison to untreated controls. This result was in agreement with the reported toxicity of Neoral1. CsA-encapsulated PLGA nanoparticles resulted in higher serum drug concentration than CsA-encapsulated PLGA microparticles, probably because the smaller particle size facilitates absorption. Therefore, these nanoparticles allow an increase in the therapeutic dose range without increasing toxicity [90].

A similar study demonstrated that the pharmacokinetic profile of tacrolimus formulated in PLGA nanoparticles was maintained for at least 14 days after a single subcutaneous or intramuscular

administration. It was reported that altering the PLGA/PLA ratio could help to enhance the period of tacrolimus release from the microspheres. The graft survival time in this rat model of heart transplantation was prolonged by tacrolimus-loaded PLGA nanoparticle administration [91].
Bryant et al. investigated the induction of transplant tolerance in full MHC-mismatched murine allogeneic islet transplantation through using PLGA nanoparticle encapsulated 1-ethyl-3-(30-dimethyl aminopropyl)-carbodiimide (donor ECDI-SP) as a synthetic, cell-free delivery system. Their results showed that the administration of donor antigen-encapsulated PLGA nanoparticles induced tolerance in approximately 20% of recipient mice by modulation of the indirect pathway of allorecognition. They also demonstrated that the combination of donor antigen-encapsulated PLGA nanoparticles with a short-term delivery of a low dose of rapamycin at the time of transplant significantly improved the effectiveness of tolerance to 60% [92].
Ischemia-reperfusion injury (IRI) induces intragraft inflammatory responses (increase in DC autophagy and production of IL-6) and can result in chronic rejection. Solhjou et al. demonstrated that local antagonism of the effect of IL-6 in the the donor heart, by anti-IL-6 loaded PLGA nanoparticles significantly reduced chronic rejection with a considerably smaller amount administered than other delivery approaches [93].
Uehara et al. used MMF-loaded PEG–PLGA nanoparticles (MMF-NPs) to deliver the MMF directly to the heart graft ex vivo. As previously discussed, one of the most important stimuli of early alloimmune reactivity and chronic allograft rejection is inflammation occurring within the transplanted organ from the moment of harvest. These results showed that perfusion of a donor mouse heart with MMF-NPs prior to transplantation suppressed cardiac transplant vasculopathy through repressing pro-inflammatory cytokines and chemokines within the graft.[94] A summary of studies explained in this section is provided in Table 1.

Several varieties of nanotechnology have been applied to overcome the obstacles that organ and cell transplantations present. This research has been extensively reviewed by Tasciotti et al . Nanocomposite polymer scaffolds offer an innovative approach to the synthesis of a successfully implanted artificial trachea. Furthermore, targeted and controlled delivery of immunosuppressive drugs by nanotechnology has been demonstrated to play a significant role in assuring successful transplants, by overcoming the problems related to drug solubility and improving drug efficacy in patients at a high risk of chronic rejection [95].

Nanotechnology could also be utilized to encapsulate therapeutic cells, such as pancreatic islets, blood cells, hepatocytes, and stem cells. Cell-based therapies have potential to treat a wide range of human diseases, such as diabetes, blood disorders, acute liver failure, spinal cord injury, and several types of cancer. Utilizing nanotechnology in the context of cell-based therapy can help to limit immune rejection and to enable the controlled release of active agents to cells, providing a supportive environment, enabling cells to function effectively to enable the retrieval of the graft [96].

8. Use of PLGA delivery systems to decrease the side-effects of common immunosuppressive drugs

Many studies have been conducted to evaluate the efficiency of PLGA-micro/nanoparticle delivery systems compared with free immunosuppressant drugs to decrease various side effects and to overcome other disadvantages.

Wang et al. compared the effects of tacrolimus-loaded PLGA nanoparticles and free tacrolimus on insulin secretion. They reported lower pancreatic concentrations tacrolimus when tacrolimus-loaded PLGA nanoparticles were used in a mouse islet allograft models, compared to the free drug. They additionally reported that a single injection of 30 mg/kg of free tacrolimus can suppress insulin secretion after 24 hours, whereas administration of the equivalent dose of tacrolimus-loaded PLGA nanoparticles did not decrease the insulin secretion. Consequently, tacrolimus-loaded PLGA nanoparticles prolonged islet graft survival more effectively with diminished side-effects on insulin secretion and therefore have potential use in the context of islet transplantation [64].

In another study, Junhua et al. reported that the formulation of liraglutide (an antidiabetic agent) and sirolimus in islet transplanted diabetic mice resulted in normal glucose levels and sustained appropriate concentration of insulin. [97].

Meissner et al. studied nephrotoxiciy as the main adverse effect of tacrolimus in inflammatory bowel disease. They compared tacrolimus-loaded PLGA nanoparticles with pH-sensitive delivery of tacrolimus. At the end of the treatment period, they observed that nanoparticles treatment was associated with less-marked increases of BUN and serum creatinine [66].

It has been demonstrated that, compared with free drug, nanoparticle delivery of mycophenolic acid, results in a lower total drug load but nevertheless, prolongs allograft survival whilst avoiding systemic toxic effects such as anemia and splenic cytopenia [72].

The results of another study with daily dosing of CsA-loaded PLGA nano/microparticles did not show any deleterious general health effects and no significant hematological or biochemical changes. However, the high dose of CsA-nano/micro-particles (45mg/kg) resulted in significant loss of body weight. Nevertheless, CsA-nanoparticles could be loaded with a higher dose range of drug and resulted in higher serum concentrations of drug without exhibiting toxicity.[90]
Another approach to improve drug-targeting through galactosylated of PLGA nanoparticles was evaluated by Mistry et al. They demonstrated that tacrolimus-galactosylated PLGA nanoparticles provided more specific targeted delivery of tacrolimus to the liver compared with Tac- PLGA nanoparticles. This occurred via facilitation of receptor-mediated endocytosis via asialoglycoprotein receptors on liver cells. Furthermore, Tac-Gal PLGA nanoparticles were found to be less abundant in the kidney, suggesting the possibility of decreased nephrotoxicity [98].

PLGA nanoparticles have also been investigated as an approach to avoid fluctuations in the circulating concentrations of tacrolimus. Zamorano-Leon et al. reported that a single-dose subcutaneous administration of tacrolimus-loaded PLGA nanoparticles resulted in notable immunosuppression in rats. This occurred due to increased plasma concentrations of tacrolimus and a subsequent decrease of both circulating IL-2 levels and calcineurin phosphatase activity in mononuclear cells. Delivery of tacrolimus with PLGA nanoparticles reduced the side effects of gastrointestinal drug absorption and alterations of metabolism [99]. Italia et al. demonstrated that the rapamycin-loaded PLGA nanoparticles exhibited higher intestinal uptake, bioavailability, and controlled release over 5 days. This was associated with significantly lower nephrotoxicity in rats compared with Sandimmune Neoral® and cyclosporine suspension [100].

9. Drawbacks associated with PLGA drug delivery systems

PLGA nanoparticle-based drug delivery systems have many advantages, but as with any medical technology, they are have limitations and problems [101,102]. The instability of encapsulated proteins is one of the most significant concerns when the controlled release system is loaded with proteins. These problems occur as a result of mechanical and chemical stresses or pH sensitivity during the manufacturing processes. Indeed, the dehydration process, which removes organic solvents during the process of encapsulation can affect the secondary structure of proteins, resulting in the formation of partially unfolded aggregates. Furthermore, degradation of polymers to lactic and glycolic acid acidifies the interior of the nanoparticles, where the protein resides, to as much as pH 3. These acidic conditions lead to disturbances in the structure of proteins and hence result in protein instability and the formation of aggregates [103,104]. Many of these issues may be overcome by modifying the drug release process or by modifying the carrier systems [105]. Various methods have been proposed to overcome these difficulties, by preventing the generation of the acidic microenvironment [106,107]. These methods include continuous flow-through cells in which the buffer is continuously replenished, membrane diffusion technique through a dialysis bag which removes acidic by-products, and the addition of stabilizers. The interaction of protein molecules with the hydrophobic surface of the polymer during processing, storage, and release results in incomplete release because of protein unfolding and aggregation and reduced activity of the protein [105,107,108]. Also, the interaction of proteins with hydrophobic polymer surfaces results in several interactions including adhesion, spreading and proliferation [109]. The hydrophilic and hydrophobic environments can be modified to decrease undesirable interactions between the polymeric system and proteins. This can be achieved with a range of techniques, including chemical treatment, electrospinning with suitable polymers such as Pluronics®, high-energy radiation and high-energy modification [103,104,110].

The burst release of the drugs from PLGA matrices due to rapid diffusion of protein adsorbed at the surface of the polymer and high mobility of drug molecule during the hydration of PLGA is another problem associated with PLGA based nanoparticle delivery. It has been demonstrated that the application of certain additives, including PEG 400 can resolve this problem by improving the stability of encapsulated drugs [106]. Additionally, the enhanced permeability and retention (EPR)

effect is often misunderstood as one of the major drawbacks of this system. EPR is a heterogeneous phenomenon that differs from model to model, and from patient to patient. Active drug targeting to improve EPR is not very effective due to immunogenicity and protein adsorption following the introduction of targeting moieties [111]. PLGA-based nanoparticles are associated with weak drug loading, despite having high encapsulation efficiency. This results in increased costs of production and difficulties in scaling production [112,113].

Another major obstacle is the poor knowledge around the various processes parameters that affect nanoparticle preparation [113] and particle size and morphology of the nanoparticles which are affected by the conditions during homogenization and droplet size distribution. The in vivo heterogeneous expression levels of the targeted receptor in target cells, several physiological barriers and in vivo different behavior of these nanoparticles can prevent the efficient accessibility of these nanoparticles to target tissues [114]. Consequently, in vitro and animal studies of specific drug delivery systems cannot be generalized due to the complexity of drug release and other concerns discussed above [115].

10. Expert opinion

One of the main concerns of allotransplantation is overcoming and modulating the immune responses to improve the survival time of allografts. Common immunosuppressant drugs such as corticosteroids and azathioprine cause adverse effects because of their general and non-specific impacts on the body. This is made worse by the fact that so many of these drugs require high doses and long periods of administration in order to be effective. Therapy can therefore be improved by specific targeting of these agents to the immune system, especially T cells. The development of nano/microparticles such as PLGA, a biomimetic system, can improve drug stability and enhance the pharmacokinetic and pharmacodynamic profiles of drugs, enabling sustained release of the therapeutic agent. Decoration of particles with specific targeting agents, can improve penetration into specific tissues, and enable the use of lower doses of drugs, reducing toxicity without compromising the clinical effectiveness of immunosuppressant drugs. Interestingly, immunosuppressant drugs, immune system modulating agents (cytokines and donor-specific antigens to induce tolerance) can be loaded onto PLGA nano- or mocro-particles to enhance the efficiency of allo-transplant rejection therapy. Currently, attention is focused on the development of artificial antigen-presenting cells (aAPCs) which are decorated with functional groups (e.g. cell receptors, aptamers, antibodies, etc) to enable them to interact with specific cell types. The lower toxicity of drug-encapsulated PLGA nanoparticles than the soluble form, and the potential for combination therapy with additional induction therapies are the main advantages of nano/microparticle PLGA delivery systems.

Compliance with Ethical Standards

Conflict of interests: Dr. XXXXX owns four shares in Astra Zeneca PLC and has received travel/speaker's fees from Amgen Inc. There are no other competing interests to disclose.

Ethical Approval: Not applicable.

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Table 1. Studies of Drugs-loaded-PLGA MNP

Nanoparticle	**Animal models/Cell culture**	**Clinical outcomes**	**Ref**
CSA-loaded PLGA MP/NP	Mice	-Nanoparticles and 1-μm microparticle demonstrated a higher	[56,60,61]

		humoral immunosuppressive response initially (0-24 h) -30-µm microparticle led to a great level of immunosuppression on day 7	
CsA-loaded PLGA nanoparticles	Mice	-Sustained and high level of CSA in the inguinal lymph nodes up 1 month and nearly 20 times higher than soluble systemic CsA	[62]
FK506-encapsulated PLGA MP/NP	Rat liver transplantation model	-sustained delivery of FK506 from micro/nanoparticles for 10 days -Regional lymph node of the subcutaneously injected site was the main site of particle uptake	[63]
FK506-encapsulated PLGA particles	Islet transplantation mice models	-Improved immunosuppressant effects -decreased side-effects on insulin secretion	[64]
FK506 -encapsulated PLGA particles	Male Wistar rats suffering from preexisting experimental colitis	-Enhanced and selective drug delivery into the region of inflammation to about 3-fold higher than in healthy tissue	[65]
Tacrolimus-loaded PLGA	Dextran sulfate mouse model of colitis	-Oral tacrolimus-loaded nanoparticles were less efficient in alleviating the experimental colitis compared to subcutaneous drug solution but preferred to oral administration of drug solution. –Less toxicity (including nephrotoxicity) compared with free drug solution	[66]
Tacrolimus-loaded PLGA nanoparticles entrapped into pH-sensitive microspheres	Experimental rat model of colitis	-Selective delivery of the drug to their site of action (colon) - Significant improvement in clinical activity index after 3 days of treatment	[67]
Rapamycin-encapsulated PLGA microparticles	DC cell culture	-Weak ability of DC to activate T cells compared to DC treated with soluble rapamycin - Modulate DC function	[69]
Rapamycin-encapsulated PLGA nanoparticle	DC cell culture	-Reduced expression of all maturation markers like MHC class II, CD86, ICAM-I and CD40 in DC cells	[70,71]

		-Suppressor effect on the cytokine production and functional effects on the proliferation of T cells more than soluble rapamycin	
Mycophenolic acid (MMF)-PLGA nanoparticle	Murine skin transplants models	-Prolonged survival of allograft compared with daily systemic injection of MMF -Upregulation of programmed death ligand-1 (PD-L1) and decreasing the priming of alloreactive T cells -No toxicity observed	[72]
LIF-loaded nanoparticles (NPs)	- Non-human primate cells -Murine Heart Allograft Transplant model	-Suppression of IL6-driven Th17 development -Increased FOXP3+ CD4+ T cell numbers -Prolonged survival of vascularized heart grafts in mice	[75]
The "islets stealth" by PEGylation decorated with LIF-encapsulated PLGA nanoparticles	Islet transplanted diabetic mice	-Prolonged islet viability and functionality in vitro/in vivo -Improved the long-term normoglycemia -Developed the capability of this innovative therapeutic strategy to diabetes cell therapy	[76]
(IL-2, TGF-β plus rapamycin)-encapsulated PLGA nanoparticles		- In vitro FoxP3+ Treg induction in human cells	[38]
CCL22-loaded PLGA nanoparticle	Mice	-Promoted the in vivo site-specific recruitment of endogenous Tregs -The adoptively transferred Treg cells in a mouse model migrated to the site of microparticle injection -Delayed rejection of transplanted allogeneic cells which were also implanted at the site of microparticle injection	[79]
(Rapa/IL-10, Rapa/TGFβ, RA/IL-10, and RA/TGFβ)-encapsulated PLGA microparticle	DC cell culture	-Decrease of the surface levels of expression of MHC II, CD80 and CD86 on DC cells -Resistance of DC to LPS stimulation in comparison to untreated DCs and soluble proteins controls	[116]

		- RA/IL-10 MPs: inhibition of the allogeneic T cell proliferation and shift T cells toward a regulatory phenotype	
aAPC on a biodegradable PLGA microparticle that targeted by (anti-CD3 and anti-CD28 ligands) and loaded by IL-2.	Mice	-Stimulates and develops the polyclonal and specific T-cells -Improves the stimulatory capacity of these acellular systems through sustained release of IL-2 in the region of T-cell contacts	[82]
aAPCs: PLGA NP that decorated by a soluble MHC-Ig and anti-CD3 and CD28	Cell line	-Induction and development of specific CTLs for cytomegalovirus (CMV) or melanoma	[83]
aAPC :IL-2-encapsulated PLGA that coupled to anti-CD3 and CD28	Cell line	- Significant differentiation of CD8+ and CD4+ T cells through the paracrine release of IL-2 - Induction of notable IL-2 accumulation in the synaptic contact region -Sustained release and accumulation of IL-2 enhanced CD25 (the inducible IL-2 Rα chain) on responding T cells -Increased proliferation of CD8+ T cells in vitro - Activation-induced apoptosis in CD4+populations	[84]
CsA encapsulated PLGA Nano/microparticle	Sprague Dawley (SD) rats	- Serious side effects were not observed except at high dose (45 mg/kg) that induce significant loss of body weight and diminished food consumption and lymphocyte numbers	[90]
Tacrolimus-Galactosylated PLGA nanoparticle	Rat	-More specific targeted delivery of tacrolimus to liver through facilitating of receptor-mediated endocytosis via asialoglycoprotein receptor on liver cells	[98]

		- Decreased nephrotoxicity due to lower distribution in the kidney	
Tacrolimus- PLGA nanoparticle	Rat model of heart transplantation	-The extended action of drug in this formulation (at least 14 days) -Prolonged graft survival	[91]
PLGA NP encapsulated 1-ethyl-3-(30-dimethyl aminopropyl)-carbodiimide (donor ECDI-SP)	Full MHC-mismatched murine allogeneic islet transplantation model	-Induction of tolerance ~20% of recipient mice - Significant development of the tolerance effectiveness to 60% when combined with a short-term delivery of a low dose of rapamycin at the time of transplant	[92]
Tacrolimus-loaded PLGA NP	Rat	-Development of a notable immunosuppressive response -Increased plasma levels of tacrolimus -Decrease of both circulating IL-2 levels and calcineurin phosphatase activity in mononuclear cells. -Reduced side effects of gastrointestinal drug absorption and metabolism modifications	[99]
Rapamycin loaded PLGA NP	Wistar Kyoto Rat	Improved intestinal uptake, bioavailability, controlled release over 5 days -Significant lower nephrotoxicity	[100]
Anti-IL-6 loaded PLGA NP	Mice/ Heterotopic intra-abdominal cardiac transplantation	-Local antagonism of the IL-6 that effect in the donor heart before transplantation – Significant decrease of chronic rejection with a considerably lower amount administered	[93]
PEG–PLGA nanoparticles(MMF-NPs)	Heart graft under the ex vivo situation	- Suppressed cardiac transplant vasculopathy through repressing intragraft pro-inflammatory cytokines and chemokines after perfusion of a donor mouse heart	[94]

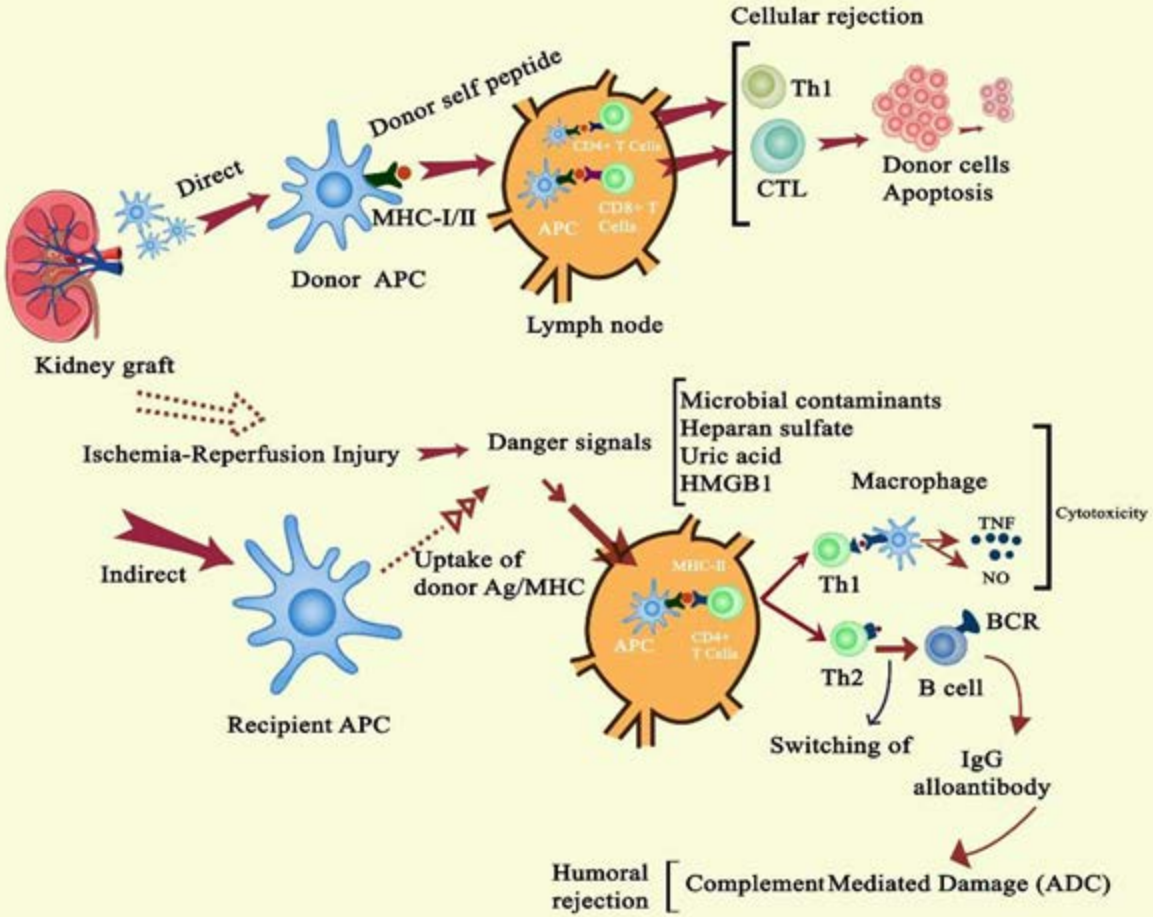
		with MMF-NPs prior to transplantation	

Figure legends

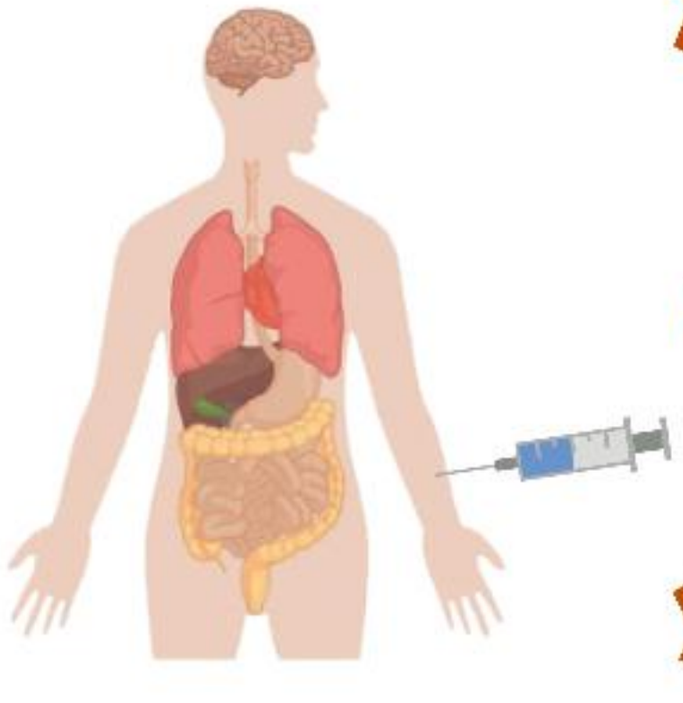
Figure 1. The main pathways of alloantigen recognition. In the direct presentation pathway, donor resident DCs in the allograft migrate to the draining lymph nodes and present their alloantigens to alloreactive recipient T cells that result in the cellular rejection process. In indirect presentation, recipient DCs process alloantigens (donor MHC molecules) as exogenous antigens in a self-MHC context and present to alloreactive recipient T cells.

Figure 2. The development of immunosuppressant therapy in organ transplantation.
The systemic administration of immunosuppressive drugs (Corticosteroids, Mycophenolic acid (MMF), Calcineurin inhibitors—FK506 and Cyclosporine and Rapamycin) was the conventional organ transplant treatment, but they have many serious side effects that have prompted the development of novel treatments based on immunomodulation or induction of tolerance. To develop specific and safer treatments, micro/nano particles have been decorated with targeting agents and loaded with immunosuppressant drugs.

Figure 3. The immunomodulation drugs mechanism. These drugs can modulate the immune system via induction of tolerance and other biologic functions.


Cellular rejection
Th1
CTL
Donor cells Apoptosis
Donor self peptide
Direct
MHC-I/II
Donor APC
CD4+ T Cells
CD8+ T Cells
APC
Lymph node
Kidney graft
Ischemia-Reperfusion Injury
Danger signals
Microbial contaminants
Heparan sulfate
Uric acid
HMGB1
Macrophage
TNF
NO
Cytotoxicity
Indirect
Uptake of donor Ag/MHC
Recipient APC
MHC-II
APC
CD4+ T Cells
Th1
Th2
BCR
B cell
Switching of
IgG alloantibody
Humoral rejection
Complement Mediated Damage (ADC)

Drugs administration



1.Immunosuppression Drugs

Disadvantage:

High dose of drug

Global toxicity

2. Immunomodulation drug

Tolerance induction

Biologic Therapy

[Cytokine → LIF/TGF- β
[pro-regulaory Factor

Disadvantage: Drug interactions

3. MNP-loaded drugs delivery

Drugs-loaded-PLGA MNP

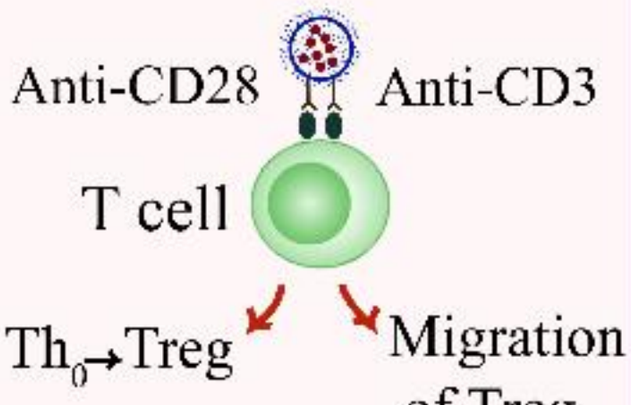
Loaded-by :
Immunosuppression drugs
Immunoregulatory factors like cytokine

aAPC

- Decorated by Anti- CD3/CD28/CD25
- Loaded by cytokine

Advantage:

- Sustained local Delivery
- High local concentration
- Lower toxicity
- Specific targeting
- Lower effective dose
- Intracellular targeting



Mechanisms of immunologic drugs