Novel targeted drug delivery systems to minimize systemic immunosuppression in vascularized composite allotransplantation

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Purpose of review
The long-term adverse effects of immunosuppressive treatment, the high rate of acute rejection and the development of chronic rejection are the main factors preventing a wider clinical application of vascularized composite allotransplantation (VCA). Targeted immunosuppression using innovative drug delivery systems (DDS) may help to overcome these hurdles, increasing therapeutic efficacy while reducing systemic toxicity. This review provides a summary of the recently developed strategies for targeted delivery of immunosuppressive drugs in VCA.

Recent findings
Currently, several innovative strategies for targeted immunosuppression have been designed based on the anatomy and function of the target organ. Site-specific DDS have been developed both for directly accessible organs (i.e. skin, eye and lung) and internal organs (i.e. lymph nodes, liver, nervous system, etc.). In preclinical models, DDS designed for sustained, 'on demand,' or 'on cue' drug release has been shown to promote VCA survival while reducing systemic toxicity. These findings suggest that targeted delivery could increase patient compliance and potentially decrease toxicity in VCA recipients.

Summary
Targeted immunosuppression in VCA represents a promising approach for improving patient compliance and graft survival while reducing off-target toxicity, intensity and frequency of acute rejection episodes and risk of chronic rejection.

Video Abstract
http://links.lww.com/COOT/A1

Keywords
drug delivery system, immunosuppression, immunosuppression toxicity, vascularized composite allotransplantation

INTRODUCTION

Vascularized composite allotransplantation (VCA) is an increasingly performed reconstructive procedure to restore the appearance, anatomy and function in patients suffering major tissue loss and not candidates for conventional reconstruction [1–3]. The success achieved in upper extremity and face transplantation has fueled a rapid expansion of the VCA field and a host of other types of VCA being performed around the world, including transplantation of abdominal wall, bone and joint, laryngotrachea, uterus, penis, tongue, ear, scalp and lower extremity [4].

Long-term adverse effects, however, of immunosuppressive treatment (IST) are the main factors preventing a wider clinical application of VCA. Chronic immunosuppression using this protocol is associated with diabetes mellitus, nephrotoxicity, osteonecrosis, leukopenia, hypertension, hyperlipidemia, opportunistic infections, higher cancer risk as well as psychological sequelae and increased financial burden [3,5]. Moreover, a high rate of acute rejection, with 88% of the patients experiencing at least one episode in the first posttransplant year [6▪], and development of chronic rejection [7] leading to

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KEY POINTS

- Targeted drug delivery systems can increase the therapeutic index in VCA.
- Implantable and nanocarrier-based drug delivery systems have shown promising results for site-specific immunosuppression in skin, eye, lung and lymph nodes.
- On demand, inflammatory-responsive hydrogels represent a promising therapeutic approach for the treatment of inflammatory disease and VCA rejection.
- Sustained site-specific immunosuppression can reduce side effects increasing patient compliance.
- Validation of targeted immunosuppression: exploration in large animal models is urgently required.

graft vasculopathy and often to graft loss, are others major problems in VCA management that strongly decrease the risk-to-benefit ratio of this procedure.

Several strategies have been investigated to boost the therapeutic index of immunosuppression (i.e. increasing efficacy and reducing toxicity). Among them, the development of drug delivery systems (DDS) has been relentlessly investigated and a plethora of DDS have been described for controlled drug release, enhanced bioavailability and selective organ targeting [8,9]. Due to its accessibility, VCA offers unique opportunities for site-specific delivery of immunosuppressive medications directly to the graft [10]. The rationale for such site-specific, transplant-targeted IST is to reduce systemic exposure and global collateral or end-organ adverse effects. Moreover, site-specific treatment may be used to increase patient compliance. Finally, because of the high availability of immunosuppressive drug where needed, targeted IST can reduce the number of systemic drugs required for desired efficacy, as well as the dose and frequency of IST.

In this review, we will summarize recently developed strategies for targeted delivery of immunosuppressive drugs. We will start by describing DDS developed for inflammatory diseases and solid organ transplantation (SOT) and will then focus on DDS developed specifically for targeted delivery in VCA.

TARGETED IMMUNOSUPPRESSION IN SOLID ORGAN TRANSPLANTATION AND INFLAMMATORY DISEASES

Several DDS for delivery of IST have been described in the fields of inflammatory disease and transplantation. An overview is presented in Tables 1 and 2. Readers are directed to recent reviews that comprehensively describe the different strategies for drug delivery [54–56].

Strategies for targeted delivery should always be designed based on the anatomy and function of the target organ. Indeed, organs such as the skin, the eye or the lung offer unique opportunities for targeted delivery (e.g. topical administration, eye drops, inhalation of aerosolized medication). Conversely, visceral organs such as the liver, lymph nodes and kidney require DDS containing specific targeting moieties capable of directing the DDS to the target organ after systemic application.

Site-specific administration of IST has a long history in the management of inflammatory skin disease [57]. Considering that the skin represents the primary target in VCA rejection [58], DDS developed for targeted IST delivery in the skin are of outstanding interest to the VCA community. Several topical formulations of calcineurin inhibitors have been described so far and include Food and Drug Administration (FDA)-approved commercial formulations such as tacrolimus ointment, pimecrolimus cream and clobetasol propionate cream [10]. Recently, innovative nanocarrier-based topical formulations of tacrolimus have been developed with the aim to increase skin penetration while reducing systemic exposure. Topical application of a liposomal tacrolimus formulation has been shown to increase drug availability in the skin and delay skin allograft rejection in a mouse model [11]. In addition, smart DDS that are able to respond to the tissue environment have been described for IST delivery into the skin. Thermoresponsive nanogels loaded with tacrolimus [37,38] or pH-sensitive dexamethasone-loaded nanoparticles [16▪▪] are only some of the examples showing improved skin penetration and efficacy.

Similar to the skin, the eye and the lung offer the possibility to use topical application for targeted delivery of immunosuppressive drugs. Topical instillation of eye drops, intravitreal delivery methods and periocular routes of drug delivery are some of the described routes for targeted delivery of medications directly to the eye [41▪]. Moreover, intraocular implantable DDS for targeted delivery of dexamethasone [59,60] and other corticosteroids [41▪] as well as tacrolimus [42] have been developed and are used in a wide range of chronic ocular inflammatory diseases to deliver drugs in a sustained fashion. Aerosolized calcineurin inhibitors have been developed and their efficacy has been tested in clinical trials. Although these formulations failed to show efficacy in disease-free survival or overall mortality, high concentration of the drug was observed in the lung with minimal systemic

Table 1. Targeted immunosuppression using nanoparticles and microparticles in solid organ transplantations and inflammatory diseases

DDS description	Target organ (mechanism)	Immuno-suppressive drug	Testing model	DDS administration	Reference
Liposome	Skin (site-specific administration)	Tacrolimus	Mouse skin allograft	Topical application	[11]
Neutral multilamellar liposomes	Skin (site-specific administration)	Tacrolimus	Rat allergic contact dermatitis	Topical application	[12]
Methoxy-PEG-dihexyl substituted PLA micelles	Skin (site-specific administration and improved deposition)	Tacrolimus	In-vitro human skin	Topical application	[13]
Transfersomes	Skin (site-specific administration)	Tacrolimus	Mouse atopic dermatitis	Topical application	[14]
Rhamnolipid-based nanoparticles	Skin (site-specific administration)	Dexamethasone or Tacrolimus	In-vitro human skin	Topical application	[15]
pH-sensitive nanoparticles (various formulation)	Skin (site-specific administration)	Dexamethasone	In-vitro evaluation	In solution	[16▪▪]
Liposomes containing bile salts	Eye (site-specific administration)	Tacrolimus	Naïve rabbit cornea	Eye instillation	[17]
Liposomes	Eye (site-specific administration)	Tacrolimus	Rat uveoretinitis	Intravitreal injection	[18]
PLGA nanoparticles	Eye (site-specific administration)	Dexamethasone	Naïve rabbit	Intravitreal injection	[19]
PLGA nanoparticles	Eye (site-specific administration)	Tacrolimus	Naïve rabbit	Instillation in conjunctival sac	[20]
Proniosome-derived niosomes	Eye (site-specific administration)	Tacrolimus	Rat corneal allotransplantation	Eye instillation	[21]
Chitosan PLA nanoparticles	Eye (site-specific administration)	Rapamycin	Rabbit corneal allotransplantation	Eye instillation	[22]
Chitosan-coated PLGA nanoparticles	Lung (site-specific administration)	Tacrolimus	Mouse pulmonary fibrosis	Intratracheal aerosol	[23]
PLGA nanoparticles	Lymphatic system (physicochemical characteristics of the particles)	Mycophenolic acid	Mouse skin allograft	Intraperitoneal injection	[24]
PLGA and PLGA-PEG nanoparticles	Lymphatic system (physicochemical characteristics of the particles)	Tacrolimus	Naïve rats	Intravenous injection	[25]
PEG-*bl*-PPS micelles	Draining lymph node (physicochemical characteristics of the particles)	Tacrolimus and Rapamycin	Mouse skin allograft	Intradermal injection	[26]
PLGA nanoparticles	Draining lymph node (physicochemical characteristics of the particles)	Cyclosporine	Naïve mouse	Intramuscular injection	[27]
Polylactic (PLA)-based microparticles	Draining LN (particle conjugation with MECA-79 antibody to target peripheral node addressin)	Tacrolimus	Mouse heart allograft	Intravenous injection	[28]
PEG-PLA nanoparticles	Liver (site-specific administration)	Tacrolimus	Rat liver allograft	Gastric perfusion	[29]
Liposome	Liver (high uptake by reticuloendothelial system)	Cyclosporine	Rat liver allograft	Intravenous injection	[30]
Galactosylated PLGA nanoparticles	Liver and spleen (high uptake by reticuloendothelial system)	Tacrolimus	Naïve rats	Intravenous injection	[31]
PLA nanoparticles	Liver and spleen (high uptake by reticuloendothelial system)	Tacrolimus	Naïve rats	Intravenous injection	[32]
Poly-d,l-lactic acid (PDLLA) microspheres	Peyer's patch (high uptake by macrophages)	Tacrolimus	Porcine small bowel transplantation	Oral administration	[33]
Liposome	Brain (site-specific administration)	Tacrolimus and Rapamycin	Rat dopaminergic graft	With the implanted cells	[34]
Eudragit P-4135F nanoparticles	Intestine (sensitivity to luminal pH during intestinal passage)	Tacrolimus	Mouse colitis	Oral administration	[35]
PEG-PE-amine and *N*-palmitoyl homocysteine micelles	Endothelial cells (using cyclic Arginine-Glycine-Aspartate, cRGD moieties to target αVβ3 integrin)	Rapamycin	In-vitro and ex-vivo EC culture	In-vitro culture	[36]

PEG, poly(ethylene glycol); PLC, poly(D,L-lactide-co-e-caprolactone); PLGA, poly(lactide-*co*-glycolide); PPS, poly(propylene sulfide).

Table 2. Targeted immunosuppression using controlled delivery systems and special formulations in solid organ transplantations and inflammatory diseases

DDS description	Target organ (mechanism)	Immuno-suppressive drug	Testing model	DDS administration	Reference
Polyglycerol-based thermoresponsive nanogels	Skin (site-specific administration)	Dexamethasone or Tacrolimus	In-vitro human skin	Topical application	[37,38]
PLC microfilms	Eye (site-specific administration)	Prednisolone acetate or Tacrolimus	Rat corneal transplantation Mouse allergic conjunctivitis	Subconjunctival implanted	[39,40]
Intravitreal implants (various formulations)	Eye (site-specific administration)	Corticosteroids	In clinical use	Intravitreal implant	[41▪]
PLGA scleral plug	Eye (site-specific administration)	Tacrolimus	Rabbit uveitis	Intravitreal implant	[42]
Propylene glycol suspension	Lung (site-specific administration)	Cyclosporine	Human clinical trial	Inhalation	[43,44,45]
3D-macroporous polydimethylsiloxane (PDMS) scaffold	Pancreatic islets (site-specific administration)	Dexamethasone	Diabetic mouse model	Seeding of the islets on the scaffold	[46]
Fibrin gel reservoirs containing solubilized, particulated, and PLGA-microspheres-encapsulated drug	Peripheral nerve (site-specific administration)	Tacrolimus	In-vitro neurite extension	In-vitro culture	[47]
Electrospun poly(ester urethane) urea and tacrolimus elastomeric matrix (PEUU-Tac)	Central nervous system (site-specific administration)	Tacrolimus	Rat acute central nervous system ischemia	Wrapped and sutured around the nerve injury	[48▪▪]
Ascorbyl palmitate inflammation-targeting hydrogel	Inflammation (negative surface charge to facilitate adhesion to the positively charged inflamed colon epithelium)	Dexamethasone	Mouse colitis models	Rectal administration	[49]
Triglycerol monostearate inflammation-responsive hydrogel	Joint (site-specific administration)	Triamcinolone acetonide	Mouse inflammatory arthritis	Intra-articular injection	[50▪▪]
Ointment and cream	Skin (site-specific administration)	Tacrolimus or pimecrolimus or clobetasol propionate	Clinical use	Topical application	[10]
Inhalation solution	Lung (site-specific administration)	Tacrolimus	Rat lung allograft	Inhalation	[51]
Nanostructured aggregates	Lung (site-specific administration)	Tacrolimus	Rat lung allograft	Inhalation	[52]
Solutions and ointment	Eye (site-specific administration)	Tacrolimus or Cyclosporine	Clinical	Topical application	[53]

PEG, poly(ethylene glycol); PLC, poly(D,L-lactide-co-e-caprolactone); PLGA, poly(lactide-co-glycolide); PPS, poly(propylene sulfide).

exposure [43–45]. Furthermore, encouraging results have been obtained in preclinical models for the prevention of acute lung rejection using inhalation [51] of nanostructured aggregates [52] of tacrolimus. Although DDS specifically designed for IST targeting the eye and lung may have limited applications in upper extremity and face transplantations, these studies have shown that site-specific immunosuppression is an effective method to control allograft rejection providing new means and ideas for the generation of innovative DDS in other VCA procedures.

Draining lymph nodes are the main site of immune activation after transplantation. Therefore, many studies have focused on delivering IST directly to the lymphatic system and to the draining lymph node [61▪▪]. Passive accumulation of nanoparticles or microparticles into the lymph node after systemic administration (i.e. subcutaneous, intravenous or intraperitoneal injections) has been reported by several groups [24,25,62]. Shirali *et al.* [24] developed poly-lactide-co-glycolide (PLGA) nanoparticles loaded with mycophenolic acid (MPA) that

accumulated into the lymph node prolonging murine skin allograft survival without detectable toxicity. Dane *et al.* [26] demonstrated that encapsulated tacrolimus and rapamycin inside micelles drain to the lymph nodes following intradermal injection and promote allograft survival in an allogeneic skin transplantation model. In order to improve the trafficking of nanoparticles to lymph nodes, Azzi *et al.* [28] designed microparticles containing tacrolimus coated with an anti-MECA-79 antibody for specific delivery to lymph node. Treatment using these microparticles achieved prolongation of heart allograft survival with low circulatory levels of tacrolimus. At present, to the best of our knowledge, none of these targeted IST delivery modalities to the lymphatic system have been investigated in VCA models.

Site-specific delivery of tacrolimus has also been investigated as a means to provide protective and regenerative benefits to neurons. Fibrin hydrogel reservoirs containing solubilized, particulate or PLGA microsphere-encapsulated tacrolimus could be utilized for enhancing peripheral nerve regeneration as shown by in-vitro dorsal root ganglion neurite extension assays [47]. The beneficial effect of locally delivered tacrolimus has recently been confirmed *in vivo*. A biodegradable and elastic matrix of poly(ester urethane) urea (PEUU)-loaded tacrolimus wrapped around the nerve injury was shown to decrease astrogliosis and increase axon growth signaling pathways, confirming the potential of site-specific delivery of tacrolimus to improve nerve repair while minimizing adverse side effects [48▪▪]. The beneficial effect of locally delivered tacrolimus on nerve regeneration may contribute to the process of neural repair after VCA. As functional recovery is one of the most important determinants of clinical success in VCA, more studies are warranted to investigate this intriguing possibility.

'Smart' materials that can respond to environmental stimuli such as biological signals, pathological abnormalities or exogenous signals are appealing therapeutic platforms for targeted IST delivery [63]. Considering that inflammation is a driving force for many chronic diseases, inflammation-responsive hydrogels represent an ideal candidate for such a material. It has been demonstrated that ascorbyl palmitate hydrogels loaded with dexamethasone have preferential adhesion to inflamed epithelial surfaces and result in a significant reduction of inflammation with lower drug serum concentrations in murine colitis models [49]. More recently, the same group reported the development of a triglycerol monostearate hydrogel loaded with triamcinolone acetonide, demonstrating inflammation-dependent disassembly and reduction of arthritis activity in a mouse model [50▪▪]. These reports confirm that inflammation-responsive hydrogels are promising next-generation DDS for the treatment of inflammatory diseases and transplant rejection.

TARGETED IMMUNOSUPPRESSION IN VASCULARIZED COMPOSITE ALLOTRANSPLANTATION

VCA is still a young field in transplantation. After an initial phase in which the experience gained in SOT was the driving force for improving outcomes in VCA patients, researchers have started to develop VCA-specific strategies to address specific problems presented by composite grafts. Immunosuppression obviously represents one of the most pressing issues in VCA [64]. Consequently, the field has started to develop approaches for targeted IST (Fig. 1) using the argument that these highly visible grafts allow easy monitoring of rejection episodes and provide the ideal setting for the use of site-specific immunosuppression [65].

Data generated in rodent models suggest that topical tacrolimus [66,67] and clobetasol [67], can prolong survival without systemic levels of immunosuppressive drugs. Tacrolimus and clobetasol ointments are already used clinically in episodes of acute rejection [6▪]. A topical formulation of MPA with high local but low systemic exposure in patients has recently been reported, further expanding the options for topical application of IST in VCA [68]. A drawback of using ointments and creams in the clinical setting is the twice-daily application that demands high patient compliance. Moreover, the skin penetration of ointments is limited and there are no commercially available topical formulations of widely used drugs such as rapamycin [10]. Therefore, although topical applications can be helpful in the immediate treatment of acute rejection episodes, their use as an alternative to systemic immunosuppression is unlikely.

Recently, our group reported that an intra-graft injection of high-dose tacrolimus may induce long-term survival with half of the treatment group of rats reaching 200 days' survival without signs of rejection [69]. Intra-graft tacrolimus application immediately after transplantation increased tissue drug availability, promoting the establishment of transient donor-cell chimerism and thus long-term graft acceptance. Recently, the beneficial effect of peri-transplant high-dose tacrolimus for VCA survival have also been reported in a swine model [70].

In an earlier work, our group reported the use of an innovative DDS to achieve long-term (>100 days) VCA survival with reduced systemic exposure

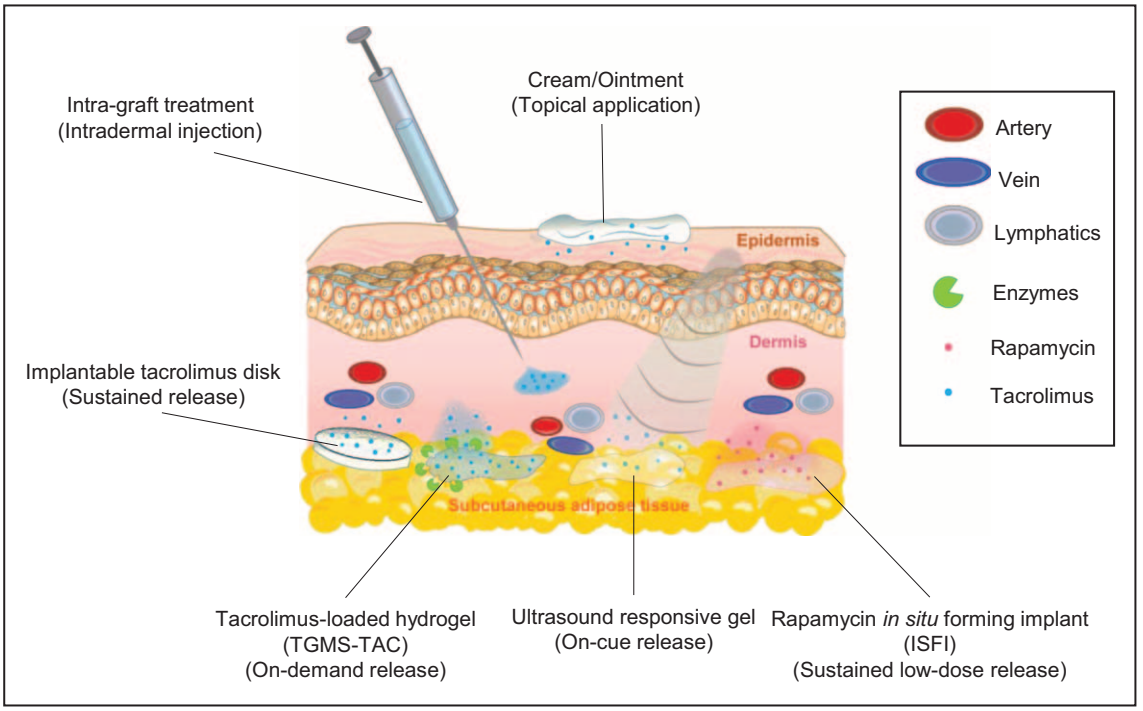

FIGURE 1. Targeted immunosuppression in vascularized composite allotransplantation. Drug delivery systems specifically developed for the control of VCA rejection. All the delivery systems described so far have the skin as the main target tissue. Targeting mechanisms are reported in the bracket. Topical application of tacrolimus cream and ointment has been reported for immediate, local release of the immunosuppressive drug. Intra-graft injection of tacrolimus in the peri-transplant period increased drug availability and improves graft survival. An implantable disk containing tacrolimus-loaded microparticles has been designed for sustained drug release into the graft and draining lymph nodes. Tacrolimus-containing hydrogels (TGMS-TAC) represent 'on-demand' delivery systems that can release the drug in response to inflammatory enzymes, indefinitely prolonging graft survival with marked reduction of systemic immunosuppression toxicity. Ultrasound responsive alginate-gels guarantee sustained baseline and 'on-demand' release of drugs upon ultrasound stimulation. Low-dose delivery of rapamycin for controlling rejection and inducing immunoregulatory mechanisms has been achieved using in-situ forming implants. Figure design by Catherine Tsai. VCA, vascularized composite allotransplantation.

in a rat model [71]. We demonstrated that triglycerol monostearate (TGMS), an agent generally recognized as well tolerated by the FDA, could self-assemble into hydrogels and disassemble in response to proteolytic enzymes that are over-expressed during inflammation, providing 'on-demand' drug release. Building upon this study, we recently investigated the long-term outcomes and the immunological and toxicological impacts of this approach [72▪▪]. Our data showed that periodic TGMS loaded with tacrolimus (TGMA-TAC) injections (every 70 days) promoted long-term graft survival up to 280 days. Systemic drug exposure was significantly reduced and TGMS-TAC-treated rats showed decreased toxicity compared with systemic tacrolimus-treated rats, the latter group showing increased creatinine, increased blood urea nitrogen levels, appearance of opportunistic infections and aggressive tumors. These results clearly show the advantages of our 'on-demand' release system for the reduction of immunosuppression toxicity. However, while providing better recipient outcomes, TGMS-TAC treatment resulted in inferior graft outcomes with TGMS-TAC-treated rats experiencing at least one rejection episode. Therefore, further studies are warranted to understand if these rejections are because of low intra-graft tacrolimus levels, and if they can be avoided by increasing frequency or dose of the 'on-demand' DDS.

The Pittsburg group recently developed a biodegradable disk containing tacrolimus-loaded microspheres for sustained regional immunosuppression in VCA [73▪▪]. As the disk slowly degrades, tacrolimus-laden microspheres are released to act in solution or are actively broken down and phagocytosed by macrophages. The published results showed sustained tacrolimus release from the disk with steady systemic levels and significant accumulation of tacrolimus in the groin lymph nodes. A single injection of the tacrolimus disk in hind limb-transplanted rats promoted allograft survival for more than 180 days. Moreover, as compared with TGMS-

TAC, burst release of tacrolimus was reduced in animals injected with the tacrolimus disk. However, long-term graft outcomes and the toxicity profile of this sustained-release DDS were not explored.

The same group has recently presented an abstract describing the development of a DDS drug that not only provides sustained IST release but also 'on-cue' triggered drug release upon ultrasound stimulation (USS) [74]. The study reported that alginate gels loaded with tacrolimus, rapamycin or both, released the immunosuppressive drug in response to USS. When used *in vivo* in a rat model of hind limb transplantation, alginate gels promoted long-term allograft survival (>100 days) in rats receiving tacrolimus-containing gels. However, although concentration in allograft tissues was higher than in the blood and the contralateral limb, sustained drug release occurred from alginate gels in the absence of ultrasound, prompting the authors to devote further efforts to optimizing the on-cue drug release.

The potential of locally delivered immunosuppression to promote VCA survival has also led us to investigate whether the administration of immunosuppressive drugs directly into the graft may reduce potential side effects as well as directly influence the magnitude and nature of an allogeneic immune response. We have designed an in-situ forming implant (ISFI) loaded with the immunoregulatory drug rapamycin [75]. A single injection of the rapamycin-loaded ISFI (Rapa-ISFI) in close proximity to the transplant prolonged VCA survival up to 100 days. Importantly, rats treated with Rapa-ISFI had significantly higher levels of multilineage chimerism and T_{reg} in peripheral blood and transplanted skin compared with untreated rats. This study demonstrates that targeted IST delivery can be used not only to promote less toxic immunosuppressive protocols and patient compliance but also to favor the reprogramming of the local response toward regulatory function.

LIMITATIONS AND FUTURE PERSPECTIVES

All these preclinical experiments confirm that site-specific immunosuppression is a feasible and promising approach in VCA. However, thus far the success of this therapeutic approach has been proven only in rodent models. Targeted IST therapies need to be validated in large animal models that generate solid preclinical data in order to substantiate the notion that targeted immunosuppression has real advantages in VCA. Evaluation of long-term graft and toxicity outcomes should be the main focus. Moreover, in order to determine the right dose of local immunosuppression needed, more efforts should be devoted to building a graft-specific 'therapeutic window' rather than relying on systemic drug levels.

On the other hand, multidrug immunosuppressive protocols are currently used in human patients to guarantee an effective level of immunosuppression. Therefore, localized immunosuppression should further evolve to include multiple drugs to control graft rejection. Eventually, combined use of minimized systemic immunosuppression and targeted immunosuppression might be envisaged to balance graft and toxicity outcomes.

Importantly, despite promising results, limitations associated with implantable DDS such as foreign body reaction, pro-inflammatory microenvironment promoted by the biomaterials, activation of the complement system and immunogenicity, particularly following consecutive implantations, should be carefully evaluated in specifically designed studies.

CONCLUSION

Targeted immunosuppression in VCA represents a promising new approach for improving patient compliance and graft survival while reducing off-target toxicity, intensity and frequency of acute rejection episodes and risk of chronic rejection. More studies are needed to generate solid preclinical data on the modalities of application, drug-distribution, toxicity profile and immunological parameters of DDS-based approaches. Such studies will foster the development of effective delivery platforms, paving the way for the design of clinical trials in VCA patients.

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Conflicts of interest

There are no conflicts of interest.

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