12-20-2017

Nanomedicine for Immunosuppressive Therapy: Achievements in Pre-Clinical Research

Hanan Al-Lawati
University of Alberta

Hamidreza Montazeri Aliabadi
Chapman University, montazer@chapman.edu

Behzad Sharif Makhmalzadeh
Ahvaz Jundishapur University of Medical Sciences

Afsaneh Lavasanifar
University of Alberta

Recommended Citation

Al-Lawati H, Aliabadi HM, Makhmalzadeh BS, Lavasanifar A. Nanomedicine for immunosuppressive therapy: achievements in pre-clinical and clinical research. Expert Opinion on Drug Delivery. 2018;15(4):397-418. doi: 10.1080/17425247.2018.1420053

Nanomedicine for Immunosuppressive Therapy: Achievements in Pre-Clinical Research

Nanomedicine for Immunosuppressive Therapy: Achievements in Pre-Clinical Research

Journal:	*Expert Opinion on Drug Delivery*
Manuscript ID	EODD-2017-0137
Manuscript Type:	Review
Keywords:	Immunosuppression, Drug delivery, Nanoparticles, Cyclosporine A, Tacrolimus, Corticosteroids, Methotrexate, Sirolimus, mycophenolic acid, mycophenolate mofetil

Nanomedicine for Immunosuppressive Therapy: Achievements in Pre-Clinical Research

ABSTRACT

Introduction: Immunosuppression has been the mainstay therapy in organ transplantation and autoimmune diseases. Different classes of drugs have been used in such disease conditions, but their effective clinical application has been limited by the emergence of systemic immunosuppression and/or individual drug side effects. Nanotechnology approaches may be used to modify the mentioned shortcomings of immunosuppressive therapy by either enhancing the delivery of immunosuppressant to their target cells of the immune system, and/or reducing their distribution to normal tissues, thus decreasing drug toxicity.

Areas Covered: In this manuscript, we will provide an overview on the development of nanotechnology products for the delivery of most commonly used immunosuppressive agents. At first, the rationale for the use of nanoparticles as means for immunosuppressive therapy in different disease condition will be discussed. This will be followed by a review of the major accomplishments in this area, particularly in preclinical *in vivo* studies.

Expert Opinion: The results of research conducted in this area to date, points to a great promise for nano-medicine in increasing the bioavailability, reducing the toxicity, and/or potentiating the activity of immunosuppressive agents. It is; therefore, safe to speculate the more rapid translation of nanotechnology in clinical immunosuppressive therapy in near future.

Keywords: Immunosuppression; Drug delivery; Nanoparticles; Cyclosporine A; Tacrolimus; Corticosteroids; Methotrexate; Sirolimus; mycophenolic acid; mycophenolate mofetil;

Contents

List of Abbreviations

AD	Atopic dermatitis
AUC	Area under the concentration-time curve
BSA	Bovine serum albumin
BUD	Budesonide
C_{max}	Maximum concentration
COPD	Chronic obstructive pulmonary disease
CyA	Cyclosporin A
CyD	Cyclodextrin
CYP3A	Cytochrome P-450 III-A enzyme subfamily
DMAB	Didodecylmethylammonium bromide
DMARD	Disease modifying anti-rheumatic drugs
DSP	DEX sodium phosphate
EPR	Enhanced permeation and retention
GC	Glucocorticoid;
GI	Gastrointestinal
GVHD	Graft-versus-host disease
HAS	Human serum albumin
HC	Hydrocortisone
IBD	Inflammatory bowel disease
IH	Intimal hyperplasia
IL-2, 4, 5, 10	Interleukin-2, 4, 5, 10
INF-γ	Interferon-γ
KCS	Keratoconjunctivitis sicca, or dry eye
MHC	Major histocompatibility complex
MiHA	Minor histocompatibility antigen
NMF	Mycophenolate mofetil
MPA	Mycophenolate acid
MPS	Methylprednisolone hemisuccinate
MRT	Mean residence time
MTX	Methotrexate
NP	Nanoparticle
NSAID	Non-steroidal anti-inflammatory drug
PBA	Phenylboronic acid
PCL	Poly-ε-caprolactone
PEG	Poly(ethylene glycol)
P-gp	P-glycoprotein

PK	Pharmacokinetics
PLA	Polylactide
PLGA	Poly(lactide-co-glycolide)
PVA	Polyvinyl alcohol
RA	Rheumatoid arthritis
RES	Reticuloendothelial system
SiRNA	Short interfering RNA
SLE	Systemic lupus erythematosus
SR	Sirolimus (rapamycin)
SLN	Solid lipid nanoparticle
SMEDDS	Self-microemulsifying drug-delivery system
TAC	Tacrolimus
Th1, 2	T-helper lymphocytes 1, 2
t_{max}	Time of maximum concentration
TPGS	D-alpha-tocopheryl poly-ethylene glycol succinate

1 Introduction

In early 20th century, Paul Ehrlich, the director of the Royal Institute for Experimental Therapy at Frankfort-on-Main, coined the phrase “magische Kugel” or “magic bullet” to explain his ideal drug that can specifically and exclusively target the diseased tissue without affecting the healthy organs of the body [1]. His vision has been inspirational and led to numerous research efforts in creating novel drug delivery systems at nanoscopic dimensions. In practice, many of the developed nano-delivery systems were capable of improving the performance of encapsulated drugs when compared to their conventional dosage forms, but did not completely fit the definition of a “magic bullet”.

One of the challenges with traditional dosage forms is the high proportion of the drug that is “lost” enroute to the systemic circulation. Different factors that contribute to this loss include poor drug solubility, incomplete drug permeability, or both. Nano-delivery systems have been used as effective solubilizing agents because of their nano-dimensions, or as tools to enhance drug permeability through different routes of administration. Another obstacle in obtaining the required drug concentration for therapeutic effect, is the early enzymatic degradation or elimination of the drug through kidneys [2]. For these drugs, the necessity for a substantial increase in the required doses of medication due to drug loss in body possesses direct risks. The emergence of potentially toxic metabolite(s) of the active ingredient can pose additional risks [3, 4]. Such risks can be mitigated by entrapment and protection of the drug in nano-drug formulations. Uncontrolled distribution of the drug to non-target organs is a main reason for unwanted side-effects [5].

Reducing the non-specific distribution of drugs to healthy organs and reduce their side effects by doing so has been the major focus of nanotechnology research in the past few decades. To this effect, several successful examples of nano-formulations of different anti-cancer drugs and antifungals achieving reduced drug toxicity are currently in use in clinic. Enhanced distribution of a drug to the site of the drug action can potentiate its therapeutic activity and fulfil an important part of Ehrlich's vision. In this context, passive targeting of anti-cancer agents to the tumor tissue based on enhanced permeation and retention (EPR) effect through the use of nano particulate delivery systems has attracted the most attention [6].

Research on the use of nanoparticuate delivery systems for modifying the therapeutic index of drugs in cancer has been the subject of intensive research. The potential benefits of nanotechnology approaches for the delivery of therapeutic agents in diseases other than cancer are explored to a lesser extent and deserve more attention.

This manuscript will focus on the use of nanotechnology in the delivery of immunosuppressive agents and the effect of this strategy on the pharmacokinetic profile as well as pharmacodynamics of the encapsulated drugs. Nanoparticles (NP)s have been used to increase the solubility, enhance the oral absorption [7] or modify the skin permeation profile of immunosuppressant drugs for local or systemic effects. The use of nanoparticulate delivery systems with a capability of reducing drug exposure and undesired effects to other tissues has attracted significant attention in the field of immunosuppressive therapy. The nanoparticulate delivery systems may also permit the administration of higher than currently used doses of the immunosuppressant drugs leading to better ultimate therapeutic outcome.

2 Rationale for the use of NPs in the delivery of immunosuppressive agents in clinic

The immune system is a crucial defense mechanism in the body. Random non-specific systemic suppression of the immune system throughout the body can significantly increase the risk of infection. Non-targeted immunosuppression could particularly be risky in patients with compromised immune system such as HIV or cancer patients. Nanoparticulate delivery of immunosuppressants can potentially elucidate specificity for these agents to the diseased organs (Figure 1).

Systemic immunosuppression is extensively used in organ transplantation. Transplantation was introduced into medical practice in 1953 as a strategy for end stage organ disease [8]. However, graft rejection from mismatched donors limited the potential benefits of this strategy. This type of rejection is the result of a complex immune response to alloantigens expressed on the grafted cells which include the major histocompatibility complex (MHC) and the minor histocompatibility antigens (miHAs) [9]. In addition to T cells activation, many reports indicate a role for B-cells in acute cellular and chronic humoral rejection [10]. Immunosuppressive agents have played an essential role in moderating the immune response to help prevent the rejection and loss of the allograft and in increasing the survival of transplanted patients. However, severe side-effects is usually associated with the long-term use of immunosuppressants. Graft-versus-host disease (GVHD) is a specific case in which donor cells attack the immunocompromised host, and is a major cause of mortality in hematopoietic stem-cell transplantation [11]. Even though the exact mechanism of this reaction is not fully understood, activation of donor T cells against host antigens and release of inflammatory cytokines seem to be involved [12]. Infection by opportunistic organisms and primary pathogens, which also get a chance for growth due to

general immunosuppression, are among the most common causes of death in transplant patients. In this context, the use of NPs to achieve targeted immunosuppression can play a positive and crucial role in reducing the above-mentioned complications.

Another clinical setting in which targeted immunosuppression might be of potential benefit is in the management of organ specific autoimmune disorders. Autoimmune disorders could be categorized as local conditions that affect a specific organ in the body or systemic disorders which affect multiple organs and systems. It has been shown that local disorders are usually caused by cell-mediated mechanisms for attacking intracellular pathogens, and therefore, develop with T-helper lymphocyte-1 (Th1) cytokines (e.g., interleukin-2 (IL-2) and interferon-γ (INF-γ)) predominance [13]. On the other hand, systemic immunosuppression is usually associated with robust humoral responses (antibody-based reactions) that are accompanied with an increase in the levels of Th2 cytokines (e.g., IL-4, IL-5, and IL-10), complement-mediated cell targeting, and over-production of autoantibodies [14, 15]. Rheumatoid arthritis (RA) is the most common autoimmune disease affecting an estimated 0.5–2 % of the world population, and is characterized by a chronic and systemic inflammation that is mainly destructive to the joints, due to inflammation of synovial tissue [16]. The contribution of genetic and environmental factors to RA has been reported [17] [18]. RA treatment usually relies on anti-inflammatory strategies (e.g., non-steroidal anti-inflammatory drugs (NSAID)s or corticosteroids) and/or on disease modifying anti-rheumatic drugs (DMARDs) including traditional agents such as methotrexate and cyclosporine as well as biologic agents such as etanercept and infliximab. Factors including fast systemic clearance and/or low affinity and non-specificity to the target inflamed joints, demand that these agents be given at relatively high and frequent dose to achieve therapeutic level. This

comes at the expense of exposing the patients to serious side effects. Intra-articular injection of glucocorticoids has been shown to produce clinically meaningful pain relief in RA [19], which shows the importance of direct drug delivery to the site of action in the management of RA. The same rationale would apply to DMARDs. Nanoparticulate delivery systems are found to naturally accumulate in the organs that comprise the reticuloendothelial system (RES), including the spleen and liver, and passively target residing macrophages involved in the inflammatory response, in these organs [20]. Enhanced permeability at the inflamed sites attributed to an increase in the size of the gap between endothelial cells with dilation, can lead to the accumulate of NPs in inflamed joints through passive diffusion or convection [21].

Inflammatory bowel disease (IBD) is another example of a localized autoimmune disease for which different immunosuppressant agents are used orally [22, 23]. Macrophages and neutrophils present in the inflamed tissue can easily identify and uptake NPs, which allows for NP drug delivery systems to passively target those immune-related cells [24]. Moreover, it has been reported that NPs with bioadhesive properties (e.g., chitosan NPs) in the thickened mucosal layer in inflamed tissues can enhance the transmucosal delivery of immunosuppressant drugs [25, 26]. The extent of muco-adhesion is size-dependent [27]. Therefore, a NP delivery system can play a major role in enhancing the immunosuppressant drug exposure to the target cells and in prolonging the interaction of the incorporated drug with the site of drug action in IBD [28].

Among other immune-related disorders that might benefit from nano-delivery of immunosuppressive agents, are immune related pulmonary disorders, such as chronic obstructive pulmonary disease (COPD). COPD is a progressive pulmonary disorder that could

cause immunogenic cell death in the airway followed by the release of damage-associated molecular patterns. This can activate innate and adaptive immune responses [29]. The pulmonary delivery of immunosuppressive agents encapsulated in NPs seems to be a safe and effective option for localized therapy in the lungs [30]. Success in this case following systemic administration of an immunosuppressive agents such as cyclosporine or tacrolimus has been limited due to their excessive toxicity profile, narrow therapeutic index, and a great inter-subject variability in the pharmacokinetics (PK), especially in the absorption phase when given orally. The pulmonary delivery of NP formulations of these agents applied by inhalation through nebulizers or dry powder inhalers can eliminate these obstacles by providing a sustained release of the active molecules in the airways with minimal systemic drug exposure and toxicity as well as less frequent administrations [31, 32]. It is worth to note that sustained release formulations are not feasible for oral cyclosporine due to a narrow window of absorption in the gastrointestinal (GI) tract for this drug [33].

Immunosuppressant drugs have also been used for the treatment of many ophthalmic immune-related conditions through systemic or local administration. Corneal graft rejection [34], non-infectious autoimmune uveitis [35], vitreous inflammation [36], and scleritis [37] are among these conditions. While intraocular fluids and extra-ocular organs (e.g., cornea, conjunctiva and lachrymal glands) can be reached and therapeutic concentrations of drugs be maintained through the systemic administration of these drugs, the random distribution of the administered dose to non-target organs, and toxic adverse effects are among drawbacks of this approach. Ocular administration can overcome some of these problems [34]. Preparation of ophthalmic dosage forms for immunosuppressant agents which can reach the intraocular space has proven

to be a challenge. This is due to the need for excessive amounts of surfactants and oils for the solubilization of these agents, which in turn adversely affects the ocular tolerance to the formulations. NPs can provide viable options in this case as delivery systems that can enhance the retention and penetration of the medication in the eye but at the same time does not obstruct or significantly blur vision.

3 Nanoparticulate delivery of major immunosuppressant drugs

Different categories of pharmacological agents have been applied for immune suppression, which include calcineurin inhibitors (cyclosporin and tacrolimus) and their newer analogues (sirolimus and everolimus), inhibitors of nucleotide synthesis (e.g., azathioprine, mycophenolate mofetil, leflunomide), cytostatic agents affecting T cells and B cells division (e.g., methotrexate, mercaptopurine and gemcitabine), phosphodiesterase-4 (PDE4) inhibitors (mostly used as anti-inflammatory agents in COPD and autoimmune diseases), antibodies, and other biological approaches. In the following subsection, the use of nanotechnology for the delivery of major immunosuppressive agents for different indications will be reviewed (Fig 2).

3.1 Cyclosporine A

Isolation of cyclosporin A (CyA) from *Tolypocladiuminflattum gams* in 1971 has been one of the greatest discoveries in the treatment of organ transplant rejection [38]. This highly lipophilic cyclic undecapeptide selectively targets the proliferation of helper T-cells but not the suppressor T-cells [39]. Cyclosporin A is indicated for the prevention of rejection following transplantation of heart, lung and kidney; treatment of GVHD with bone marrow transplant; and treatment of various autoimmune conditions such as RA, nephrotic syndrome and psoriasis [40]. It is available

for intravenous (IV) infusion but the oral administration as soft gelatin capsules or solution is more commonly used. The original CyA product, Sandimmun® (Novartis AG, Basel, Switzerland), is an oil-in-water emulsion. A newer micro-emulsion formulation, Neoral® (Novartis AG, Basel, Switzerland) which improves the oral bioavailability of CyA is also approved for use [41]. An ophthalmic emulsion of CyA, Restasis® (Allergan Inc., Irvine, CA), is also approved by FDA for the treatment of inflammation in the dry eye syndrome.

Cyclosporin A, being highly lipophilic, distributes widely into tissues. The primary route of its elimination is by metabolism in the liver and excretion of the drug and metabolites by the biliary system [42]. The key catalyst for the biotransformation of CyA in the liver is the cytochrome P-450 III-A enzyme subfamily (CYP3A). Cyclosporin A is also eliminated through urinary excretion, however, to a lesser extent (less than 10% in humans) and only a fraction of that being as unchanged drug [43]. Cyclosporin A suffers from a narrow therapeutic index and a great intra and inter subject variability of its pharmacokinetic (PK), especially in the absorption phase, which greatly complicate therapy [44]. This means that even at the same dose within the recommended range, CyA therapy could be sub-therapeutic for certain patients leading to therapy failure or toxic for other patients [45]. A number of serious side effects have been reported with the use of CyA, including nephrotoxicity and hypertension [46]. Because of its very low water solubility (23 µg/mL), various solubilizing agents, such as Cremophor EL® have been used in CyA formulation. The solubilizing agents themselves can cause adverse effects and add to the burden of CyA therapy [47].

Encapsulation of CyA in NPs has been investigated for different routes of administration as detailed below. The results of these studies show in general, properly designed NPs can effectively solubilize CyA in aqueous formulations reducing the need for other excipients. The NP formulations of CyA have shown to be feasible for drug use via ocular or topical routes of administration while enhancing drug delivery to the diseased site. The sustained release of drug that is usually accomplished with many NP formulations can help maintaining consistent therapeutic CyA concentrations, while reducing the frequency of CyA dosing. Moreover, NPs can limit the exposure of CyA to sites of drug toxicity, e.g., kidneys particularly when given by the IV route. There are a number of papers showing that the oral administration of NP formulations of CyA can also lead to a reduction of its nephrotoxicity, but the reason for this effect is not elucidated.

Parenteral administration- Our research group have reported designing micellar structures based on self-assembly of poly(ethylene oxide)-*block*-poly(ε-caprolactone) (PEO-*b*-PCL) co-polymers for solubilization of CyA [48]. These NPs not only increased CyA's aqueous solubility by ~ 100-folds, but also sustained the release of CyA *in vitro* [49]. This formulation showed a significant reduction in nephrotoxicity of CyA compared to Sandimmune® in healthy Sprague Dawley rats, despite achieving significantly higher CyA blood concentrations [50]. Pharmacokinetic and biodistribution studies demonstrated a 6.1-fold increase in the area under the blood concentration-time curve (AUC), and 10- and 7.6-fold reduction in the volume of distribution and systemic clearance of CyA, respectively, in healthy rats. The NP formulation also restricted the distribution of CyA to liver, kidneys, and spleen [51]. Owing to reduced distribution in kidneys, the NP formulation of CyA was found to attenuate the nephrotoxic effects of this drug

as evidenced by a non-significant change in the creatinine clearance after multiple doses of the formulation in rats compared to control animals receiving saline injection. This was in contrast to the commercial formulation of CyA, i.e., Sandimmune®, which led to a significant decrease in creatinine clearance following identical dosing schedule [50]. The NP formulation showed a potent immunosuppressive effect similar to that of Sandimmune® both *in vitro* and *in vivo* [52].

Mondon *et al* prepared micellar formulation of CyA based on block copolymers of PEO-block-hexyl-substituted poly(lactides)(hexPLA) (PEO-b-hexPLA) [53]. At the block copolymer concentration of 20 mg/mL, the prepared nano-formulation increased the CyA solubility up to 500-fold, and as a result, was able to provide the clinically used CyA concentrations while requiring four times less excipients compared to Sandimmune®. The low toxicity of this formulation was established using 3 different cell lines and on a chick embryo chorioallantoic membrane model.

Poly(ethylene glycol) (PEG)-grafted chitosan, in combination with lecithin (as vesicle-forming lipid) and poloxamer, has also been investigated for the systemic delivery of CyA *in vitro* and *in vivo* [54]. The resulting NPs were ~ 90 nm in size; close to neutral in charge and demonstrated a sustained-release pattern *in vitro*. Systemic administration of these NPs via IV injection significantly decreased the apparent volume of distribution of CyA by 3-folds and its clearance by 33-folds, prolonged the half-life of elimination phase by 21 folds, and increased the AUC of the encapsulated drug by over 25 folds in comparison to a control solution of CyA dissolved in ethanol and Cremophor EL®. The important role of the PEG block in this approach became more apparent

when the same NPs but without the PEG coating were proven to be inefficient in changing the AUC of CyA.

Oral administration- Poly(lactide-co-glycolide) (PLGA) NPs has been studied for the encapsulation and oral delivery of CyA alone or in combination with other components (**Table 1**). Italia *et al.* reported the formulation of PLGA NPs with an average size of ~ 140 nm, that increased the CyA solubility up to ~ 644.2 µg/mL [55]. When orally administered to Sprague Dawley rats, these NPs increased the bioavailability of CyA reaching a relative bioavailability of 119.2% as compared to the commercially available Neoral® formulation, while decreasing the associated nephrotoxicity. Similar approach has been reported by Ankola *et al.* who formulated CyA-loaded PLGA NPs with slightly smaller particle size (~ 110 nm), which could match the bioavailability and maximum blood concentration (C_{max}) of Neoral® with significantly lower nephrotoxicity [56].

Wang *et al.* have also evaluated the efficacy of PLGA-based NPs for oral delivery of CyA as well as solid lipid nanoparticles (SLN)s composed of Precirol® ATO 5 and Captex® 100 and a self-microemulsifying drug-delivery system (SMEDDS) made from a mixture of Labrafil® M 1944 CS, Cremophor EL®, and Transcutol® P in a side-by-side *in vivo* study in beagle dogs [57]. While each of the SLNs and SMEDDS formulations achieved bioavailability that was not statistically different from the reference Neoral®, PLGA NPs only produced a relative bioavailability of 22.7%. These results for CyA loaded PLGA NPs are not in line with the reports discussed earlier. The exact reason for this controversy is not clear but may be attributed to the use of the polyvinyl alcohol (PVA) solution as a stabilizer here instead of Didodecylmethylammonium bromide (DMAB) used in the other studies [55].

The effect of varying the emulsifying agent used in the formulation of oral lipid NPs of CyA on the bioavailability of CyA has been recently investigated [58]. Three different formulations were developed, using Percirol® as a lipid phase and Tween® 80 as an emulsifier in the first formulation and a mixture of phosphatidylcholine or Pluronic® F127 with taurocholate in the other two. An *in-vivo* pharmacokinetic study following a single oral dose in Balb/c mice revealed the formulation which used Tween® 80 achieved higher C_{max} and AUC resulting in a relative CyA bioavailability of 149.1% and 133.5% for the freshly prepared and the lyophilized forms when compared to the Neoral®, respectively, while the other two formulations matched the bioavailability of Neoral®. This effect is suggested to be due to a higher stability of the formulation which used Tween® 80 in gastric and intestinal pH over time.

The enhancement of oral absorption of CyA (along with other hydrophobic drugs, e.g., griseofulvin) has also been reported by using chitosan modified with hydrophobic moieties such as quaternary ammonium palmitoyl glycol. The use of nanostructures increased the C_{max} of CyA compared to Neoral® by 6-folds after oral administration in male Wistar rats [7]. An increase in the dissolution rate of CyA, adherence and penetration of NPs in mucus layer, and enhancement of the trans-cellular transport of CyA was hypothesized to be the reason behind this observation.

Designing pH-sensitive NPs for oral delivery of CyA is another approach. The idea is to create a delivery system that has a stable association with its cargo in acidic conditions of stomach, but readily releases CyA in the pH of the small intestine. If this is accomplished, the drug is protected and contained in the upper parts of the GI tract that is not the main absorption site for CyA; it is, however, released later when it arrives to the absorption site [59]. An *in vivo* study performed

in Sprague-Dawley rats to compare NPs prepared with Eudragit® (poly(methacrylic acid and methacrylate) copolymer) showed that the bioavailability of the NPs was comparable to that of Neoral® [60]. The same research group also reported a similar study using hydroxypropyl methylcellulose phthalate as the pH-sensitive component of the NPs. In this study, using the same animal model, NPs increased the mean residence time (MRT) and decreased the elimination constant of CyA in the central compartment, when compared to Neoral® [59].

Ocular administration- Various NP systems have recently been developed trying to improve the efficacy of ocular CyA treatment. In this context, PEG-PLGA NPs of CyA were found to have an equivalent activity in T-cell proliferation suppression compared to that of free CyA [61]. CyA-loaded NPs using PLGA alone, mixed with Eudragit® RL, or coated with Carbopol® have been evaluated for ocular administration in dry eye syndrome and inflammation of eye surface. These formulations demonstrated an average particle size of ~ 148 nm (which increased significantly with Carbopol® coating), extended-release profiles totaling 75-90% drug release in 24 h, and increased ocular retention times [62]. Similarly, hyaluronic acid-coated nanospheres formed from PCL/ benzalkonium chloride have shown an increase in corneal CyA concentration in rabbit corneas at various time points during 1-24 h, reaching 6-8 folds of the concentrations reached by a control solution of CyA dissolved in castor oil [63].

Chitosan nanocarriers were also used for ocular delivery of CyA. It has been hypothesized that since mucin is negatively charged in physiologic pH, using a cationic carrier could enhance the bioavailability of ocular formulations [64]. CyA-loaded chitosan NPs prepared by a spray-drying technique have been reported to prolong the residence time of the formulation on the corneal

and conjunctival surfaces, which resulted in CyA detection in both aqueous and vitreous humour samples for 72 h in sheep [65]. Chitosan-coated PLGA NPs have also been designed for ocular delivery of CyA. *In vitro* experiments have confirmed a sustained-release profile and maintained efficacy [66]. Chitosan has also been used in association with SLNs. Ex vivo experiments on pig's cornea showed biocompatibility of these particles and enhanced permeation and/or penetration of CyA via increased cellular internalization [67].

Liu *et al* reported the formulation of CyA NPs based on poly(D,L-lactide)-b-Dextran with surfaces functionalized with the mucoadhesive ligand, phenylboronic acid (PBA). These NPs (with an average diameter of ~ 36 nm and an encapsulation efficiency of ~ 30%) sustained the release of CyA at clinically relevant doses for up to five days [68]. *In vivo* studies in dry eye induced female C57BL/6 mice showed that a once weekly application of these NPs resulted in elimination of the inflammatory infiltrates and a full recovery of the ocular surface, which compared favourably to a 3 times daily application of Restasis®. Prosperi-Porta *et al* also investigated the encapsulation of CyA in a series of (poly(L-lactide)-b-poly(methacrylic acid-co-PBA)) block copolymer mucoadhesive micelles [69]. These formulations achieved a high encapsulation efficiency of over 99.8% and a slow release within 14 days.

Tommaso et al developed a micellar formulation for CyA which provided a success rate of cornea graft transplantation of 73% in treated animals, equivalent to that achieved with systemic CyA, compared to 25% for the control group [70]. SLNs have also been extensively studied, for CyA delivery. The reports on these formulations mostly include only *in vitro* testing of the formulations, though [71-73].

In 2008, Gokce *et al* reported preparation of CyA-loaded SLNs, with average size of 225 nm and zeta potential of ~ - 17 mv, that showed significant cellular internalization of CyA *in vitro* and *ex vivo* (in excised pig cornea) and seemed promising for ocular delivery [74]. The same group later reported *in vivo* delivery of the same SLNs to cul-de-sac of rabbits, and showed aqueous humor drug levels as high as 50.53 ng/mL without the emergence of any serious ocular irritation [75].

Topical administration- Nanoparticle delivery systems have also been used for the topical delivery of CyA in inflammatory conditions of the skin such as psoriasis. They can help achieve and maintain clinically relevant levels of CyA at affected sites, thereby eliminating the need for systemic administration of CyA. Several research groups have reported skin penetration and absorption of CyA with similar approaches that have been studied for systemic delivery of CyA. SLNs prepared with ticaprin (1,2,3-tridecanoylglycerol), L-α-phosphatidylcholine, and Tween® 80 have been evaluated for topical application using murine skin and Franz diffusion cells, as well as an *in vivo* murine model. The SLN formulation of CyA not only increased the skin penetration by 2-folds compared to CyA/oil mixture, but also relieved the dermatitis symptoms in the murine model by demonstrating an inhibiting effect on cytokines IL-4 and -5 [76]. Lopes *et al.* reported a reverse hexagonal phase nano-dispersion system using monoolein, oleic acid, and poloxamer that solubilized CyA up to 6 mg/mL, increased skin penetration *in vitro*, and created 1.5- and 2.8-fold higher concentrations in stratum corneum and epidermis/dermis, respectively, compared to CyA dissolved in olive oil in male hairless mice [77]. In order to increase the dermal penetration of CyA, Romero *et al* formulated an amorphous suspension of CyA NPs which was prepared by wet bead milling using kolliphor® tocopheryl poly-ethylene glycol succinate (TPGS) as a stabilizer [78]. The NPs showed a size of ~350 nm. The efficacy of the formulation was investigated using

fresh pig skin and the tape stripping method. Incorporated in hydroxypropylcellulose gel as a vehicle, the NPs at tape 30 were able to penetrate a cumulative CyA lrvel that was 6.3-folds higher than that achieved with a micrometer-sized CyA powder in the same vehicle.

NPs formed with PCL and CyA by a solvent evaporation method have also been reported [79]. In this *in vitro* study using human skin organ cultures, penetration of the fluorescently labeled NPs through epidermis and dermis within 2 and 24 hours, respectively, was revealed. The NPs reduced the secretion of IL–1β, IL–6, IL–8, IL–20 and IL–23 in a psoriasis model.

Topical application of CyA by NPs has also been reported on mucus membrane, and in particular, for inflammatory conditions such as oral mucosal ulceration. Karavana *et al.* have recently reported a bio-adhesive gel for buccal administration in the treatment of aphthous stomatitis [80]. In this study, CyA was first incorporated in SLNs formed with Compritol® 888 ATO in water using poloxamer 188 and Tween® 80. The SLNs dispersion was then converted to a gel by adding Carbopol® 974 P NF and hydroxypropylmethylcellulose K 100M. The *in vivo* studies in rats showed ~ 65% of formulation to remain on the buccal mucosa 6 hours after the application, which confirmed the bio-adhesive properties of the formulation. Using adult male New Zealand rabbit models of oral ulcer on the gingiva, rapid healing of the ulcers upon use of CyA NPs was observed compared to control groups receiving gel alone or no treatment.

3.2 Tacrolimus

Tacrolimus (TAC) is a macrolide calcineurin inhibitor isolated from a strain of *Streptom*yces *tsukubaensis.* It is used as an effective immunosuppressant with a mechanism of action similar to that of CyA [81]. In fact, multiple clinical trials showed more efficacy and less severe systemic

side effects for TAC compared to CyA [82-85]. TAC has been widely used in transplant patients, ocular immunologic disorders, and atopic dermatitis (AD). Although, TAC is approximately 99% protein bound, it widely distributes in the body [86]. TAC has a narrow therapeutic window and its half-life ranges from 11.7 to 34.8 h [87]. Moreover, it is a substrate of P-glycoprotein (P-gp) and cytochrome P450 3A4 (CYP3A4). Thus, any modulator of P-gp and CYP3A4 can change its PK [88]. TAC is poorly water soluble (4-12 μg/mL), its oral bioavailability is poor and shows high intra and inter-subject variability ranging from 4-93% (with a mean bioavailability of 17-22%) [87, 89]. Low aqueous solubility, site dependent permeability, extensive first pass metabolism in the gut and liver, P-gp mediated drug efflux and influence of food are the most important reasons for low and variable oral bioavailability of TAC [89]. NPs may be used to correct some of the shortcomings of TAC such as poor water solubility, large volume of distribution and low GI or skin permeability, and as a result enhance its bioavailability and/or therapeutic activity. While TAC is available as an injection, Prograf® (Astellas Pharma, Tokyo, Japan), the intravenous administration of TAC is usually limited to early stages of organ transplantation and to cases where oral administration is not feasible.

Parenteral administration- Thao *et al* reported incorporation of TAC with human serum albumin NPs (~186 nm in diameter). This approach, increased TAC water solubility by 46-folds and resulted in a sustained release of TAC for 24 h [90]. The NPs showed an anti-proliferative activity on activated T cells, but not on normal cells *in-vitro*, and significantly reduced the arthritis clinical score by 2- and 3-folds compared to TAC injected solution or orally administered suspension, respectively, in a mice model of collagen-induced arthritis.

The solubility of TAC has been reported to increase with cyclodextrin (CyD) derivatives such as heptakis(2,6-di-*O*-methyl)-β-CyD (DM-β-CyD) [91]. In male Wister rats this formulation provided a time to maximum concentration (t_{max}) of 8 h and MRT of 9.72 h compared to t_{max} of 30 and 15 min; and MRT of 8.76 and 7.65 h for TAC loaded bovine serum albumin (BSA) NPs and a reference TAC solution, respectively [91]. TAC loaded BSA NPs have shown favorable PK profile compared to Prograf®, the commercial formulation of TAC [92]. A single IV dose of TAC loaded BSA resulted in 1.8-fold increase in TAC AUC compared to Prograf® in Sprague Dawley rats, and a 1.2-1.8-fold less drug accumulation in the kidneys within 24 h.

A nanosomal formulation of TAC for IV use has also been prepared using soy phosphatidylcholine and alpha-tocopherol [93]. This formulation showed similar PK profile to that of marketed polyoxyl 60 hydrogenated castor oil formulation in healthy human subjects following 4 h intravenous infusion.

Polymeric micelles have also been applied in several studies as a delivery vehicle for TAC. Allen *et al* prepared PEO-b-PCL block copolymer micelles containing TAC for the treatment of peripheral nerve injury [94]. Sprague Dawley rats with lesioned sciatic nerves injected subcutaneously once every 6 days with the micellar TAC showed full function recovery in 16 days. This was achieved despite subcutaneous administration of only 20% of effective TAC daily doses. Wang *et al* also reported on the use of same TAC micellar formulations for the management of ulcerative colitis [95]. *In-vivo* results in mice with dextran sulfate sodium induced colitis showed that the micellar formulation administered intravenously or orally, provided effective yet safer

treatments than the free TAC as measured by body weight loss, colon length and pathological changes in colon specimens.

Oral administration- Self-emulsifying delivery systems can enhance the oral absorption of TAC through its solubilization in the GI tract, inhibition of P-gp drug efflux and pre-absorptive metabolism, increasing lymphatic transport and permeabilization of GI membrane. Optimized SMEDDS formulations for TAC using Capmul® MCM C8 as the oil phase, Cremophor® EL as surfactant and Carbitol™ as co-surfactant were prepared and shown to significantly reduce lymphocyte count in peripheral blood when compared to TAC capsule, Pangraf® (Panacea Biotec Ltd., India) upon oral administration for 10 days in Swiss albino mice [96]. This observation was attributed to a more efficient drug absorption in the GI tract. An *in-vivo* study in Sprague Dawley rats, following administration of SMEDDS using ethyl oleate as the oily phase; Solutol HS 15 as the surfactant; and glycofurol as the co-surfactant, showed a 3-fold increase in TAC C_{max} and a 3-fold decrease in t_{max}. This formulation resulted in more TAC accumulation in RES organs including liver, spleen, lung and small intestine at 15 min following administration; but less TAC was accumulated in kidneys at 3 h post-dose when compared to Prograf® [97]. In another study, two kinds of SMEDDS were prepared for TAC [98], with Miglyol® 840 and Transcutol® P as oil phase and co-surfactant, respectively, in both formulations. The two formulations differed in the use of either TPGS or Cremophor® EL 40 as surfactant. These formulations increased TAC relative bioavailability 7-8 folds when compared to TAC solution. The authors suggested that excipients and not TAC solubilization play an important role in increasing drug bioavailability, perhaps through interfering with the P-gp efflux or CYP450 mediated drug elimination.

pH-sensitive microspheres have been used for the local delivery of TAC to the colon for the treatment of IBD [99]. To achieve this, the authors prepared TAC loaded NPs of PLGA and then embedded the NPs in Eudragit® P-4135F, a pH-sensitive polymer proven to release its cargo in the lower intestine. In an *in vitro* release study in pH= 7.4, an immediate release of nearly 100 % of incorporated drug was observed. In contrast, at pH of 4.0 the NPs and the drug were both retained within the Eudragit® coating. This design led to an improvement in the therapeutic activity in male Wistar rats with induced colitis. The difference in therapeutic outcome became significant on day 9 compared to the untreated control group and TAC solution treated animals.

Topical administration- Li *et al* prepared ethosomal formulations of TAC with lower particle size (~76-104 nm) and higher encapsulation efficiency (~77-80%) as compared with a traditional liposomal formulation of the drug [100]. The ethosomal carriers demonstrated superior penetration of TAC through the skin *in vitro*, with high TAC concentrations in the epidermis of excised rat skin, over 4-fold increase in comparison to the reference, Protopic® ointment (Astellas Pharma, Tokyo, Japan). *In-vivo* studies on Balb C mice with 2,4-Dinitrofluorobenzene induced dermatitis revealed improved inhibitory action of the ethosomal formulation on mouse ear swelling compared to Protopic® ointment. In another study, Erdogan *et al* prepared a liposomal TAC lotion and used it in a skin graft murine model [101]. Based on radiolabeling studies, the TAC level in the skin was 9 times higher at 24 h following a topical administration of the liposomal TAC compared to the TAC IV administration. The difference persisted at 96 h. A 3-day course of topical TAC liposomal administration significantly prolonged skin graft survival (by 2 days when given alone, or by 6 days when combined with systemic administration of the liposomal

formulation), while the systemic administration of either TAC or the liposomal TAC formulation alone, was unable to delay skin graft rejection.

Pulmonary administration- Seo *et al* formulated TAC inhalable albumin NPs for the treatment of pulmonary fibrosis [102]. The NPs (with a particle size of ~182 nm and a zeta potential of -34.5 mv) showed a sustained release of TAC for ~24 h. Histopathological evaluations of excised lung tissues from mice with bleomycin-induced pulmonary fibrosis revealed a markedly lower degree of lung inflammation for NP treated animals compared to untreated mice, and mice treated with intraperitoneal TAC.

3.3 Methotrexate

Methotrexate (MTX) is an antifolate agent that has shown anti-proliferative, anti-inflammatory and immunosuppressive activity. It is clinically used as an antineoplastic drug for the treatment of various types of cancer and as a DMARD for the treatment of many autoimmune diseases. MTX demonstrates low permeability (LogP of 0.94) and poor aqueous solubility (0.01 mg/mL) but the sodium salt of MTX is soluble in water [103]. MTX can be given orally or by injection. Oral MTX is absorbed in the GI tract by an active transport mediated mechanism [104]. Its oral absorption is highly variable between individuals and the absolute mean oral bioavailability is found to range from 30-90%. The bioavailability of subcutaneously administered MTX is significantly higher and less variable than oral MTX [105]. A single-dose administration of MTX subcutaneously has resulted in a higher relative bioavailability and fewer GI adverse effects than oral MTX in human [106]. MTX shows high tissue distributions; its plasma protein binding is in the range of 20-57% and its plasma half-life is in the range of 5-8 hours [107]. Therapeutic

application of MTX induces adverse effects such as acute and chronic hepatotoxicity, bone marrow suppression, nephrotoxicity and chronic interstitial obstructive pulmonary disease [108]. Moreover, GI adverse effects such as diarrhea, ulcerative stomatitis, haemoragic enteritis and perforation have also been reported following MTX administration. Encapsulation of MTX in nano-delivery systems may be used to increase its bioavailability, reduce adverse effects and at the same time increase its accumulation in the diseased site [109].

Parenteral administration- Garg *et al* developed SLN formulation of MTX for IV injection using Gelucire® 50/13, [110]. In Sprague Dawley rats, SLN formulations increased the MTX half-life by 3-folds and its AUC by 3.6-folds compared to the marketed MTX injection. An organ distribution study in female Wistar rats with DMBA induced breast cancer showed less accumulation of MTX as part of NPs in the heart, kidneys, or liver. Another SLN formulation for the IV administration of MTX was prepared by stearic acid and soya lecithin as a surfactant [111]. This formulation was shown to increase the half-life of MTX by 76% (from 8.2 to 14.5 h) and its MRT by 49% (from 16.1 to 23.9 h) compared to a MTX solution.

Chen *et al*. reported the formulation of MTX-loaded Pluronic® P105/F127 mixed micelles for IV application [112]. The micellar formulation prolonged the systemic circulation of MTX *in vivo* in Sprague Dawley rats with the MTX half-life increasing by 2.2 folds and the AUC by 4.5 folds compared to the free MTX injection. In a different study, MTX was encapsulated in a micellar nano-network of polyethyleneimine ionomer containing redox-sensitive cross-link [113]. The formulation displayed a prolonged *in vitro* swelling-controlled release of MTX over 24 with no initial burst when compared with the free MTX.

Williams *et al*, reported liposomally conjugated MTX, that provided better anti-inflammatory effects in male Lewis rats with established adjuvant arthritis and displayed less haematotoxicy compared to the free drug when administered IV [114]. Free MTX, on the other hand was shown to be more effective in controlling the progression of the disease in a preventative setting. Gottschalk *et al* considered encapsulating MTX in cationic liposomes and have found the formulation to provide a significantly superior reduction in leucocyte- and platelet-endothelial cell interaction, functional capillary density, and knee joint diameter when given IV to arthritic female C57/BI6 mice compared to control [115]. Mice treated with Free MTX or empty liposomes did not achieve significant reductions in these parameters. Hong *et al.* prepared PEGulated liposomes of MTX, and compared the PK and bio-distribution of these formulations to free drug and MTX formulations in conventional liposomes in Sprague Dawley rats following iv administration [116]. The results of their study showed stealth liposomes containing dipalmitoylphosphatidylcholine/cholesterol/distearoyl phosphatidylethanolamine-N-PEG (DPPC /CH/DSPE-PEG) to decrease MTX clearance by 53.1-fold compared to free MTX, effectively prolong the blood circulation and reduce hepatosplenic and kidney uptake of MTX. A different study by Prabhu, *et.al* investigated PEGylated, conventional and chitosan coated liposomal formulations of MTX and found a significant reduction in edema volume by these formulations in complete Freund's adjuvant arthritis models in Wistar-Lewis rats compared to that observed for control or free drug treated animals following IV administration [117] .

Albumin based delivery systems have also been investigated for injectable MTX. A human serum albumin (HSA) conjugate of MTX was shown to reduce the onset of arthritis appearance in a collagen induced arthritis rat model following IV injection. This was attributed to the

accumulation of encapsulated MTX in inflamed paws. MTX levels were reported to be 17-fold higher for encapsulated MTX compared to that achieved with free MTX [118]. The MTX-HSA was about 5-folds more potent than free MTX. The plasma half-life of HSA and MTX-HSA were shown to be ~19 days in a phase I clinical trial of the formulation in cancer patients [119], which is much longer than the half-life of a low dose MTX given IV to patients with RA (~6–8 h).

MTX loaded poly(L-lactic acid) microspheres were developed by Liang *et al*, and shown to reduce the plasma concentration of MTX by 10-folds compared to free MTX in white New Zealand rabbits injected intra-articularly, indicating a decreased clearance of MTX from the joint cavity [120]. Niosomal formulations of MTX have also been developed and shown to prolong MTX circulation in the blood and increase the MTX uptake in the liver and brain, due to increased permeability, following IV administration [121]. When given orally, the niosomal formulation significantly improved the absorption. In both routes of administration higher levels of the drug were accumulated in the liver.

Topical administration- Misra *et al* formulated SLNs of MTX as a topical gel to improve the therapeutic index of MTX and to replace or supplement oral MTX therapy [122]. Their formulation, which was clinically evaluated on psoriasis patients, has shown improved drug accumulation in human skin; average percent improvement in healing (APIH) of lesions, and reduction in average score of erythema and scaling in psoriasis. Trotta *et al*. developed an oil in water (o/w) microemulsion of MTX and reported a 6-fold increase in the flux of MTX from the microemulsion compared to MTX solutions resulting in improved permeation from the skin of hairless mouse [123]. Amarji *et al* developed a different MTX microemulsion based hydrogel and

showed the formulation to possess improved penetration in different skin layers reducing drug's systemic absorption [110]. A better therapeutic activity was observed *in vivo* in C57BL/6 mice with imiquimod induced psoriasis model by the MTX microemulsion hydrogel compared to its control cream formulation.

Pulmonary administration- Doddoli *et al* administered liposomal or free MTX by pulmonary instillation and analyzed its distribution in male Wistar rats [124]. The liposomal formulation was found to significantly increase MTX accumulation in the lungs (by 4-fold at 180 min post-dose) compared to free drug. Liposomal MTX was found to cross the lung barrier, but to a lesser extent than free MTX, suggesting better local action as well as a delayed systemic distribution by this formulation.

3.4 Glucocorticoids

Glucocorticoids (GCs) are a class of steroid hormones that have a wide range of physiological activities including inhibition of antigen presentation, cytokine production, and proliferation of lymphocytes [125]. These agents are frequently used as anti-inflammatory drugs for various conditions including asthma, dermatitis and RA. They are also used as immunosuppressants for autoimmune diseases such as systemic lupus erythematosus. These agents also have application in organ transplantation. The most commonly used systemic GCs include budesonide, dexamethasone (DEX), fludrocortisone, hydrocortisone (HC), methylprednisolone (MPS), and prednisolone, which are usually given orally (and often as adjunctive therapy), but dosage forms for their IV, intramuscular, intra articular, intralesional, topical, ophthalmic, and otic applications are also available. Most GCs have high apparent permeability but low aqueous solubility and

therefore prodrugs of established GCs are usually used in intravenous formulations to improve their solubility [126]. Glucocorticoids are well absorbed and show an absolute bioavailability of 60-100% when given orally, a moderate apparent volume of distribution (27 L for HC, 26 L for prednisolone succinate and 3.6 L for DEX sodium phosphate, given IV), and moderate protein binding (~86% for oral prednisone and 75% for methylprednisone) [127]. Glucocorticoids are metabolized mainly in the liver and kidney. The long term use of GCs is associated with serious side effects including osteoporosis, glucose intolerance, serious infections, diabetes, hypertriglyceridemia, gastritis, peptic ulcer disease, GI bleeding, skin thinning, purpura, cataracts, glaucoma, accelerated atherosclerosis, hypertension and cardiovascular diseases [128]. Nanoparticulate delivery of GCs can improve the relatively poor biodistribution of these agents and help reduce the associated side effects. Moreover, nanoparticle delivery systems can improve MTX permeability across biological membranes [129].

Parenteral administration- Quan *et al* compared four different nano-formulations of DEX, namely DEX encapsulated liposomes, DEX conjugated cross-linked micelles, and two polymeric prodrugs of DEX, a slow releasing and a fast releasing formulation for their anti-inflammatory activity in an adjuvant model of arthritis in rats [130]. Their results showed that a single equivalent IV dose of all four formulations resulted in improvements in the signs of joint inflammation which were statistically significant compared to the two control groups receiving free DEX or saline. However, the micellar formulation and the slow releasing polymeric prodrug formulations, both of which had DEX covalently conjugated to the carrier, provided prolonged duration of therapy and a more efficacious joint protection than the other two formulations.

Methylprednisolone loaded in PEGylated liposomes once weekly for 18 weeks was compared to a daily application of this drug for systemic lupus erythematosus *in vivo* in an MRL-lpr/lpr murine model [131]. The liposomal formulation resulted in superior suppression of anti-dsDNA antibody levels by ~ 2.5 folds, reduction in spleen size by 2.7 folds and a decrease in serum urea levels by 2.2 folds compared to free MPS. Moreover, the liposome treated mice had a higher survival rate up to the duration of the study, and showed a decrease in body weight gain and renal damage. PEGylated liposomes encapsulating prednisolone phosphate (PLP) have also been studied in animals [132], and in human [133]. The PK profiling following IV administration of liposomal formulation in human, showed a dose-dependent prolongation of drug half-life to 45-63 h by 7-15 folds compared to free PLP. Moreover, when studied in a double-blinded and placebo controlled setting, the liposomal PEG appeared in 75% of the macrophages isolated from iliofemoral atherosclerotic plaques of patients. However, no evidence for anti-inflammatory effect for the liposomal formulation was found based on multimodal imaging.

Kenyon *et al.* developed a NP formulation composed of PEG-dendritic block copolymers loaded with DEX, for systemic administration to treat asthma [134]. Their results showed that, unlike free DEX, treatment with the DEX NPs reduced the total inflammatory cells recovered by lung lavage by 2.12-folds and the lung lavage eosinophil counts by 2.7-folds in Balb/c mice with ovalbumin (OVA) induced airway inflammation compared to untreated mice. Moreover, the DEX nanoparticle resulted in significantly lower levels of the two inflammatory cytokines; i.e., IL-4 by 2.5-folds and MCP-1 by 2.2-folds in the lungs compared to the control untreated group. The cytokine levels were not lower in the free DEX group.

Ocular administration- Dexamethasone has been encapsulated in mixed micelles based on polyoxyl 40 stearate and polysorbate 80 as a non-invasive approach of delivering the drug to the back of the eye in conditions such as posterior uveitis [135]. The formulation, when administered as single and multiple topical doses in New Zealand albino rabbits, resulted in therapeutically relevant DEX concentrations in the retina and choroid, which is generally not achieved with conventional DEX formulations. In another study, the PLGA based NP formulation of DEX sodium phosphate (DSP), a water soluble prodrug of DEX, was shown to provide a sustained ocular release of drug for at least 7 days when administered subconjunctivally to Sprague Dawley rats with minimal systemic exposure ($1/8^{th}$ of the systemic exposure of free DSP administered through the same route at 2 h post operation) [136]. Moreover, a once weekly application of the DSP NPs in corneal transplant recipient Lewis rats prevented corneal allograft rejection for 9 weeks (duration of the study). This was not true for the DSP in solution group which demonstrated corneal graft rejection. In another study, Ali *et al* prepared two nanosuspensions of HC for ocular use and observed a sustained drug action from both products when tested on albino rats. The NP formulation resulted in a 2-fold increase in exposure as measured by AUC and was maintained for up to 9 h compared to 5 h from the HC solution [39].

Pulmonary administration- Konduri *et al* developed a stealth liposomal formulation of budesonide (BUD) and investigated its suitability as a once weekly therapy for asthma via nebulization [137]. The once weekly liposomal formulation was found to be as effective as a daily administration of a commercially available free BUD in decreasing lung inflammation and lowering the levels of immunologic markers of eosinophil peroxidase activity, peripheral blood eosinophils, and serum IgE levels. These benefits were not obtained with a once weekly

application of a conventional liposomal formulation. Beclomethasone dipropionate (BDP) has also been formulated in polymeric nanocapsules and a polymeric micelles [138, 139]. In the latter formulation, BDP was encapsulated in micelles based on a PEGylated phospholipid–polyaminoacid conjugate. The formulation was found to increase the solubility of the poorly soluble BDP by 240 folds, when compared to free BDP. It also showed high drug internalization (about 84 wt% of amount incubated with cells) in human bronchial epithelial (16HBE) cells compared to that achieved with BDP suspension.

Nasal and aural administration- Cagno *et al.* reported development of HC encapsulated phosphatidylcholine liposomes (particle size of 179.7±18.4 nm) and TPGS micelle dispersions (particle size of 17.3±2.5 nm). Both formulations improved the solubility of HC and were found to enhance its permeability through sheep nasal mucosa compared to the free drug suspension [129].

Nanoparticle formulations have been developed for the delivery of GCs to the inner ear for many disorders which cannot adequately be treated by systemic GC administration. This is largely due to the blood-cochlear barrier which limits the concentrations of drug that can reach the cells in the inner ear. El Kechai formulated a GC liposomal gel by incorporating PEGylate liposomes loaded with DSP with hyaluronic acid gel and investigated its administration by transtympanic injection in a guinea pig model [140]. The liposomal gel displayed shear-thinning which made it suitable for injection, while the auditory brainstem response (ABR) test following injection in the middle ear revealed that the injection not to significantly modify the hearing threshold. Moreover, the formulation resulted in a sustained release of the drug in the perilymph providing

therapeutically relevant amounts of the parent DEX for up to 30 days which is a 2-fold increase over the release time attained with the free drug in hyaluronic acid gel. In another study, the therapeutic and hearing protective effect of DEX encapsulated in PEG-coated Polylactide (PLA) stealth NPs administered by transtympanic injection against cisplatin induced hearing loss was investigated in a guinea pig animal model [141]. A single dose administration of the NP formulation provided a sustained release of the drug for up to 48 h which was quickly distributed in the cochlea within the first hour. The equivalent dose of free DEX was cleared within 12 h of administration. Moreover, the NP formulation provided protective effect against cisplatin induced hearing loss.

3.5 Sirolimus (Rapamycin)

Sirolimus (SR), also known as rapamycin is a macrocyclic lactone-lactam, isolated from Streptomyces hygroscopicus as an antifungal, and later found to possess potent immunosuppressive and anti-tumor properties. Sirolimus is widely used for anti-rejection therapy in organ transplantation [142], but its mechanism of action is different from the calcineurin inhibitors CyA or TAC and it is less nephrotoxic than these agents. Sirolimus acts by interacting with the FK binding proteins to form a complex that inhibits the mammalian target of rapamycin (mTOR) kinase resulting in the suppression in T cell proliferation [143]. Sirolimus is insoluble in water and is only available commercially for oral administration. It has poor bioavailability and distributes widely in tissues [142]. The bioavailability of its oral solution, Rapamune® (Wyeth, USA), is reported to be around 15%. Sirolimus exhibits a long half-life of about 60 h and shows excessive inter subject variability in serum concentrations and systemic clearance. Moreover, it has many potential adverse effects which often necessitate

discontinuation of therapy [144]. A tablet formulation of Rapamune® was designed based on NanoCrystal® technology showing a reduction in the inter-individual variations in SR concentrations in comparison to the oral solution, but the improvement in the bioavailability was not significant [145].

Parenteral administration- The nanodelivery of parenteral SR has received much research attention with applications in various clinical settings, including cardiology as part of therapeutics to control restenosis following balloon angioplasty. For example, Haeri *et al* developed two SR colloidal formulations with stealth properties for its intra-arterial delivery to control restenosis, a micellar formulation based on PEG conjugated with phosphatidylethanolamine (DSPE-PEG) and a liposomal formulation based on DSPE-PEG and cholesterol, purified egg phosphatidylcholine, and distearoyl-sn-glycerophosphoglycerol (DSPG) [146]. Both SR formulations showed efficacy in a rat carotid artery balloon injury model with a reduction in stenosis by 42% and 19% and enlargement of the lumen by 60% and 39%, respectively, in comparison to animals receiving unloaded colloidal formulations. Gasper *et al* investigated the use of an albumin-based NP formulation of SR, nab-rapamycin, for the adventitial delivery of SR in a restenotic (double-injury) swine model. The NP formulation resulted in ~ 42% reduction in lumen area stenosis when compared to the injection of saline. Shi *et al* also developed PLGA NP formulations of SR in Pluronic® gel to control restenosis and intimal hyperplasia (IH) associated with open surgical interventions for cardiovascular conditions [147]. The NP formulation and solubilized SR showed comparable therapeutic improvements up to 14 days following treatment of rats with carotid artery balloon injury. However, only the NP formulation maintained the IH inhibition and the improvements in the lumen size for an additional 14 days.

Dou *et al* developed acetalated β-CyD formulations for the subcutaneous delivery of SR [148]. A PK analysis of the formulation in C57BL/6 mice, showed a sustained release of SR extending over 15 days with almost constant levels of SR being maintained in the blood for a period of about 13 days. This contrasted with the free SR formulation given orally which showed high peak SR concentrations, but the drug was eliminated in 72 h. Moreover, in atherosclerosis ApoE−/− mice model the NPs of SR resulted in a significant drop in the formation of atherosclerotic lesion area (from 27.3% for untreated controls to 3.9%) compared to oral free SR formulation which showed atherosclerotic lesion area of 17.7 %.

A perfluorocarbon NP formulation of SR could employ the autophagy activation by SR to provide a potentially viable therapeutic option for duchenne muscular dystrophy [149]. In a defective autophagy mice model, the IV delivery of the NP formulation resulted in a significant increase of 30% in grip strength, while an equivalent oral dose of a reference SR failed to show improvements.

Matsuzaki *et al* prepared a gelatin hydrogel which contained SR encapsulated in L-lactic acid oligomers-grafted gelatin micelles and investigated its therapeutic role when given locally by intraarticular injection in a surgical model of OA in mice [147]. Their results show that the hydrogel formulation was more effective than a reference SR injection in delaying the progression of OA which was maintained for 16 weeks following the OA surgery. This was attributed to controlled release of SR.

The suppressive activity of SR encapsulated in PLGA NPs on dendritic cells was investigated *in vitro* by Haddadi *et al [150]*. The results revealed that the PLGA encapsulated SR decreased the

expression of various maturation markers studied in immature dendritic cells, while the free SR showed very little effect on the expression of these markers. The nano-delivery of SR resulted in a significant increase in the inhibitory effect of SR on the maturation of the dendritic cells (with respect to dendritic cell phenotype) and on the cytokine production, and functional effects on the proliferation of T cells.

Oral administration- Yu *et al* have prepared a SLNs based on Precirol® ATO-5 and oleic acid that achieved a high encapsulation efficiency of 99.81% for SR [151]. The formulation improved the oral bioavailability of SR by 1.81-folds relative to the Rapamune® tablets in beagle dogs. An SMEDDS formulation based on Capryol™ Propylene glycol monocaprylate as the oil phase and glycofurol as the co-solvent was reported to have achieved success in SR delivery, as well [152]. The formulation achieved a 1.6-folds increase in C_{max} at an earlier t_{max} compared to the oral Rapamune® solution in a rat model, which was not statistically significant. In a different study, solid SMEDDS formulations were prepared by varying the composition of solubilizing agents including Labrafil® M1944 CS, Cremophor® EL, Transcutol® P [153]. The optimal formulation achieved an oral bioavailability of 136% relative to the Rapamune® tablets in beagle dogs.

Bisht *et al* reported an SR NP formulations based on copolymers of N-isopropylacrylamide, acrylic acid and methylmethacrylate or vinylpyrrolidone [154]. The formulation, showed a 2.9-fold increase in the C_{max} of SR which was achieved at 1.5 h post-dose compared to free SR whose peak concentration was attained at 6.67 h. In a different study, Solymois *et al* developed NP formulations based on polyvinyl-pyrrolidone and compared it *in vivo* in a rat model to a reference dispersion of crushed Rapamune® tablets in water [155]. The NPs improved the PK of SR resulting

in an increase in the $AUC_{0-\infty}$ and C_{max} by 2- and 3.7-folds, respectively, compared to Rapamune®. Kim *et al* also reported an SR NP based on PVP K30 and Sucroeste (as stabilizer) showing an increase of 15.2- and 18.3-folds in the AUC_{0-12} and C_{max} of RS compared to free drug, respectively, in a rat model [156].

Kim and his research group also formulated an SR micellar formulation based on TPGS [157]. The formulation achieved a 400-fold increase in solubility of SR in water. The PK profile in a Sprague Dawley rats showed an increase of 13.6- and 19-fold in the AUC_{0-12} and C_{max}, respectively, compared to free SR following oral administration.

Ocular administration- Yuan *et al* investigated a chitosan and polylactic acid based NP formulation of SR as a topical immunosuppressive option in corneal transplantation [158]. The penetration of the formulation was studied using single photon emission computed tomography imaging in rabbit eyes and revealed better retention at the precorneal area when compared to the reference SR aqueous suspension. The NP formulation showed a significant increase in the survival time of the corneal allografts reaching a median of 27.2 days compared to 23.7 days for a reference SR suspension. In another study, Linares-Alba developed a liposomal SR formulation to be administered subconjunctivally for the treatment of nonresponsive keratoconjunctivitis sicca (KCS), or dry eye [159]. The formulation was preliminarily tested *in vivo* for 1.5 months in the spontaneous KCS dog model, not responding to conventional CyA or TAC treatment. Following three dosing of the NP formulation, improvements in the normal and basal lacrimal production, tear film stability, and control of conjunctival discharge was observed.

Nanodelivery systems have also attempted to improve the SR delivery to the retina and the choroid to treat conditions such as posterior uveitis. In this context, micellar formulation of SR based on methoxy PEG-PCL were developed for intravitreal injection [160]. The micelles improved the water solubility of SR by 1000-folds and revealed improved SR accumulation in the retina over 14 days, compared to a reference SR suspension, in rats. The micellar formulation was also more effective in treating intraocular inflammation than the free drug suspension.

3.6 Mycophenolic acid

Mycophenolate acid (MPA) is a potent immunosuppressive agent that acts as a non-competitive inhibitor of inosine monophosphate dehydrogenase selectively inhibiting T and B cell proliferation [161]. It is available as mycophenolate mofetil (MMF), an ester prodrug of MPA that improves MPA bioavailability and marketed as CellCept® (Genentech, San Francisco, CA); and also as mycophenolate sodium delayed release tablets, MyFortic® (Novartis Pharma AG, Basel, Switzerland). MPA is indicated for the prevention of rejection in solid organ transplant patients and is increasingly used in various autoimmune disorders. MMF has rapid and complete absorption following oral administration and the MPA bioavailability is reported to be in the range 80.7-94% following MMF administration and 72% following the administration of sodium salt tablets [162]. MPA binds extensively to serum albumin and shows high inter- and intra-subject variability in PK parameters. MPA is better tolerated than other immunosuppressants and the most common side effects are GI and hematological disorders [163]. The nanodelivery of MPA has not been studied as extensively as the other immunosuppressants.

Parentral administration- Look *et al* developed a nanogel of MPA and investigated its capabilities in targeting immune cells in systemic lupus erythematosus (SLE) [164]. The formulation was made up of a core of CyD and a lipid bilayer exterior. It achieved an MPA encapsulation efficiency of ~3.79% and showed particles with average hydrodynamic diameter of 225 nm. *In vivo* studies in lupus-prone NZB/W F1 mice following intraperitoneal administration of this formulation in a prophylactic manner, showed an improved survival by 3 months compared to the group that received only saline. A 2 month increase in survival was also observed when the NPs were given following onset of proteinuria. Free MPA formulation did not result in the enhancement of survival. A nano-formulation with CD4 antibody surface modification for active targeting showed comparable results to the non-targeted formulation. In a follow-up study, the same research group compared the nanogel formulation to an MPA NP based on PLGA which is known to target dendritic cells [165]. Using fluorescent measurements, it was found that nanogel particles internalized more effectively in bone marrow derived dendritic cells *in vitro* than the PLGA NPs. *In vivo* studies showed unlike nanogels, the PLGA NPs did not result in enhancements of survival in lupus-prone NZB/W F1 mice model.

The MPA encapsulating PLGA NPs reported above was also investigated for its activity in an earlier study by the same research group in a skin allograft mice model [166]. Intermittent ip doses of the NP formulation showed a significant enhancement of skin graft survival when compared to mice receiving a soluble MPA, even though the total MPA dose was 1000-fold lower in the NP formulation. The superior activity of NP formulation was accompanied with their better safety. This was evidenced by lower incidence of severe anemia or splenic cytopenias observed for free drug.

The nanodelivery of MMF, the ester prodrug of MPA, has also been investigated. Teng *et al* reported a liposomal formulation of MMF based on 1,2-Dioleoyl-sn-glycero-3-phospho-choline, cholesterol, and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[(polyethylene-glycol)-2000] [167]. The formulation was investigated *in vivo* in a rat model of nephrotic syndrome. While both the liposomal formulation and the free MMF improved blood biochemical parameters, liposomes resulted in an increase of 20% in plasma albumin and a decrease of 17% in total cholesterol and 21% in triacylglycerol levels when compared to free MMF formulation. Moreover, administration of the liposomal formulation resulted in an increase in body weight and urine volume, a decrease in urinary protein levels at 24 h and in the CXC chemokine ligand 16 (CXCL16) levels compared to the control and the free MMF-treated groups, indicating reduced kidney damage.

4 Conclusions

Nanotechnology has shown great potential in improving the physicochemical properties, as well as pharmacokinetics profile and therapeutic index of several major existing immunosppressive agents in preclinical models. This is largely due to the large capacity of nano-delivery systems in the solubilization of poorly soluble immunosuppressant drugs, their ability for sustaining the rate of drug release upon systemic or local administration, and/or capability of nano-carriers in redirecting the encapsulated drug from normal tissues to sites of drug action in the RES system or inflamed tissues.

5 Expert opinion

Immunosuppressive therapy plays an important role in preventing the rejection of allografts, in improving the quality of life and increasing the survival of transplanted patients.

Immunosuppressive agents are also being used effectively to treat various local or systemic autoimmune inflammatory conditions. There is; however, still a demand for the development of more effective, yet tolerable immunosuppressive agents. This has usually been tackled by either trying to develop more potent immunosuppressants, and/or use of advanced drug delivery systems for the existing or emerging immunosuppressants.

Nanotechnology approaches have proved to be promising means to modify many shortcomings of immunosuppressive agents as summarized in this review paper. Nano-delivery systems have been shown to improve the solubility of poorly soluble immunosuppressive agents leading to better bioavailability while eliminating the need for excipients imposing undesired reactions in patients. Moreover, these advanced delivery systems have been able to positively alter the PK and biodistribution of immunosuppreive drugs leading to better activity while reducing their dose- dependent toxicity. A number of nano-formulations have been able to provide a sustained or controlled release of the immunosuppressant agent which maintains therapeutically relevant concentrations of the drug, either in the systemic circulation, or at the local site of action for prolonged periods leading to less frequent dosing regimens and/or less side effects. The feasibility and advantages of nano-delivery systems for application through ocular, pulmonary, oral and other route of administration has enhanced their potential in effective immunosuppressive therapy where regional administration is desired. Nano-delivery systems have also shown to target either RES system or inflammation sites increasing the chance of drug interaction and activity on target cells that reside in these organs. Finally, nano-formulations are shown to enhance drug delivery through local administration (e.g., transdermal delivery) providing better accumulation of the drug in areas affected by the immune system.

In this context, the effect of nanotechnology in potentiating the immunosuppressive activity of certain drugs is of particular note. It is; however, not clear whether this potentiating effect is merely a result of a change in the organ distribution of immunosuppressive drug in the animal towards organs hosting the immune cells, and/or enhanced drug activity at the cellular level by its nano-carrier through unexplained mechanisms. Further studies are required to elucidate the mechanisms behind potentiating effects of nano-carries on the encapsulated immunosuppressant agents.

Nonetheless, it is clear from the literature that the development of nanomedicine for enhancing the performance of immunosuppressive agents in the treatment of different inflammatory diseases has witnessed several examples of success in preclinical stage. Based on the results of research conducted in this area to date, it is safe to speculate the more rapid translation of nanotechnology in immunosuppressive therapy in clinic in near future.

6 Conflicts of interest statement

The authors declare no conflict of interest.

References

1. Strebhardt, K. and A. Ullrich, *Paul Ehrlich's magic bullet concept: 100 years of progress.* Nat Rev Cancer, 2008. **8**(6): p. 473-80.
2. Choonara, B.F., et al., *A review of advanced oral drug delivery technologies facilitating the protection and absorption of protein and peptide molecules.* Biotechnol Adv, 2014. **32**(7): p. 1269-1282.
3. Corsini, A. and M. Bortolini, *Drug-induced liver injury: the role of drug metabolism and transport.* J Clin Pharmacol, 2013. **53**(5): p. 463-74.
4. Aliabadi, H.M., et al., *Disposition of drugs in block copolymer micelle delivery systems: from discovery to recovery.* Clin Pharmacokinet, 2008. **47**(10): p. 619-34.
5. Krishna, R. and L.D. Mayer, *Modulation of P-glycoprotein (PGP) mediated multidrug resistance (MDR) using chemosensitizers: recent advances in the design of selective MDR modulators.* Curr Med Chem Anticancer Agents, 2001. **1**(2): p. 163-74.
6. Maeda, H., H. Nakamura, and J. Fang, *The EPR effect for macromolecular drug delivery to solid tumors: Improvement of tumor uptake, lowering of systemic toxicity, and distinct tumor imaging in vivo.* Adv Drug Deliv Rev, 2013. **65**(1): p. 71-9.
7. Siew, A., et al., *Enhanced oral absorption of hydrophobic and hydrophilic drugs using quaternary ammonium palmitoyl glycol chitosan nanoparticles.* Mol Pharm, 2012. **9**(1): p. 14-28.
8. Merrill, J.P., et al., *Successful homotransplantation of the human kidney between identical twins.* J Am Med Assoc, 1956. **160**(4): p. 277-82.
9. Monguio-Tortajada, M., R. Lauzurica-Valdemoros, and F.E. Borras, *Tolerance in organ transplantation: from conventional immunosuppression to extracellular vesicles.* Front Immunol, 2014. **5**: p. 416.
10. Zarkhin, V. and M.M. Sarwal, *The coin toss of B cells in rejection and tolerance: danger versus defense.* Semin Immunol, 2012. **24**(2): p. 86-91.
11. Garnett, C., J.F. Apperley, and J. Pavlu, *Treatment and management of graft-versus-host disease: improving response and survival.* Ther Adv Hematol, 2013. **4**(6): p. 366-78.
12. Ferrara, J.L., R. Levy, and N.J. Chao, *Pathophysiologic mechanisms of acute graft-vs.-host disease.* Biol Blood Marrow Transplant, 1999. **5**(6): p. 347-56.
13. Smith, D.A. and D.R. Germolec, *Introduction to immunology and autoimmunity.* Environ Health Perspect, 1999. **107 Suppl 5**: p. 661-5.
14. Street, N.E. and T.R. Mosmann, *Functional diversity of T lymphocytes due to secretion of different cytokine patterns.* FASEB J, 1991. **5**(2): p. 171-7.
15. Mosmann, T.R., et al., *Diversity of cytokine synthesis and function of mouse CD4+ T cells.* Immunol Rev, 1991. **123**: p. 209-29.
16. Korczowska, I., *Rheumatoid arthritis susceptibility genes: An overview.* World J Orthop, 2014. **5**(4): p. 544-9.
17. Okada, Y., et al., *Genetics of rheumatoid arthritis contributes to biology and drug discovery.* Nature, 2014. **506**(7488): p. 376-81.
18. Hunt, L. and P. Emery, *Defining populations at risk of rheumatoid arthritis: the first steps to prevention.* Nat Rev Rheumatol, 2014. **10**(9): p. 521-30.
19. Blyth, T., J.A. Hunter, and A. Stirling, *Pain relief in the rheumatoid knee after steroid injection. A single-blind comparison of hydrocortisone succinate, and triamcinolone acetonide or hexacetonide.* Br J Rheumatol, 1994. **33**(5): p. 461-3.
20. Ye, J., et al., *Injectable actarit-loaded solid lipid nanoparticles as passive targeting therapeutic agents for rheumatoid arthritis.* Int J Pharm, 2008. **352**(1-2): p. 273-9.

21. Brigger, I., C. Dubernet, and P. Couvreur, *Nanoparticles in cancer therapy and diagnosis.* Adv Drug Deliv Rev, 2002. **54**(5): p. 631-51.
22. Hooper, L.V. and J.I. Gordon, *Commensal host-bacterial relationships in the gut.* Science, 2001. **292**(5519): p. 1115-8.
23. Hooper, L.V., et al., *Molecular analysis of commensal host-microbial relationships in the intestine.* Science, 2001. **291**(5505): p. 881-4.
24. Meissner, Y., Y. Pellequer, and A. Lamprecht, *Nanoparticles in inflammatory bowel disease: particle targeting versus pH-sensitive delivery.* Int J Pharm, 2006. **316**(1-2): p. 138-43.
25. Cui, F., F. Qian, and C. Yin, *Preparation and characterization of mucoadhesive polymer-coated nanoparticles.* Int J Pharm, 2006. **316**(1-2): p. 154-61.
26. Yang, R., et al., *Enhanced electrostatic interaction between chitosan-modified PLGA nanoparticle and tumor.* Int J Pharm, 2009. **371**(1-2): p. 142-7.
27. Lamprecht, A., U. Schafer, and C.M. Lehr, *Size-dependent bioadhesion of micro- and nanoparticulate carriers to the inflamed colonic mucosa.* Pharm Res, 2001. **18**(6): p. 788-93.
28. Krishnaiah, Y.S., et al., *In vivo pharmacokinetics in human volunteers: oral administered guar gum-based colon-targeted 5-fluorouracil tablets.* Eur J Pharm Sci, 2003. **19**(5): p. 355-62.
29. Pouwels, S.D., et al., *DAMPs activating innate and adaptive immune responses in COPD.* Mucosal Immunol, 2014. **7**(2): p. 215-26.
30. Willis, L., D. Hayes, Jr., and H.M. Mansour, *Therapeutic liposomal dry powder inhalation aerosols for targeted lung delivery.* Lung, 2012. **190**(3): p. 251-62.
31. Chougule, M., B. Padhi, and A. Misra, *Nano-liposomal dry powder inhaler of tacrolimus: preparation, characterization, and pulmonary pharmacokinetics.* Int J Nanomedicine, 2007. **2**(4): p. 675-88.
32. Behr, J., et al., *Lung deposition of a liposomal cyclosporine A inhalation solution in patients after lung transplantation.* J Aerosol Med Pulm Drug Deliv, 2009. **22**(2): p. 121-30.
33. Drewe, J., C. Beglinger, and T. Kissel, *The absorption site of cyclosporin in the human gastrointestinal tract.* British journal of clinical pharmacology, 1992. **33**(1): p. 39-43.
34. Lallemand, F., et al., *Cyclosporine A delivery to the eye: a pharmaceutical challenge.* Eur J Pharm Biopharm, 2003. **56**(3): p. 307-18.
35. Gomes Bittencourt, M., et al., *New treatment options for noninfectious uveitis.* Dev Ophthalmol, 2012. **51**: p. 134-61.
36. Wang, J., et al., *Drug delivery implants in the treatment of vitreous inflammation.* Mediators Inflamm, 2013. **2013**: p. 780634.
37. Beardsley, R.M., et al., *Pharmacotherapy of scleritis: current paradigms and future directions.* Expert Opin Pharmacother, 2013. **14**(4): p. 411-24.
38. Liu, H., Y. Wang, and S. Li, *Advanced delivery of ciclosporin A: present state and perspective.* Expert Opin Drug Deliv, 2007. **4**(4): p. 349-58.
39. Von Wartburg, A. and R. Traber, *Chemistry of the natural cyclosporin metabolites.*, in *ciclosporin*. 1986, Karger: Basel. p. 28-45.
40. Beauchesne, P.R., C.N.S. C, and K.M. Wasan, *Cyclosporine A: A Review of Current Oral and Intravenous Delivery Systems.* Drug Development and Industrial Pharmacy, 2007. **3**(211-220): p. 33.
41. Noble, S. and A. Markham, *A Review of the Pharmacokinetic Properties, Clinical Efficacy and Tolerability of a Microemulsion-Based Formulation (Neoral®).* Drugs, 1995. **50**(5): p. 924-941.
42. Venkataramanan, R., et al., *Biliary excretion of cyclosporine in liver transplant patients.* Transplantation proceedings, 1985. **17**(1): p. 286-289.
43. Bleck, J.S., et al., *Urinary excretion of ciclosporin and 17 of its metabolites in renal allograft recipients.* Pharmacolog, 1989. **39**(3): p. 160-164.

44. Kahan, B.D., et al., *Therapeutic drug monitoring of immunosuppressant drugs in clinical practice.* Clinical therapeutics, 2002. **24**(3): p. 330-350.
45. Kahan, B.D., *Therapeutic Drug Monitoring of Cyclosporine: 20 Years of Progress.* Transplantation Proceedings, 2004. **36**(Suppl 25): p. 378S-391S.
46. Thompson, M.E., et al., *The contrasting effects of cyclosporin-A and azathioprine on arterial blood pressure and renal funfunction following cardiac transplantation.* International journal of cardiology, 1986. **11**(2): p. 219-229.
47. Vader, P., et al., *Taxol((R))-induced phosphatidylserine exposure and microvesicle formation in red blood cells is mediated by its vehicle Cremophor((R)) EL.* Nanomedicine (Lond), 2013. **8**(7): p. 1127-35.
48. Aliabadi, H.M., et al., *Micelles of methoxy poly(ethylene oxide)-b-poly(epsilon-caprolactone) as vehicles for the solubilization and controlled delivery of cyclosporine A.* J Control Release, 2005. **104**(2): p. 301-11.
49. Aliabadi, H.M., et al., *Encapsulation of hydrophobic drugs in polymeric micelles through co-solvent evaporation: the effect of solvent composition on micellar properties and drug loading.* Int J Pharm, 2007. **329**(1-2): p. 158-65.
50. Aliabadi, H.M., et al., *Polymeric micellar delivery reduces kidney distribution and nephrotoxic effects of Cyclosporine A after multiple dosing.* J Pharm Sci, 2008. **97**(5): p. 1916-26.
51. Aliabadi, H.M., D.R. Brocks, and A. Lavasanifar, *Polymeric micelles for the solubilization and delivery of cyclosporine A: pharmacokinetics and biodistribution.* Biomaterials, 2005. **26**(35): p. 7251-9.
52. Hamdy, S., et al., *The immunosuppressive activity of polymeric micellar formulation of cyclosporine A: in vitro and in vivo studies.* AAPS J, 2011. **13**(2): p. 159-68.
53. Mondon, K., et al., *Novel cyclosporin A formulations using MPEG-hexyl-substituted polylactide micelles: a suitability study.* Eur J Pharm Biopharm, 2011. **77**(1): p. 56-65.
54. Zhang, L., et al., *Preparation and in vitro and in vivo characterization of cyclosporin A-loaded, PEGylated chitosan-modified, lipid-based nanoparticles.* Int J Nanomedicine, 2013. **8**: p. 601-10.
55. Italia, J.L., et al., *PLGA nanoparticles for oral delivery of cyclosporine: nephrotoxicity and pharmacokinetic studies in comparison to Sandimmune Neoral.* J Control Release, 2007. **119**(2): p. 197-206.
56. Ankola, D.D., R.M. Wadsworth, and M.N. Ravi Kumar, *Nanoparticulate delivery can improve peroral bioavailability of cyclosporine and match Neoral Cmax sparing the kidney from damage.* J Biomed Nanotechnol, 2011. **7**(2): p. 300-7.
57. Wang, K., et al., *Enhancement of oral bioavailability of cyclosporine A: comparison of various nanoscale drug-delivery systems.* Int J Nanomedicine, 2014. **9**: p. 4991-9.
58. Guada, M., et al., *Lipid nanoparticles enhance the absorption of cyclosporine A through the gastrointestinal barrier: In vitro and in vivo studies.* Int J Pharm, 2016. **500**(1-2): p. 154-61.
59. Wang, X.Q., et al., *Bioavailability and pharmacokinetics of cyclosporine A-loaded pH-sensitive nanoparticles for oral administration.* J Control Release, 2004. **97**(3): p. 421-9.
60. Dai, J., et al., *pH-sensitive nanoparticles for improving the oral bioavailability of cyclosporine A.* Int J Pharm, 2004. **280**(1-2): p. 229-40.
61. Tang, L., et al., *Immunosuppressive Activity of Size-Controlled PEG-PLGA Nanoparticles Containing Encapsulated Cyclosporine A.* J Transplant, 2012. **2012**: p. 896141.
62. Aksungur, P., et al., *Development and characterization of Cyclosporine A loaded nanoparticles for ocular drug delivery: Cellular toxicity, uptake, and kinetic studies.* J Control Release, 2011. **151**(3): p. 286-94.
63. Yenice, I., et al., *Hyaluronic acid coated poly-epsilon-caprolactone nanospheres deliver high concentrations of cyclosporine A into the cornea.* Exp Eye Res, 2008. **87**(3): p. 162-7.

64. De Campos, A.M., A. Sanchez, and M.J. Alonso, *Chitosan nanoparticles: a new vehicle for the improvement of the delivery of drugs to the ocular surface. Application to cyclosporin A.* Int J Pharm, 2001. **224**(1-2): p. 159-68.
65. Basaran, E., et al., *Chitosan nanoparticles for ocular delivery of cyclosporine A.* J Microencapsul, 2014. **31**(1): p. 49-57.
66. Hermans, K., et al., *Full factorial design, physicochemical characterisation and biological assessment of cyclosporine A loaded cationic nanoparticles.* Eur J Pharm Biopharm, 2012. **82**(1): p. 27-35.
67. Sandri, G., et al., *Chitosan-associated SLN: in vitro and ex vivo characterization of cyclosporine A loaded ophthalmic systems.* J Microencapsul, 2010. **27**(8): p. 735-46.
68. Liu, S., et al., *Phenylboronic acid modified mucoadhesive nanoparticle drug carriers facilitate weekly treatment of experimentally-induced dry eye syndrome.* Nano Research, 2015. **8**(2): p. 621-635.
69. Prosperi-Porta, G., et al., *Phenylboronic-Acid-Based Polymeric Micelles for Mucoadhesive Anterior Segment Ocular Drug Delivery.* Biomacromolecules, 2016. **17**(4): p. 1449-57.
70. Di Tommaso, C., et al., *Novel micelle carriers for cyclosporin A topical ocular delivery: in vivo cornea penetration, ocular distribution and efficacy studies.* Eur J Pharm Biopharm, 2012. **81**(2): p. 257-64.
71. Urban-Morlan, Z., et al., *Preparation and characterization of solid lipid nanoparticles containing cyclosporine by the emulsification-diffusion method.* Int J Nanomedicine, 2010. **5**: p. 611-20.
72. Potta, S.G., et al., *Development of solid lipid nanoparticles for enhanced solubility of poorly soluble drugs.* J Biomed Nanotechnol, 2010. **6**(6): p. 634-40.
73. Gallarate, M., et al., *Solid Lipid Nanoparticles Loaded with Fluorescent-labelled Cyclosporine A: Anti-Inflammatory Activity In Vitro.* Protein Pept Lett, 2014. **21**(11): p. 1157-62.
74. Gokce, E.H., et al., *Cyclosporine A loaded SLNs: evaluation of cellular uptake and corneal cytotoxicity.* Int J Pharm, 2008. **364**(1): p. 76-86.
75. Gokce, E.H., et al., *Cyclosporine a-loaded solid lipid nanoparticles: ocular tolerance and in vivo drug release in rabbit eyes.* Curr Eye Res, 2009. **34**(11): p. 996-1003.
76. Kim, S.T., et al., *Topical administration of cyclosporin A in a solid lipid nanoparticle formulation.* Pharmazie, 2009. **64**(8): p. 510-4.
77. Lopes, L.B., et al., *Reverse hexagonal phase nanodispersion of monoolein and oleic acid for topical delivery of peptides: in vitro and in vivo skin penetration of cyclosporin A.* Pharm Res, 2006. **23**(6): p. 1332-42.
78. Romero, G.B., et al., *Amorphous cyclosporin A nanoparticles for enhanced dermal bioavailability.* Int J Pharm, 2016. **498**(1-2): p. 217-24.
79. Frusic-Zlotkin, M., et al., *Penetration and biological effects of topically applied cyclosporin A nanoparticles in a human skin organ culture inflammatory model.* Exp Dermatol, 2012. **21**(12): p. 938-43.
80. Karavana, S.Y., et al., *A new approach to the treatment of recurrent aphthous stomatitis with bioadhesive gels containing cyclosporine A solid lipid nanoparticles: in vivo/in vitro examinations.* Int J Nanomedicine, 2012. **7**: p. 5693-704.
81. Zhai, J., et al., *Tacrolimus in the treatment of ocular diseases.* BioDrugs, 2011. **25**(2): p. 89-103.
82. Friemann, S., et al., *Improvement of nephrotoxicity, hypertension, and lipid metabolism after conversion of kidney transplant recipients from cyclosporine to tacrolimus.* Transplant Proc, 1998. **30**(4): p. 1240-2.
83. Ligtenberg, G., et al., *Cardiovascular risk factors in renal transplant patients: cyclosporin A versus tacrolimus.* J Am Soc Nephrol, 2001. **12**(2): p. 368-73.

84. Textor, S.C., et al., *Systemic and renal hemodynamic differences between FK506 and cyclosporine in liver transplant recipients.* Transplantation, 1993. **55**(6): p. 1332-9.
85. Vincenti, F., et al., *A long-term comparison of tacrolimus (FK506) and cyclosporine in kidney transplantation: evidence for improved allograft survival at five years.* Transplantation, 2002. **73**(5): p. 775-82.
86. Scott, L.J., et al., *Tacrolimus: a further update of its use in the management of organ transplantation.* Drugs, 2003. **63**(12): p. 1247-97.
87. Christians, U., T. Pokaiyavanichkul, and L. Chan, *Tacrolimus*, in *Applied Pharmacokinetics and Pharmacodynamics: Principals of Therapeutic Drug Monitoring.* 2005, Lippincott Williams & Wilkins: Philadelphia. p. 529-562.
88. van Gelder, T., *Drug interactions with tacrolimus.* Drug Saf, 2002. **25**(10): p. 707-12.
89. Tamura, S., et al., *Tacrolimus is a class II low-solubility high-permeability drug: the effect of P-glycoprotein efflux on regional permeability of tacrolimus in rats.* J Pharm Sci, 2002. **91**(3): p. 719-29.
90. Thao le, Q., et al., *Pharmaceutical potential of tacrolimus-loaded albumin nanoparticles having targetability to rheumatoid arthritis tissues.* Int J Pharm, 2016. **497**(1-2): p. 268-76.
91. Gao, S., et al., *Preparation, characterization and pharmacokinetic studies of tacrolimus-dimethyl-beta-cyclodextrin inclusion complex-loaded albumin nanoparticles.* Int J Pharm, 2012. **427**(2): p. 410-6.
92. Zhao, L., et al., *Bovine serum albumin nanoparticles for delivery of tacrolimus to reduce its kidney uptake and functional nephrotoxicity.* Int J Pharm, 2015. **483**(1-2): p. 180-7.
93. Ali, S.M., et al., *Polyoxyl 60 hydrogenated castor oil free nanosomal formulation of immunosuppressant Tacrolimus: pharmacokinetics, safety, and tolerability in rodents and humans.* Int Immunopharmacol, 2010. **10**(3): p. 325-30.
94. Allen, C., et al., *PCL-b-PEO micelles as a delivery vehicle for FK506: assessment of a functional recovery of crushed peripheral nerve.* Drug Deliv, 2000. **7**(3): p. 139-45.
95. Wang, Y., et al., *Micelles of methoxy poly(ethylene glycol)-poly(epsilon-caprolactone) as a novel drug delivery vehicle for tacrolimus.* J Biomed Nanotechnol, 2013. **9**(2): p. 147-57.
96. Borhade, V., H. Nair, and D. Hegde, *Design and evaluation of self-microemulsifying drug delivery system (SMEDDS) of tacrolimus.* AAPS PharmSciTech, 2008. **9**(1): p. 13-21.
97. von Suesskind-Schwendi, M., et al., *Pharmacokinetics of a self-microemulsifying drug delivery system of tacrolimus.* Biomed Pharmacother, 2013. **67**(6): p. 469-73.
98. Wang, Y., et al., *Enhanced oral bioavailability of tacrolimus in rats by self-microemulsifying drug delivery systems.* Drug Dev Ind Pharm, 2011. **37**(10): p. 1225-30.
99. Lamprecht, A., et al., *A pH-sensitive microsphere system for the colon delivery of tacrolimus containing nanoparticles.* J Control Release, 2005. **104**(2): p. 337-46.
100. Li, G., et al., *Tacrolimus-loaded ethosomes: physicochemical characterization and in vivo evaluation.* Eur J Pharm Biopharm, 2012. **82**(1): p. 49-57.
101. Erdogan, M., J.R. Wright, Jr., and V.C. McAlister, *Liposomal tacrolimus lotion as a novel topical agent for treatment of immune-mediated skin disorders: experimental studies in a murine model.* Br J Dermatol, 2002. **146**(6): p. 964-7.
102. Seo, J., et al., *Therapeutic advantage of inhaled tacrolimus-bound albumin nanoparticles in a bleomycin-induced pulmonary fibrosis mouse model.* Pulm Pharmacol Ther, 2016. **36**: p. 53-61.
103. Kasim, N.A., et al., *Molecular properties of WHO essential drugs and provisional biopharmaceutical classification.* Mol Pharm, 2004. **1**(1): p. 85-96.
104. van Roon, E.N. and M.A. van de Laar, *Methotrexate bioavailability.* Clin Exp Rheumatol, 2010. **28**(5 Suppl 61): p. S27-32.

105. Bianchi, G., et al., *Methotrexate and Rheumatoid Arthritis: Current Evidence Regarding Subcutaneous Versus Oral Routes of Administration.* Adv Ther, 2016. **33**(3): p. 369-78.
106. Pichlmeier, U. and K.U. Heuer, *Subcutaneous administration of methotrexate with a prefilled autoinjector pen results in a higher relative bioavailability compared with oral administration of methotrexate.* Clin Exp Rheumatol, 2014. **32**(4): p. 563-71.
107. Edno, L., et al., *Total and free methotrexate pharmacokinetics in rheumatoid arthritis patients.* Ther Drug Monit, 1996. **18**(2): p. 128-34.
108. Inc., P.C. *Pr METHOTREXATE*. 2015 11 June 2016]; Available from: http://www.pfizer.ca/sites/g/files/g10028126/f/201511/Methotrexate_PM_E.pdf.
109. Abolmaali, S.S., A.M. Tamaddon, and R. Dinarvand, *A review of therapeutic challenges and achievements of methotrexate delivery systems for treatment of cancer and rheumatoid arthritis.* Cancer Chemother Pharmacol, 2013. **71**(5): p. 1115-30.
110. Amarji, B., et al., *Microemulsions mediated effective delivery of methotrexate hydrogel: more than a tour de force in psoriasis therapeutics.* J Drug Target, 2016. **24**(2): p. 147-60.
111. Ruckmani, K., M. Sivakumar, and P.A. Ganeshkumar, *Methotrexate loaded solid lipid nanoparticles (SLN) for effective treatment of carcinoma.* J Nanosci Nanotechnol, 2006. **6**(9-10): p. 2991-5.
112. Chen, Y., et al., *Pluronic mixed micelles overcoming methotrexate multidrug resistance: in vitro and in vivo evaluation.* Int J Nanomedicine, 2013. **8**: p. 1463-76.
113. Abolmaali, S.S., et al., *Sequential optimization of methotrexate encapsulation in micellar nano-networks of polyethyleneimine ionomer containing redox-sensitive cross-links.* Int J Nanomedicine, 2014. **9**: p. 2833-48.
114. Williams, A.S., et al., *Differential effects of methotrexate and liposomally conjugated methotrexate in rat adjuvant-induced arthritis.* Clin Exp Immunol, 1995. **102**(3): p. 560-5.
115. Gottschalk, O., et al., *Therapeutic effect of methotrexate encapsulated in cationic liposomes (EndoMTX) in comparison to free methotrexate in an antigen-induced arthritis study in vivo.* Scand J Rheumatol, 2015. **44**(6): p. 456-63.
116. Hong, M.S., et al., *Prolonged blood circulation of methotrexate by modulation of liposomal composition.* Drug Deliv, 2001. **8**(4): p. 231-7.
117. Prabhu, P., et al., *Investigation of nano lipid vesicles of methotrexate for anti-rheumatoid activity.* Int J Nanomedicine, 2012. **7**: p. 177-86.
118. Wunder, A., et al., *Albumin-based drug delivery as novel therapeutic approach for rheumatoid arthritis.* J Immunol, 2003. **170**(9): p. 4793-801.
119. Hartung, G., et al., *Phase I trial of methotrexate-albumin in a weekly intravenous bolus regimen in cancer patients. Phase I Study Group of the Association for Medical Oncology of the German Cancer Society.* Clin Cancer Res, 1999. **5**(4): p. 753-9.
120. Liang, L.S., et al., *Methotrexate loaded poly(L-lactic acid) microspheres for intra-articular delivery of methotrexate to the joint.* J Pharm Sci, 2004. **93**(4): p. 943-56.
121. Azmin, M.N., et al., *The effect of non-ionic surfactant vesicle (niosome) entrapment on the absorption and distribution of methotrexate in mice.* J Pharm Pharmacol, 1985. **37**(4): p. 237-42.
122. Misra, A., Kalariya, M., Padhi, B.K. and Chougule, M, *Methotrexate-loaded solid lipid nanoparticles for topical treatment of psoriasis: formulation and clinical implications.* Drug Del Tech, 2004. **4**: p. 1-12.
123. Trotta, M., F. Pattarino, and M.R. Gasco, *Influence of counter ions on the skin permeation of methotrexate from water--oil microemulsions.* Pharm Acta Helv, 1996. **71**(2): p. 135-40.
124. Doddoli, C., et al., *In vitro and in vivo methotrexate disposition in alveolar macrophages: comparison of pharmacokinetic parameters of two formulations.* Int J Pharm, 2005. **297**(1-2): p. 180-9.
125. Smith, S.L., *Immunosuppressive therapies in organ transplantation.* Organ Transplant, 2002.

126. Derendorf, H. and G.n. Hochhaus, *Handbook of pharmacokinetic/pharmacodynamic correlation*. 1995, Boca Raton: CRC Press. 483 p.
127. Czock, D., et al., *Pharmacokinetics and pharmacodynamics of systemically administered glucocorticoids.* Clin Pharmacokinet, 2005. **44**(1): p. 61-98.
128. Saag, K.G., et al., *Low dose long-term corticosteroid therapy in rheumatoid arthritis: an analysis of serious adverse events.* Am J Med, 1994. **96**(2): p. 115-23.
129. di Cagno, M. and B. Luppi, *Drug "supersaturation" states induced by polymeric micelles and liposomes: a mechanistic investigation into permeability enhancements.* Eur J Pharm Sci, 2013. **48**(4-5): p. 775-80.
130. Quan, L., et al., *Nanomedicines for inflammatory arthritis: head-to-head comparison of glucocorticoid-containing polymers, micelles, and liposomes.* ACS Nano, 2014. **8**(1): p. 458-66.
131. Moallem, E., et al., *A liposomal steroid nano-drug for treating systemic lupus erythematosus.* Lupus, 2016. **25**(11): p. 1209-16.
132. Lobatto, M.E., et al., *Pharmaceutical development and preclinical evaluation of a GMP-grade anti-inflammatory nanotherapy.* Nanomedicine, 2015. **11**(5): p. 1133-40.
133. van der Valk, F.M., et al., *Prednisolone-containing liposomes accumulate in human atherosclerotic macrophages upon intravenous administration.* Nanomedicine, 2015. **11**(5): p. 1039-46.
134. Kenyon, N.J., et al., *Self-assembling nanoparticles containing dexamethasone as a novel therapy in allergic airways inflammation.* PLoS One, 2013. **8**(10): p. e77730.
135. Patel, S., et al., *Development and evaluation of dexamethasone nanomicelles with potential for treating posterior uveitis after topical application.* J Ocul Pharmacol Ther, 2015. **31**(4): p. 215-27.
136. Pan, Q., et al., *Corticosteroid-loaded biodegradable nanoparticles for prevention of corneal allograft rejection in rats.* J Control Release, 2015. **201**: p. 32-40.
137. Konduri, K.S., et al., *Efficacy of liposomal budesonide in experimental asthma.* J Allergy Clin Immunol, 2003. **111**(2): p. 321-7.
138. Chassot, J.M., et al., *Beclomethasone Dipropionate-Loaded Polymeric Nanocapsules: Development, In Vitro Cytotoxicity, and In Vivo Evaluation of Acute Lung Injury.* J Nanosci Nanotechnol, 2015. **15**(1): p. 855-64.
139. Craparo, E.F., et al., *Phospholipid-polyaspartamide micelles for pulmonary delivery of corticosteroids.* Int J Pharm, 2011. **406**(1-2): p. 135-44.
140. El Kechai, N., et al., *Hyaluronic acid liposomal gel sustains delivery of a corticoid to the inner ear.* J Control Release, 2016. **226**: p. 248-57.
141. Sun, C., et al., *A single dose of dexamethasone encapsulated in polyethylene glycol-coated polylactic acid nanoparticles attenuates cisplatin-induced hearing loss following round window membrane administration.* Int J Nanomedicine, 2015. **10**: p. 3567-79.
142. Mahalati, K. and B.D. Kahan, *Clinical pharmacokinetics of sirolimus.* Clin Pharmacokinet, 2001. **40**(8): p. 573-85.
143. Sehgal, S.N., *Sirolimus: its discovery, biological properties, and mechanism of action.* Transplant Proc, 2003. **35**(3 Suppl): p. 7S-14S.
144. Merkel, S., et al., *Side effects of sirolimus.* Transplant Proc, 2006. **38**(3): p. 714-5.
145. Shen, L.J. and F.L. Wu, *Nanomedicines in renal transplant rejection--focus on sirolimus.* Int J Nanomedicine, 2007. **2**(1): p. 25-32.
146. Haeri, A., et al., *Sirolimus-loaded stealth colloidal systems attenuate neointimal hyperplasia after balloon injury: a comparison of phospholipid micelles and liposomes.* Int J Pharm, 2013. **455**(1-2): p. 320-30.
147. Matsuzaki, T., et al., *Intra-articular administration of gelatin hydrogels incorporating rapamycin-micelles reduces the development of experimental osteoarthritis in a murine model.* Biomaterials, 2014. **35**(37): p. 9904-11.

148. Dou, Y., et al., *Sustained delivery by a cyclodextrin material-based nanocarrier potentiates antiatherosclerotic activity of rapamycin via selectively inhibiting mTORC1 in mice.* J Control Release, 2016. **235**: p. 48-62.
149. Bibee, K.P., et al., *Rapamycin nanoparticles target defective autophagy in muscular dystrophy to enhance both strength and cardiac function.* FASEB J, 2014. **28**(5): p. 2047-61.
150. Haddadi, A., et al., *Delivery of rapamycin by PLGA nanoparticles enhances its suppressive activity on dendritic cells.* J Biomed Mater Res A, 2008. **84**(4): p. 885-98.
151. Yu, Q., et al., *Lipids-based nanostructured lipid carriers (NLCs) for improved oral bioavailability of sirolimus.* Drug Deliv, 2016. **23**(4): p. 1469-75.
152. Cho, W., et al., *Optimized formulation of solid self-microemulsifying sirolimus delivery systems.* Int J Nanomedicine, 2013. **8**: p. 1673-82.
153. Hu, X., et al., *Sirolimus solid self-microemulsifying pellets: formulation development, characterization and bioavailability evaluation.* Int J Pharm, 2012. **438**(1-2): p. 123-33.
154. Bisht, S., et al., *In vivo characterization of a polymeric nanoparticle platform with potential oral drug delivery capabilities.* Mol Cancer Ther, 2008. **7**(12): p. 3878-88.
155. Solymosi, T., et al., *Sirolimus formulation with improved pharmacokinetic properties produced by a continuous flow method.* Eur J Pharm Biopharm, 2015. **94**: p. 135-40.
156. Kim, M.S., et al., *Enhanced bioavailability of sirolimus via preparation of solid dispersion nanoparticles using a supercritical antisolvent process.* Int J Nanomedicine, 2011. **6**: p. 2997-3009.
157. Kim, M.S., et al., *Enhanced solubility and oral absorption of sirolimus using D-alpha-tocopheryl polyethylene glycol succinate micelles.* Artif Cells Nanomed Biotechnol, 2013. **41**(2): p. 85-91.
158. Yuan, X.B., et al., *Preparation of rapamycin-loaded chitosan/PLA nanoparticles for immunosuppression in corneal transplantation.* Int J Pharm, 2008. **349**(1-2): p. 241-8.
159. Linares-Alba, M.A., et al., *Preformulation Studies of a Liposomal Formulation Containing Sirolimus for the Treatment of Dry Eye Disease.* J Ocul Pharmacol Ther, 2016. **32**(1): p. 11-22.
160. Wu, W., et al., *Intravitreal injection of rapamycin-loaded polymeric micelles for inhibition of ocular inflammation in rat model.* Int J Pharm, 2016. **513**(1-2): p. 238-246.
161. Allison, A.C., *Mechanisms of action of mycophenolate mofetil.* Lupus, 2005. **14 Suppl 1**: p. s2-8.
162. Staatz, C.E. and S.E. Tett, *Clinical pharmacokinetics and pharmacodynamics of mycophenolate in solid organ transplant recipients.* Clin Pharmacokinet, 2007. **46**(1): p. 13-58.
163. Atcheson, B.A., et al., *Mycophenolic acid pharmacokinetics and related outcomes early after renal transplant.* Br J Clin Pharmacol, 2005. **59**(3): p. 271-80.
164. Look, M., et al., *Nanogel-based delivery of mycophenolic acid ameliorates systemic lupus erythematosus in mice.* J Clin Invest, 2013. **123**(4): p. 1741-9.
165. Look, M., et al., *The nanomaterial-dependent modulation of dendritic cells and its potential influence on therapeutic immunosuppression in lupus.* Biomaterials, 2014. **35**(3): p. 1089-95.
166. Shirali, A.C., et al., *Nanoparticle delivery of mycophenolic acid upregulates PD-L1 on dendritic cells to prolong murine allograft survival.* Am J Transplant, 2011. **11**(12): p. 2582-92.
167. Teng, J., et al., *Overall condition improvement in a rat model of nephrotic syndrome treated with CellCept nanoliposomes.* Artif Cells Nanomed Biotechnol, 2016: p. 1-7.
168. Han, S., et al., *Amphiphilic poly(L-aspartic acid) copolymer nanoparticles for cyclosporine A delivery.* J Nanosci Nanotechnol, 2013. **13**(2): p. 1444-7.
169. Chaw, C.S., et al., *Thermally responsive core-shell nanoparticles self-assembled from cholesteryl end-capped and grafted polyacrylamides:; drug incorporation and in vitro release.* Biomaterials, 2004. **25**(18): p. 4297-308.
170. Azzi, J., et al., *Polylactide-cyclosporin A nanoparticles for targeted immunosuppression.* FASEB J, 2010. **24**(10): p. 3927-38.

171. Park, M.J., P. Balakrishnan, and S.G. Yang, *Polymeric nanocapsules with SEDDS oil-core for the controlled and enhanced oral absorption of cyclosporine.* Int J Pharm, 2013. **441**(1-2): p. 757-64.
172. Lamprecht, A., et al., *Nanoparticles enhance therapeutic efficiency by selectively increased local drug dose in experimental colitis in rats.* J Pharmacol Exp Ther, 2005. **315**(1): p. 196-202.
173. Sinswat, P., et al., *Nebulization of nanoparticulate amorphous or crystalline tacrolimus--single-dose pharmacokinetics study in mice.* Eur J Pharm Biopharm, 2008. **69**(3): p. 1057-66.
174. Nassar, T., et al., *A novel nanocapsule delivery system to overcome intestinal degradation and drug transport limited absorption of P-glycoprotein substrate drugs.* Pharm Res, 2008. **25**(9): p. 2019-29.
175. Nassar, T., et al., *Novel double coated nanocapsules for intestinal delivery and enhanced oral bioavailability of tacrolimus, a P-gp substrate drug.* J Control Release, 2009. **133**(1): p. 77-84.
176. Shin, S.B., et al., *Preparation and evaluation of tacrolimus-loaded nanoparticles for lymphatic delivery.* Eur J Pharm Biopharm, 2010. **74**(2): p. 164-71.
177. Pople, P.V. and K.K. Singh, *Targeting tacrolimus to deeper layers of skin with improved safety for treatment of atopic dermatitis.* Int J Pharm, 2010. **398**(1-2): p. 165-78.
178. Lopez-Sanchez, A., A. Saenz, and C. Casals, *Surfactant protein A (SP-A)-tacrolimus complexes have a greater anti-inflammatory effect than either SP-A or tacrolimus alone on human macrophage-like U937 cells.* Eur J Pharm Biopharm, 2011. **77**(3): p. 384-91.
179. Pople, P.V. and K.K. Singh, *Development and evaluation of colloidal modified nanolipid carrier: application to topical delivery of tacrolimus.* Eur J Pharm Biopharm, 2011. **79**(1): p. 82-94.
180. Katiyar, S.S., et al., *Co-delivery of rapamycin- and piperine-loaded polymeric nanoparticles for breast cancer treatment.* Drug Deliv, 2016. **23**(7): p. 2608-2616.
181. Shirasu, T., et al., *Nanoparticles Effectively Target Rapamycin Delivery to Sites of Experimental Aortic Aneurysm in Rats.* PLoS One, 2016. **11**(6): p. e0157813.
182. Zandstra, J., et al., *Microsphere-Based Rapamycin Delivery, Systemic Versus Local Administration in a Rat Model of Renal Ischemia/Reperfusion Injury.* Pharm Res, 2015. **32**(10): p. 3238-47.
183. Maldonado, R.A., et al., *Polymeric synthetic nanoparticles for the induction of antigen-specific immunological tolerance.* Proc Natl Acad Sci U S A, 2015. **112**(2): p. E156-65.
184. Khondee, S., et al., *Targeted therapy of colorectal neoplasia with rapamycin in peptide-labeled pegylated octadecyl lithocholate micelles.* J Control Release, 2015. **199**: p. 114-21.
185. Zhao, Y., et al., *CD44-tropic polymeric nanocarrier for breast cancer targeted rapamycin chemotherapy.* Nanomedicine, 2014. **10**(6): p. 1221-30.
186. Ono, K., et al., *A novel strategy inducing autophagic cell death in Burkitt's lymphoma cells with anti-CD19-targeted liposomal rapamycin.* Blood Cancer J, 2014. **4**: p. e180.
187. Gonzalez-Angulo, A.M., et al., *Weekly nab-Rapamycin in patients with advanced nonhematologic malignancies: final results of a phase I trial.* Clin Cancer Res, 2013. **19**(19): p. 5474-84.
188. Gasper, W.J., et al., *Adventitial nab-rapamycin injection reduces porcine femoral artery luminal stenosis induced by balloon angioplasty via inhibition of medial proliferation and adventitial inflammation.* Circ Cardiovasc Interv, 2013. **6**(6): p. 701-9.
189. Shah, M., et al., *A rapamycin-binding protein polymer nanoparticle shows potent therapeutic activity in suppressing autoimmune dacryoadenitis in a mouse model of Sjogren's syndrome.* J Control Release, 2013. **171**(3): p. 269-79.
190. Yanez, J.A., et al., *Pharmacometrics and delivery of novel nanoformulated PEG-b-poly(epsilon-caprolactone) micelles of rapamycin.* Cancer Chemother Pharmacol, 2008. **61**(1): p. 133-44.

Table 1. Selected publications on cyclosporin delivery using nanoparticles in the last decade

Carrier Type	Ingredients	Size (nm)	Route of Administration	Type of Study	Results	Ref.
Micelle	PEO-*b*-PCL	~ 100	-	In vitro	↑ Solubility & sustained release	[48, 49]
Micelle	PEO-*b*-PCL	~ 100	IV Injection	In vivo	↓ Nephrotoxicity	[50]
Micelle	PEO-*b*-PCL	~ 100	IV Injection	In vivo	↑ AUC & ↓ CL & Vd	[51]
Micelle	PEO-*b*-PCL	~ 100	IV Injection	In vivo	Suppressed immune response	[52]
Micelle	MPEG-hexPLA	~ 30	IV Injection	In vivo	↑ Solubility & ↓ excipient use	[53]
NP	PEG-chitosan/Lecithin	~ 90	IV Injection	In vivo	↑ AUC & $t_{1/2}$ / ↓ CL & VD	[54]
NP	PLGA	~ 140	Oral	In vivo	↑ Solubility, ↓ Nephrotoxicity, ↑ Bioavailability	[55]
NP	PLGA	~ 110	Oral	In vivo	↑ Solubility, ↓ Nephrotoxicity	[56]
LN	Percirol® & Lec-TC/PL-TC/Tw	~114-209	Oral	In vivo	↑ $_{Cmax}$ & AUC, ↑ Bioavailability	[58]
NP	GCPQ	40 – 200	Oral	In vivo	↑ C_{max}	[7]
NP	HPMCP	50 – 60	Oral	In vivo	↑ MRT & ↓ Elimination constant	[59]
NP	Eudragit®	35 – 110	Oral	In vivo	↑ C_{max} and Oral Bioavailability	[60]
NP	PEG-PLGA	< 100	-	In vitro	in vitro therapeutic efficacy maintained	[61]
NP	PLGA/Eudragit®/Carbopol®	148-393	Ocular	In vivo	↑ ocular retention and drug availability	[62]
NP	HA-coated PCL/BKC	200-300	Ocular	In vivo	↑ Iris/ciliary body concentrations	[63]
NP	Chitosan	300-600	Ocular	In vivo	↑ corneal and conjunctival residence time	[65]
NP	Chitosan-coated PLGA	200-250	-	In vitro	Sustained release and maintained efficacy	[66]
SLN	C888 or Precirol® ATO 5	~ 200	Ocular	Ex vivo	↑ Corneal permeation	[67]
NP	PDLLA-b-Dextran-g-PBA	~ 36	Ocular	In vivo	Sustained release & ↓ administration frequency	[68]
Micelle	PLA-b-p(MAA-PBA)	36-64	Ocular	In vivo	Slow release within 14 days	[69]
SLN	C888 or Gelucire®	~ 300 or > 700	-	In vitro	Stable particles (with Gelucire®) & Rapid release	[71]
SLN	C888/Poloxamer 188/Tw	~ 225	-	Ex vivo	↑ Corneal permeation	[74]
SLN	C888/Poloxamer 188/Tw	~ 225	Ocular	In vivo	↑ Aqueous humor level & ocular tolerance	[75]
SLN	Ticaprin/PC/Tw	~ 75	Topical	In vivo	↑ skin penetration &↓ IL-4 and -5	[76]
RHPND	Monoolein/oleic acid/poloxamer	~ 180	Topical	In vivo	↑ skin penetration	[77]
NP	TPGS	~ 350	Topical	In vitro	↑ skin penetration	[78]
NP	PCL	~ 30	-	In vitro	↓ Secretion of inflammatory cytokines	[79]
Gel	C888/Carbopol/HPMC	~ 200	Topical	In vivo	↑ Wound healing rate	[80]
NP	PLA-DPPE	200 -375	-	In vitro	Sustained release	[168]
Micelle	Cholesteryl-modified polymers*	100-200	-	In vitro	Thermally responsive controlled release	[169]

NP	PEG-PLA	~ 85	-	In vivo	Internalization into dendritic cells	[170]
NP	PDLLA/MCM/MCT	150 – 250	Oral	In vivo	↑ AUC, MRT & Bioavailability	[171]

*: Cholesteryl end-capped P(NIPAAm-co-DMAAm) and cholesteryl grafted P(NIPAAm-co-NHMAAm)
↑: increased; ↓: decreased; AUC: Area under the curve; C888: Compritol® 888 ATO; CL: Systemic clearance; C_{max}: maximum blood concentration; GCPQ: quaternary ammonium palmitoylglycol chitosan; HA: Hyaluronic acid; HPβCD: hydroxypropyl-β-cyclodextrin; HPMC: Hydroxypropyl methyl cellulose; Lec-TC: mixture of phosphatidylcholine and taurocholate; MCT: Medium chain triglycerides; MCM: Medium chain mono-diglyceride; MPEG-hexPLA: methoxy-poly(ethylene glycol)-hexyl-substituted poly(lactides); MRT: Mean Residence Time; NP: Nanoparticle; PBA: Phenylboronic acid; PC: L-α- Phosphatidylcholine); PCL: Poly-ε-caprolactone; PCL/BKC: Poly-ε-caprolactone/ benzalkonium chloride; PDLLA: Poly-DL-lactide; PEO-*b*-PCL: Poly(ethylene oxide)-*block*-poly(ε-caprolactone); PLA-b-p(MAA-PBA): Poly(L-lactide)-b-poly(methacrylic acid-co-phenylboronic acid); PL-TC: mixture of Pluronic® F127 and taurocholate; PLA: Polylactide; PLA-DPPE: Poly(L-aspartic acid-co-L-lactic acid)- 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine; PLGA: poly(lactide-co-glycolide); P(NIPAAm-co-DMAAm): Poly(N-isopropylacrylamide-co-N,N-dimethylacrylamide); P(NIPAAm-co-NHMAAm): poly[N-isopropylacrylamide-co-N-(hydroxymethyl) acrylamide]; RHPND: Reverse Hexagonal Phase Nano-dispersion system; SLN: Solid Lipid Nanoparticle; $t_{1/2}$: drug half-life; TPGS: Vitamin E polyethylene glycol succinate; Tw: Tween® 80; Vd: Volume of distribution

Table 2. Selected publications on tacrolimus delivery using nanoparticles

Carrier Type	Ingredients	Size (nm)	Route of Administration	Type of Study	Results	Ref.
NP	HAS	185.8	IV injection	In vivo	↑ solubility, sustained release, ↓ arthritis index score	[90]
NP	DM-β-CyD BSA	148.4–262.9	IV injection	In vivo	↑ solubility, ↑ t_{max} & MRT, ↓ C_{max}	[91]
NP	BSA	~ 190	IV injection	In vivo	↑ AUC, ↓ accumulation in kidneys & nephrotoxicity	[92]
NP	SPC / α-Tocopherol	~ 40	IV injection	In vivo	↓ Toxicity	[93]
Micelles	PEO-PCL	~ 50	IV injection	In vivo	↓ doses & ↑ functional recovery of injured nerves	[94]
Micelles	MPEG-PCL	~ 25	IV injection	In vivo	Sustained release, ↑ body weight, ↑colon length	[95]
Micelles	PCL-PEG-PCL	~ 20	IV injection	In vitro	Sustained release	[98]
SMEDDS	Capmul® MCM C8, C-EL, Carbitol™	< 25	Oral	In vivo	↓ lymphocytes in peripheral blood	[96]
SMEDDS	Ethyl oleate, GF, Solutol HS 15	< 100	Oral	In vivo	↑ C_{max}, ↓ t_{max}, ↑ accumulation in RES organs	[97]
SMEDDS	Miglyol® 840, Transcutol® P, TPGS/CL-E	~ 18	Oral	In vivo	↑ C_{max}, t_{max}, AUC, & relative bioavailability	[98]
NPMS	PLGA/Eudragit®	~ 240	Oral	In vivo	↑ Colon delivery, ↓ side effects	[99]
Ethosomes	Ethanol, Propylene glycol	~ 76-104	Topical	In vivo	↑ skin penetration & ↓ mouse ear swelling	[100]
Liposomes	P90H, loralan-CH	-	Topical	In vivo	↑ skin penetration	[101]
NP	BSA	~ 182	Pulmonary	In vivo	Sustained release, ↓ degree of lung inflammation	[102]
NP or MS	PLGA or Eudragit®	455 or 469	Oral	In vivo	↓ Nephrotoxicity, ↑ Colon delivery	[24]
NP	PLGA	~ 100	Rectal	In vivo	↑ Colon delivery and tissue penetration	[172]
URF	Lactose	2570	Pulmonary	In vivo	Significant and rapid systemic absorption	[173]
NP	Eudragit®/HPMC	500 – 2000	Oral	In vivo	↓ GI degradation, ↑ cell uptake	[174]
NP	Eudragit®/HPMC	2000 – 10000	Oral	In vivo	↑ absorption and bioavailability	[175]
NP	PLGA-PEG	~ 220	IV	In vivo	↑ AUC, MRT, lymphatic accumulation, ↓ CL	[176]
NP	Glyceryltrimyristate	80 – 160	Topical	In vivo	↑ Stratumcorneum, epidermal and dermal levels	[177]
Complex	Surfactant protein A	~ 400	-	In vitro	↑ cell uptake, anti-inflammatory effect	[178]
MNLC	PGMC/Glyceryltrimyristate	20 – 150	Topical	In vivo	↑ Solubility, ↓ Skin irritation	[179]

↑: increased; ↓: decreased; AUC: Area under the curve; BSA: Bovine serum albumin; C-EL: Cremophor® EL; CL: Systemic clearance; DM-β-CyD: heptakis(2,6-di-*O*-methyl)-β-cyclodextrin; GF: glycofurol; HSA: human serum albumin; HPMC: Hydroxypropylmethylcellulose; MRT: Mean Residence Time; NPMS: Nanoparticles entrapped in pH-

sensitive microspheres; P90H: Phospholipon® 90-H; PEG: Polyethylene glycol; PGMC: Propylene glycol monocaprylate; PLGA: poly(lactide-co-glycolide); TPGS: tocopheryl polyethylene glycol succinate; SMEDDS: self-microemulsifying drug delivery system; SPC: Soy phosphatidylcholine; URF: Ultra-rapid Freezing.

Table 3. Selected publications on methotrexate delivery using nanoparticles

Carrier Type	Ingredients	Size (nm)	Route of Administration	Type of Study	Results	Ref.
SLN	Gelucire, stearic acid, P90NG, fucose	~ 163-174	IV injection	In vivo	↑ $t_{1/2}$ & AUC, ↑ accumulation in tumor site	[110]
SLN	Soya lecithin, stearic acid	~ 270-490	IV injection	In vivo	↑ $t_{1/2}$ & MRT, ↑ life-span of EAC bearing mice	[111]
Micelles	Pluronic® P105/F127	~ 23	IV injection	In vivo	↑ systemic circulation, ↑ cytotoxicity in MDR tumor	[112]
Micelles	PEI-g-mPEG, Zn^{2+}, DTDPA	117	-	In vitro	enhanced and specific antitumor activity	[113]
Liposomes	Egg lecithin, CH, PA	~100	IV injection	In vivo	↑ anti-inflammatory effect, ↓ haematotoxic activity	[114]
Liposomes	Cationic liposome	-	IV injection	In vivo	↓ leucocyte- & platelet-endothelial cell interaction	[115]
Liposomes	PC, CH w/out DSPE-PEG	105-168	IV injection	In vivo	↓ CL, ↑ circulation, ↓ hepatosplenic & kidney uptake	[116]
Liposomes	DSPE-MPEG/chitosan	210-260	IV injection	In vivo	↓ edema, ↑ anti-inflammatory effect	[117]
ABDD	HAS	n.r.	IV injection	In vivo	↓ onset of arthritis & ↑ accumulation in inflamed paws	[118]
ABDD	HAS	n.r.	IV injection	In vivo	↑ MTX potency & ↑ $t_{1/2}$	[119]
microsphere	Poly(L-lactic acid)	~83000-18700	IA injection	In vivo	↓ plasma concentration & ↓ clearance from joint	[120]
Niosomes	NIS I, CH, dicetyl phosphate	~ 115-124	IV injection/oral	In vivo	↑ circulation, ↑ uptake in liver & brain, ↑ absorption	[121]
SLN	Cetyl alcohol, stearic acid, C888, Tw	~ 123-511	Topical	In vivo	↑ accumulation in human skin and ↑ APIH	[122]
ME	Lecithin, water/PG, decanol	n.r.	Topical	In vitro	↑ solubility & skin permeation	[123]
ME	Phospholipid 90G, ethanol, Tw	~ 19-48	Topical	In vivo	↑ skin penetration & ↓ systemic absorption	[110]
Liposomes	PC, PI, CH, collagen, carrageenan	~ 138	Pulmonary	In vivo	↑ accumulation in the lungs	[124]

↑: increased; ↓: decreased; 2HBC: 2-hydroxypropyl-β-cyclodextrin; ABDD: Albumin based drug delivery; APIH: Average percent improvement in healing of psoriasis lesions; AUC: area under the curve; C888: Compritol® 888; CH: cholesterol; DSPE-PEG: distearoylphosphatidyl-ethanolamine-N-poly(ethyleneglycol) 2000; DTDPA: dithiodipropionic acid; EAC: Ehrlich Ascite Carcinoma Gelucire: Gelucire® 50/13; IA: intraarticular; IV: intravenous; LD50: The dose lethal to 50% of the experiment mice; ME: Microemulsion; MRT: Mean residence time; NIS: Non-ionic surfactant; n.r.: not reported; P90NG: Phospholipid 90 NG ;PA: Phosphatidic acid; PC: Phosphatidylcholine; PEI-g-mPEG: Polyethyleneimine grafted methoxy polyethylene glycol; PG: Propylene glycol; PI: phosphatidylinositol; $t_{1/2}$: drug half-life; Tw: Tween® 80; SLN: Solid-lipid nanoparticle;

Table 4. Selected publications on corticosteroids delivery using nanoparticles

Carrier Type	Ingredients	Size (nm)	Route of Administration	Type of Study	Results	Ref.
Liposomes / Micelles	HC, Phosphatidylcholine/ TPGS	~180/ ~ 17	-	In vitro	↑ Solubility & permeability through nasal mucosa	[129]
Liposomes / Micelles	DEX, DPPC, PEG-DSPE, CH/ DMSL3, PEG-b-pHPMAmLacn	~96/ ~ 53	IV injection	In vivo	↓ joint inflammation, maintained longer with micelles	[130]
Liposomes	MPS, HSPC, DSPE-PEG, CH	80-90	SC injection	In vivo	↓ dose frequency, ↑ efficacy & ↓ toxicity	[131]
Liposomes	PLP, DPPC, PEG-DSPE, CH	~ 100	IV injection	In vivo	↑ $t_{1/2}$,↓ side effects & showed efficacy for atherosclerosis	[132]
Liposomes	PLP, DPPC, PEG-DSPE, CH	~ 100	IV injection	In vivo	↑ $t_{1/2}$ & targeted atherosclerotic macrophages	[133]
NP	DEX, PEG-dendritic block telodendrime	10-20	Pulmonary	In vivo	↓ airway inflammation	[134]
Liposomes	BUD, PG-PC-PEG-DSPE-CH	n.r.	Pulmonary	In vivo	Maintained efficacy while ↓ dose frequency	[137]
nanocapsule	BDP, ethyl cellulose/PCL	~ 260	Pulmonary	In vivo	Sustained release & no acute pulmonary injury in rats	[138]
Micelles	PHEA-PEG-DSPE	~ 59-69	Pulmonary	In vivo	↑ solubility & drug cellular internalization	[139]
Micelles	DEX, Polyoxyl 40 stearate, polysorbate 80	~ 15	Ocular	In vivo	Therapeutic concentrations in the retina and choroid	[135]
NP	DSP, PLGA	~ 200	Ocular	In vivo	Sustained release, ↓ systemic exposure	[136]
liposomes	DSP, EPC, DSPE-PEG, CH, HA	~ 145	Otic	In vivo	Sustained release in the perilymph for up to 30 days	[140]
NP	DEX, PEG-PLA		Otic	In vivo	Sustained release & protection against hearing loss at 4 and 8 kHz	[141]

↑: increased; ↓: decreased; BDP: Beclomethasone dipropionate; CH: Cholesterol; DEX: Dexamethasone; DMSL3: A polymerizable prodrug of dexamethasone; DPPC: Colfosceril palmitate; DSP: Dexamethasone sodium phosphate; DSPE: 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine; EPC: Egg phosphatidylcholine; HA: hyaluronic acid; HC: Hydrocortisone; MPS: Methylprednisolone hemisuccinate; NP: Nanoparticles; PC: Phosphotidylcholine; PCL: Poly(ε-caprolactone); PEG: Poly(ethylene glycol); PEG-b-pHPMAmLacn: Poly(ethylene glycol)-b-poly(N-(2-hydroxypropyl)methacrylamide lactate); PG: Phosphatidylglycerol; PHEA: α,β-poly(N-2-hydroxyethyl)-dl-aspartamide; PLA: Polylactic acid; PLGA: Poly(lactic-co-glycolic acid); PLP: Prednisolone phosphate; TPGS: d-α-tocopheryl polyethylene glycol 1000 succinate

Table 5. Selected publications sirolimus (rapamycin) delivery using nanoparticles

Carrier Type	Ingredients	Size (nm)	Route of Administration	Type of Study	Results	Ref.
NP	PIP, PLGA	~134	Oral	In vivo	Sustained release, ↑ bioavailability, ↑ transport (P-gp efflux)	[180]
NLC	Precirol® ATO 5, oleic acid, Tween® 80	108.3	Oral	In vivo	Sustained release, ↑ oral bioavailability	[151]
NP	PVP K90, SDS	30	Oral	In vivo	↑ C_{max}, AUC, C_{24h} & ↓ t_{max}	[155]
NP	PEG-b-PBLG	106	IV injection	In vivo	NP accumulation in the abdominal aortic aneurysm wall	[181]
NP	Acetalated β-CyD material	185-250	SC injection	In vivo	Sustained release, ↑ anti-atherosclerotic activity	[148]
SMEDDS	Capryol™ PGMC, glycofurol, vitamin E TPGS	~108.2	Oral	In vivo	↑ absorption and oral bioavailability	[152]
Micelles	TPGS	11	Oral	In vivo	↑ absorption, AUC, C_{max} and oral bioavailability	[157]
SMEDDS	LF, CrEL, TransP, MCC, Lactose, CMS-Na	~25	Oral	In vivo	↑ absorption and oral bioavailability	[153]
NP	polyvinylpyrrolidone (PVP) K30	250	Oral	In vivo	↑ absorption, AUC, C_{max} and oral bioavailability	[156]
NP	NIPAAm, acrylic acid, MMA/or NVP	80	Oral	In vivo	↑ bioavailability, improved PK and efficacy profile	[154]
Liposomes	soybean lecithin, cholesterol	140-211	Ocular, S/C	In vivo	Clinical ↑ in tear production in dry eye	[159]
Micelles	MPEG-PCL	40	Ocular, IVI	In vivo	↑ solubility and drug localization in retinal tissue	[160]
Microsphere	20[PDLA-PEG1000]-80[PLLA]	20000	S-Cap injection	In vivo	↓ macrophage infiltration, ↓ myofibroblasts in the kidney	[182]
NP	PLGA	200	IV & SC injection	In vivo	Induced antigen-specific B-cell tolerance lasted 200 days	[183]
Micelles	PEGylated octadecyl lithocholate, LTTHYKL	121-130	IP injection	In vivo	Targeted and ↑ efficacy (adenoma regression), ↓ toxicity	[184]
Micelles HG	l-lactide monomers, gelatin	-	IA injection	In vivo	Delayed OA progression maintained for 16 weeks	[147]
NP	HA, 3-amino-4-methoxy-benzoic acid	~10	SC injection	In vivo	↑ C_{max}, $t_{½}$, AUC, & ↓ Cl, ↑ survival in BC animal model	[185]
NP Gel	PLGA, PVA, Poloxamer 407	220-350	periadventitial	In vivo	Sustained release, prolonged attenuation of IH	[147]
Liposomes	DPPC, DPPE, DCP, cholesterol, ganglioside	~140	IV injection	In vivo	Induce autophagic cell death in Burkitt's lymphoma cells	[186]
NP	PFOB, surfactant mixture including lecithin	~160-240	IV Injection	In vivo	↑ skeletal muscle strength & cardiac contractile in DMD	[149]
NP	Albumin	~100	IV injection	In vivo	improve delivery, safety, efficacy in various solid tumors	[187]
NP	Albumin	~100	adventitial inj.	In vivo	↓ in luminal stenosis and medial fibrosis at 28 days	[188]
NP	ELPs fused with FKBP12	24	IV injection	In vivo	↓ nephrotoxicity and ↓ CATS	[189]
Liposomes/ micelles	Cholesterol, EPC, DSPG and DSPE–PEG / DSPE–PEG	~90 / ~14	Local intramural	In vivo	↓ Vascular stenosis, ↑ luminal area, suppressed Ki67-positive cell proliferation	[146]
NP	chitosan/polylactic acid	300	Ocular	In vivo	↑ survival time of the corneal allografts	[158]

Micelles	PEG-b-PCL, w/ or w/o α-tocophero	37,101	IV injection	In vivo	Altered drug disposition with ↓ distribution into the brain	[190]

↑: increased; ↓: decreased; 20[PDLA-PEG1000]-80[PLLA]: 20% w/w of poly(DL-lactide)-PEG1000-poly(DL-lactide) and 80% w/w of poly(L-lactide); AUC: Area under the plasma concentration curve; BC: Breast cancer; C_{max}: The maximal plasma concentration; C_{24h}: Total blood concentration at 24 h; CATS: Cathepsin S, a tear biomarker of Sjögren's syndrome; CMS-Na: sodium carboxymethyl starch; CrEL: Cremophor EL; CyD: Cyclodextrin; DCP: Dicetylphosphate; DMD: Duchenne muscular dystrophy; DOPE: Dioleoylphosphatidylethanolamine; DPPC: Dipalmitoylphosphatidylcholine; DPPE: dipalmitoylphosphatidylethanolamine; DSPE-PEG: 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000]; DSPG: distearoyl-sn-glycerophosphoglycerol; ELPs: Elastin-like Polypeptides; EPC: Purified egg phosphatidylcholine; HA: Hyaluronic acid; HG: Hydrogel; IA: Intraarticular; IH: intimal hyperplasia; IV: Intravenous; IVI: Intravitreal injection; LF: Labrafil® M1944CS; LTTHYKL: Targeting peptide; MMA: methylmethacrylate; MCC: microcrystalline cellulose; NIPAAm: N-isopropylacrylamide; NLC: Nanostructured lipid carrier; NVP: vinylpyrrolidone; P-gp: P-glycoprotein; PEG-b-PBLG: Poly(ethylene glycol)-block-poly(γ-benzylL-glutamate); PEG-b-PCL: Poly(ethylene glycol)-block-poly(ε-caprolactone); PFOB: perfluorooctylbromide; PGMC: Propylene glycol monocaprylate; PIP: Piperine , a chemosensitizer; PLGA: Poly(D,L-lactide-co-glycolide); S/C: Subconjunctival; S-Cap: Subcapsular; SDS: Sodium dodecyl sulfate; SMEDDS: self-microemulsifying drug delivery system; t_{max}: Time at which C_{max} is achieved; TPGS: d-α-tocopheryl polyethylene glycol succinate; TransP; Transcutol® P;

Table 6. Selected publications on mycophenolic acid or its ester prodrug mycophenolate mofetil delivery using nanoparticles

Carrier Type	Ingredients	Size (nm)	Route of Administration	Type of Study	Results	Ref.
Liposomes	DOPC, Chol, DSPE-PEG-NH_2	351.3	Injection	In vivo	Effective for nephrotic syndrome & ↓ kidney damage	[167]
Nanogel	Phosphatidylcholine, Chol, DSPE-PEG-amine	225	IP injection	In vivo	↑ MST by 2-3 months in a lupus prone mice model	[164]
Nanogel/ NP	Phosphatidylcholine, Chol, DSPE-PEG-amine /PLGA	187/ 171	IP injection	In vivo	internalized by dendritic cells	[165]
NP	PLGA	171	IP Injection	In vivo	↑allograft survival & ↓ drug toxicity	[166]

↑: increased; ↓: decreased; Chol: Cholesterol; DOPC: 1,2-Dioleoyl-sn-glycero-3-phosphocholine; DSPE-PEG(2000)-amine: 1,2-distearoyl-snglycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000]; DSPE-PEG-NH_2: 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[(polyethylene glycol)-2000]; PLGA: poly(lactic-co-glycolic acid);

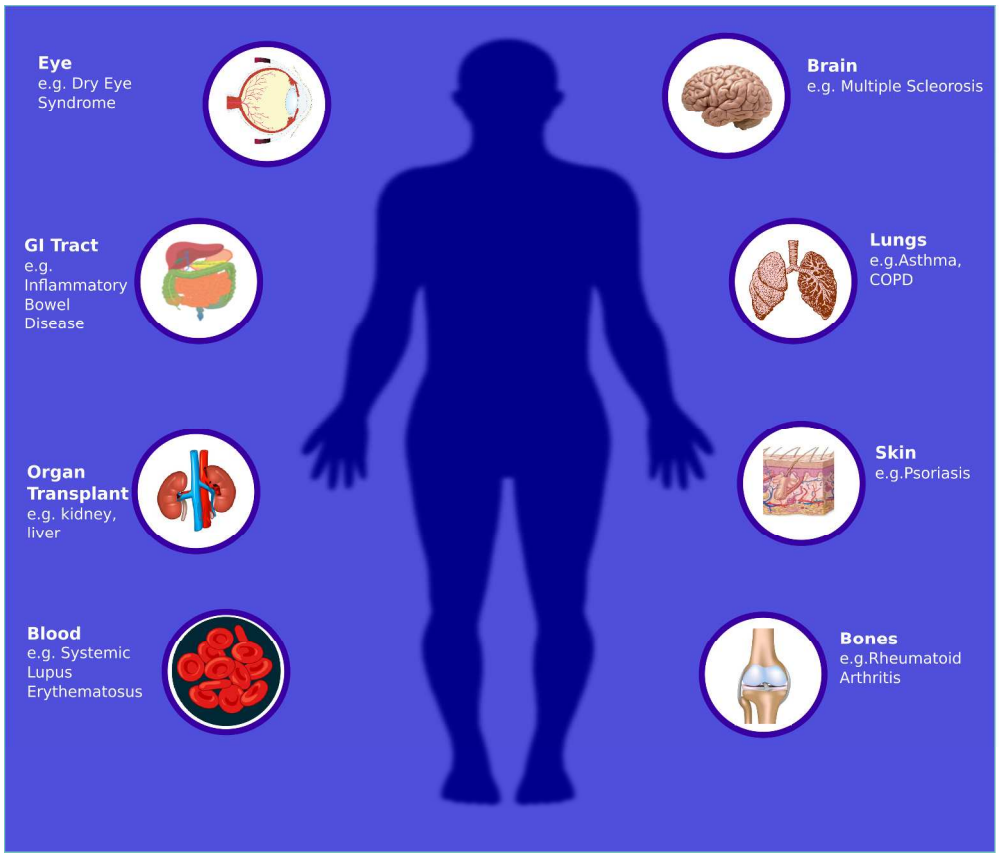

Figure 1. Body parts and organs that benefit from immunosuppressive therapy

574x486mm (144 x 144 DPI)

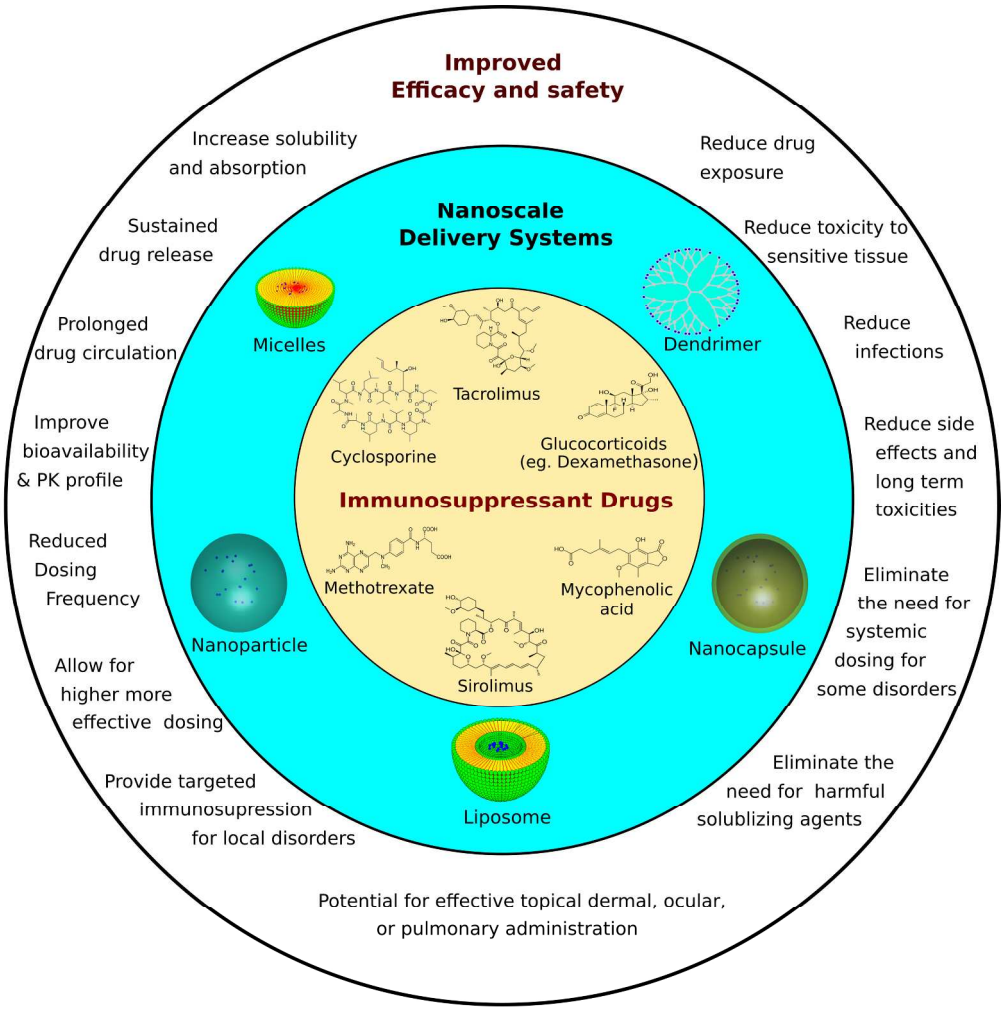

Figure 2. Nano-delivery of immunosuppressive therapy

470x470mm (144 x 144 DPI)