Review

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Tacrolimus-loaded Drug Delivery Systems in Vascularized Composite Allotransplantation: Lessons and Opportunities for Local Immunosuppression

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Abstract. Long-term systemic immunosuppression is needed for vascularized composite allotransplantation (VCA). The high rate of acute rejection episodes in the first posttransplant year, the development of chronic rejection, and the adverse effects that come along with this treatment, currently prevent a wider clinical application of VCA. Opportunistic infections and metabolic disturbances are among the most observed side effects in VCA recipients. To overcome these challenges, local immunosuppression using biomaterial-based drug delivery systems (DDS) have been developed. The aim of these systems is to provide high local concentrations of immunosuppressive drugs while reducing their systemic load. This review provides a summary of recently investigated local DDS with different mechanisms of action such as on-demand, ultrasound-sensitive, or continuous drug delivery. In preclinical models, ranging from rodent to porcine and nonhuman primate models, this approach has been shown to reduce systemic tacrolimus (TAC) load and adverse effects, while prolonging graft survival. Localized immunosuppression using biomaterial-based DDS represents an encouraging approach to enhance graft survival and reduce toxic side effects of immunosuppressive drugs in VCA patients. Preclinical models using TAC-releasing DDS have demonstrated high local immunosuppressive effects with a low systemic burden. However, to reduce acute rejection events in translational animal models or in the clinical reality, the use of additional low-dose systemic TAC treatment may be envisaged. Patients may benefit through efficient graft immunosuppression and survival with negligible systemic adverse effects, resulting in better compliance and quality of life.

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INTRODUCTION

The first vascularized composite allotransplantation (VCA) was attempted by Gilbert and his team in 1964. They transplanted a hand onto a unilateral amputee. However, the lack of efficient postoperative immunosuppressive therapies at this time failed to prevent acute rejection, and the graft needed to be amputated 3 wk after transplantation.[1] The 1980s and 1990s experienced a surge of discoveries of new immunosuppressive agents such as the calcineurin inhibitors cyclosporine and tacrolimus (TAC) or the inosine monophosphate dehydrogenase inhibitor mycophenolate mofetil (MMF). These potent immunosuppressive drugs transformed the field of solid organ transplantation (SOT) and showed in both small- and large-animal models that VCA might be doable in humans.[2-4] Thanks to these discoveries, the first successful hand transplantation could be performed by Dubernard et al[5] in 1999.[6] The clinical success of VCA was, therefore, clearly linked to the availability of potent immunosuppressive drugs.

As a young medical field, compared with SOT, with only limited numbers of clinical transplantations performed until today, VCA largely relies on immunosuppressive strategies developed for solid organ grafts. However, chronic systemic immunosuppression using standard maintenance triple therapy including corticosteroids, TAC, and MMF—as used clinically today—increases the

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long-term risks of metabolic, infectious, vascular, and malignant complications.[7] Long-term immunosuppression is among the most mentioned ethical concerns in VCA. The risks and burdens that are associated with it are major obstacles to a wider clinical application of VCA.[8-10]

To date, around 107 upper extremity and 48 face transplantations have been performed worldwide.[11,12] For comparison, in 2021, a record number of 25 487 kidneys have been transplanted in the United States only.[13] This difference shows the limited possibilities of extensive clinical research in VCA. The early research needed to focus on feasibility and surgical techniques. However, research funding in the area of VCA is episodic, no stable funding sources have been established until today.[9] Due to restricted data availability, it is difficult to compare and interpret outcomes from different patient groups. Thus, no specific guidelines for evaluation of complications, rejection episodes, and comparison to alternatives such as prosthetic fitting or extensive reconstructive surgery have been validated.[14] The same applies to postoperative immunosuppressive therapies, which are not customized for VCA but derived from SOT protocols.[15]

The International Registry on Hand and Composite Tissue Allotransplantation (IRHCTT) so far lists 81 upper limb and 39 face transplantations.[16] In 2022, the IRHCTT reported that 88% of upper extremity transplant recipients experienced at least 1 episode of acute rejection in the first year posttransplantation despite a triple immunosuppressive therapy regimen.[16] For reference, current data from the U.S. Scientific Registry of Transplant Recipients show an acute rejection rate of 5%–10% in the first year posttransplantation for kidney, 18%–32% for heart, 8%–20% for liver, and 10%–17% for lung transplantation.[13,17-19] Of the 66 upper extremity transplantations listed in the 2017 IRHCTT report, graft survival was 90.4% 1 y and 86.6% 5 and 10 y after transplantation.[7] Despite the considerably higher number of acute rejection episodes occurring during the first postoperative year, VCAs have much better 5- and 10-y graft survival than kidneys (76% and 62%, respectively, for live and deceased donors[20]), livers (67% and 54%[21]), and lungs (54% and 32% patient survival[22]). Early VCA graft losses in the first 1.5 mo occurred due to nonfunction of the graft, ischemia, sepsis, or bacterial graft infection. The following complications were reported in the first year after upper extremity transplantation[7]: 32% developed bacterial, 12% cytomegalovirus, 6% herpes simplex, 2% herpes zoster, and 12% fungal infections. Metabolic complications comprise hyperglycemia with a rate of 42% in the first posttransplant year that regressed in 21% of the cases. Neoplastic malignancies were reported in 2% of the patients. Overall, the rate of acute rejection episodes and the adverse effects that come along with VCA decrease the risk-to-benefit ratio of this—life-enhancing rather than life-saving—procedure.

Long-term immunosuppression after VCA requires higher systemic drug concentrations than, for example, kidney transplantations, resulting in toxic side effects. Recently, Rifkin et al[23] conducted a systemic literature review in which they compared VCA (upper extremity, face) and SOT (kidney) immunosuppressive therapy regimens (TAC, MMF, prednisone). Their dataset consisted of 57 VCA and 98 kidney recipients. This study showed that the enrolled VCA and kidney recipients received similar prednisone and MMF doses. However, the long-term TAC target trough levels were significantly higher in VCA recipients.[23]

To counteract the side effects that come along with lifelong immunosuppressive treatment, several approaches have been developed to increase efficacy and reduce the toxic effects of immunosuppressive drugs. In 1951, Billingham et al[24] described one of the first possibilities that local administration of a drug might prolong allograft survival. Topical application of a cortisone suspension in a rabbit skin allograft model was shown to prolong graft survival to more than twice that of systemically applied cortisone. Like skin, the eyes and lungs are optimally accessible for local delivery of immunosuppression. Topical corticosteroids are the gold standard for preventing graft rejection in corneal transplantation.[25] In pneumology, inhalation corticosteroids are well-known, site-specific drugs for the treatment of chronic inflammatory lung diseases such as bronchial asthma with significant local but limited systemic effects. For lung transplantation, inhalation of calcineurin inhibitors has been developed and investigated for >20 y.[26] Due to their anatomical location, most types of VCA grafts offer the opportunity for topical or subcutaneous immunosuppression. Preclinical studies have proven the efficacy of topical TAC application in the treatment of acute rejection by delivering high local TAC concentrations. The benefit of topical TAC application is the reduction of side effects due to reduced systemic exposure.[27,28] However, without simultaneous systemic immunosuppression, graft survival was not prolonged.[27] Other approaches include drug delivery systems (DDS) using biodegradable substances that enhance control over drug release and bioavailability.[29] In contrast to SOT, most VCA offer the possibility of local immunosuppressive drug delivery, making them optimal targets for such systems. In this review, we describe recently developed and investigated TAC-loaded, biomaterial-based DDS for local immunosuppression.

Tacrolimus

TAC (FK506) is the main immunosuppressive drug used in posttransplantation treatment to prevent and treat acute rejection in SOT or VCA.[28] Usually, it is used in a triple therapy regimen together with MMF and prednisone.[23,28] TAC is an immunosuppressant that acts like cyclosporine but is 10–100 times more potent. It inhibits the cytoplasmic phosphatase calcineurin, which leads to the activation of nuclear factor of activated T cells (NF-AT). NF-AT is involved in the synthesis of interleukin-2 by activated T cells, which is crucial for T-cell survival. The half-life of TAC in humans is about 9–12 h if given intravenously. It is mainly metabolized by P450 enzymes in the liver; thus, there may be a risk of interactions with other medications.[30] The oral bioavailability of TAC is around 25% due to hepatic and intestinal metabolism, and it binds extensively to red blood cells.[31] The therapeutic levels range from 5 to 20 ng/mL for liver, heart, or kidney transplantation.[32-34]

Chronic systemic use of TAC leads to adverse effects that include nephrotoxicity, metabolic disturbances—such as diabetes—opportunistic infections, and malignancy.[7] TAC-induced nephrotoxicity is proposed to be caused in different ways. It has been demonstrated that TAC induces kidney damage on one hand by upregulation of nicotinamide adenine dinucleotide phosphate

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oxidases that increase production of reactive oxygen species (ROS) and on the other hand by downregulation of antioxidant defense mechanisms that remove ROS.[35] It has also been shown that increased production of ROS takes place in glomerular endothelial cells leading to endothelial dysfunction and glomerular injury.[36] Another study suggested that TAC has a direct effect on proximal tubular epithelial cells by promoting their apoptosis and enforcing the apoptotic effect of the nitric oxygen oxidase.[37] Several studies showed a nephroprotective effect of renin-angiotensin-aldosterone system inhibitors, such as angiotensin II type 1 or angiotensin-converting enzyme inhibitors, when used together with TAC.[36,38] Their anti-nephrotoxic effect is proposed to be based on the inhibition of nicotinamide adenine dinucleotide phosphate oxidases and thus reduced ROS production.[36,38]

Treatment with high doses of TAC is known to have a role in a higher incidence of posttransplant diabetes mellitus (PTDM) than cyclosporine.[39] Thanks to adjustments and refinements of the immunosuppressive therapy, PTDM incidence could be reduced in kidney recipients.[40,41] It decreased from 10% in 2007 to <4% in 2016 in the first year after transplantation.[42] The 5-y PTDM incidence decreased from 12% in 2005 to roughly 5% in 2012.[40] TACs effect on the formation of PTDM is based on its interaction with pancreatic targets. The phosphatase calcineurin is expressed in pancreatic β cells that secrete insulin.[43] It has 2 molecular targets in the pancreas: the cAMP-responsive element binding protein and the NF-AT family of transcription factors.[43] These factors are activated during hyperglycemia and promote gene transcription of insulin and expression of specific factors to maintain β-cell mass and function.[43,44] Thus, inhibition of calcineurin by TAC leads to reduced insulin expression and secretion favoring a diabetogenic metabolic state. As VCA recipients need higher doses of TAC than kidney recipients in the long term,[23] the risk of PTDM development is higher.

BIOMATERIAL-BASED DRUG DELIVERY SYSTEMS FOR LOCAL IMMUNOSUPPRESSION IN VCA

A summary of the TAC-encapsulated DDS discussed below is provided in Table 1.

Hydrogels

Hydrogels are matrix-like structures formed by low-molecular-weight amphiphilic molecules that self-assemble in water by weak noncovalent intermolecular interactions and encapsulate hydrophobic drugs.[45,55] The hydrogel-encapsulated drugs can then be released progressively by different triggers such as ROS or enzymes.[55] In 2014, Gajanayake et al[45] developed an enzyme-responsive hydrogel from amphiphilic components recognized as safe by the U.S. Food and Drug Administration (FDA). They showed that self-assembling triglycerol monostearate (TGMS) could encapsulate relevant doses of TAC and be degraded by proteolytic enzymes present during inflammatory conditions, leading to release of TAC (Figure 1). In a Brown Norway-to-Lewis rat hindlimb VCA model, a single, subcutaneous injection of 7 mg of the TAC-encapsulated TGMS hydrogel (TGMS-TAC) could prolong graft survival to >100 d compared with 33.5 d in local graft injection of free TAC and 11 d with a single injection of TGMS hydrogel alone. Systemic levels of TAC were shown to have a significantly lower peak after administration as compared with nonencapsulated TAC but remained detectable for a longer time in the TGMS-TAC group as compared with the

TABLE 1.

TAC-loaded drug delivery systems in VCA

Study	Model	Biomaterial	Drug system application	Mechanism of drug delivery
Hydrogels				
Gajanayake et al[45]	Brown Norway-to-Lewis rat hindlimb	TGMS hydrogel	Subcutaneous	On-demand, enzyme responsive
Dzhonova et al[46]	Brown Norway-to-Lewis rat hindlimb	TGMS hydrogel	Subcutaneous	On-demand, enzyme responsive
Fries et al[47]	Porcine hindlimb	TGMS hydrogel	Subcutaneous	On-demand, enzyme responsive
Feturi et al[48]	Brown Norway-to-Lewis rat hindlimb	Alginate hydrogel	Subcutaneous	On-demand, ultrasound responsive
Lin et al[49]	In vitro and Lewis rats	Mixed hydrogel	Subcutaneous	Continuous release
Wu et al[50]	Brown Norway-to-Lewis rat skin	PEG-polyester hydrogel	Subcutaneous	Continuous release
Nanoparticles				
Gama et al[51]	Nonhuman primates, fasciocutaneous flaps	Tyrosine-derived triblock copolymer nanoparticles	Topical	Continuous release
Lellouch et al[52]	Nonhuman primates, partial face VCA	Tyrosine-derived triblock copolymer nanoparticles	Subcutaneous	Continuous release
Disks				
Unadkat et al[53]	Brown Norway-to-Lewis rat hindlimb	PLGA-PLLA disk	Subcutaneous	Continuous release
Feturi et al[54]	Brown Norway-to-Lewis rat hindlimb	PCL disk	Subcutaneous	Continuous release

PCL, polycaprolactone; PEG, polyethylene glycol; PLGA, poly(lactic-co-glycolic acid); PLLA, poly(L-lactic acid); TAC, tacrolimus; TGMS, triglycerol monostearate; VCA, vascularized composite allotransplantation.

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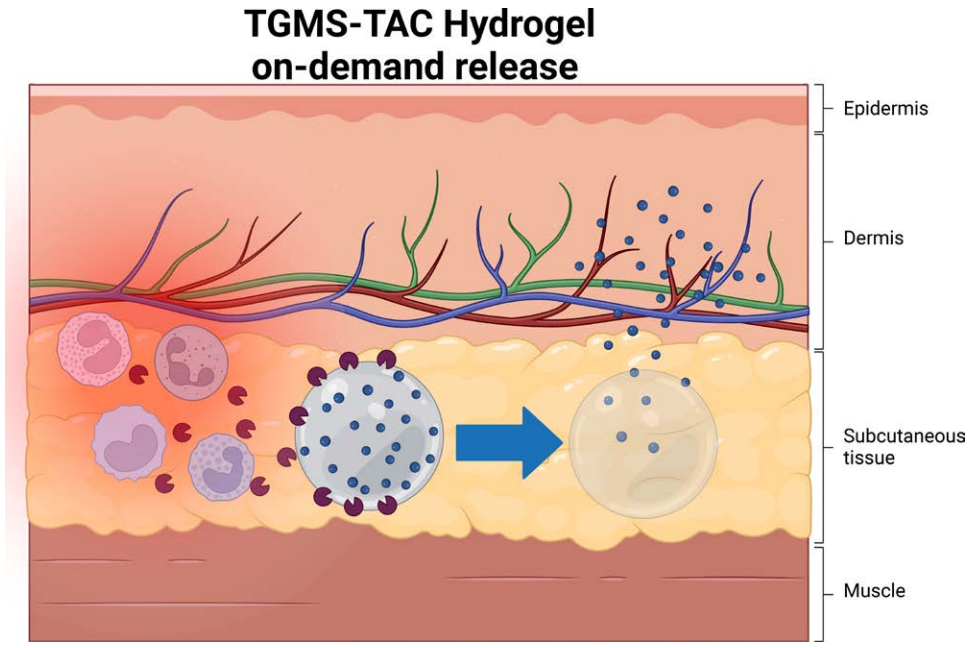

FIGURE 1. Mechanism of action of TGMS-TAC encapsulated hydrogel. Development of rejection leads to recruitment of pro-inflammatory immune cells that secrete enzymes. These enzymes degrade the hydrogel leading to the release of TAC for local immunosuppression. Created with BioRender.com. TAC, tacrolimus; TGMS, triglycerol monostearate.

group that received a local injection of free TAC. A similar trend was seen in local graft concentrations of TAC.[45]

Building on these findings, Dzhonova et al[46] investigated the long-term outcome and immunological and toxicological impacts of this strategy in the same VCA model. Both groups—daily systemic injections of TAC (1 mg/kg) and subcutaneous intragraft injections of 7 mg TGMS-TAC every 70 d—showed graft survival of up to 280 d. Most graft recipients treated with TGMS-TAC experienced rejection episodes graded between 1 and 3 starting from postoperative day (POD) 149. Occasionally, grade 1 rejection resolved in around a month's time but reappeared a few months later. Two of 6 animals of the TGMS-TAC group did not show signs of rejection. Histopathological analysis of graft tissue revealed that all observed signs of rejection were restricted to skin. Graft muscle tissue did not show signs of rejection. The 6 animals that got systemic TAC treatment did not show any signs of rejection, neither macroscopically nor microscopically. Systemic TAC concentrations in the TGMS-TAC group were significantly lower compared with the animals that got daily TAC injections. The lower systemic TAC levels of TGMS-TAC treated rats resulted in preserved kidney and hematological parameters as compared with systemic TAC therapy. Moreover, of the systemically treated graft recipients, one developed an infected pseudocyst with commensal skin bacteria, and another one got an aggressive lymphoma. TGMS-TAC-treated animals did not show development of any opportunistic infection or malignancy.[46]

To confirm that the TGMS-TAC DDS is enzyme responsive in vivo, and thus acts in an on-demand manner, Dzhonova et al[56] conducted a further study using naive Lewis rats. Two groups were formed with both receiving 4 depots of TGMS-TAC subcutaneously. While the first group served as control, the second group received an inflammatory challenge on POD 7 through subcutaneous injection of 100 µg lipopolysaccharide (LPS) near the TGMS-TAC depot. Systemic TAC levels peaked in the TGMS-TAC group and stayed >20 ng/mL until POD 4. TAC levels remained detectable until POD 50. In the TGMS-TAC-LPS group, TAC levels were similar for the first 7 d. After LPS challenge on POD 7, blood TAC values significantly increased to 18.7 ± 3.3 ng/mL on POD 7 and 24.4 ± 3.5 ng/mL on POD 12. The further development of TAC blood levels followed the same pattern of decrease as in the group without LPS but with slightly higher TAC concentrations. In total, more TAC was released in the TGMS-TAC-LPS group as compared with the TGMS-TAC group with 342.1 ± 13.3 ng/mL and 262.7 ± 14.6 ng/mL, respectively. Local tissue analysis also revealed significantly higher TAC levels in skin and muscle of LPS-treated animals as compared with the control group. This study confirmed that TAC is released upon inflammatory stimulus from the TGMS-TAC hydrogel.[56]

Fries et al[47] applied the same DDS in an orthotopic porcine forelimb VCA model. To evaluate the impact of TGMS-TAC, they compared single injections of low-dose (49 mg) and high-dose (91 mg) TAC. The low-dose group achieved significantly longer survival (between POD 56–93) as compared with the high-dose group, in which the animals failed to thrive and needed to be euthanized early between POD 24 and 42. Necropsy revealed that these pigs suffered from pancreatitis. The control group that received no treatment reached POD 6–7 until grade 4 graft rejection. Local levels of TAC in graft tissue were 100–1000-fold higher than systemic levels. Systemic levels of TAC were similar in the low dose and in the high-dose group with a similar concentration pattern over time. TAC concentration reached a first peak (30–40 ng/mL) at POD 1 and a second peak (10–20 ng/mL) at POD 7–10. The initial peak coincided with inflammation due to surgery and ischemia/reperfusion injury. The second peak corresponded with acute rejection. The first signs of rejection were detected on POD 4 in the low dose group where 2

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of the 4 grafts developed grade 1 rejection. The 2 other pigs had first signs of rejection at POD 7, graded at level 2. During the experiment, the rejection grade oscillated between 1 and 2 in 2 pigs and progressed to grade 4 on POD 70 and 72, respectively. The other 2 pigs reached the defined endpoint of the experiment without progressing to grade 4 rejection. In the high-dose group, acute rejection started at the same time but was more severe with grade 2 and 3 rejection in 2 of 3 pigs.[47]

Using another hydrogel DDS, Feturi et al[48] described an injectable, reloadable drug eluting ultrasound responsive hydrogel made from alginate gels in a Brown Norway-to-Lewis rat hindlimb VCA model (Figure 2). The animals received a single dose of the gel subcutaneously, containing either 10 mg TAC, 10 mg rapamycin or 10 mg TAC + 10 mg rapamycin. Long-term allograft survival of >100 d was observed for gels containing TAC and TAC + rapamycin. Limbs containing gels with rapamycin only developed grade 3 rejection on POD 21. In absence of ultrasound, the gels showed a sustained release of the drugs within the therapeutic range. Upon ultrasound stimulus, drug release was triggered, leading to increased drug levels. The drug concentration was significantly higher in the graft than in blood or the contralateral limb. No significant systemic adverse effects were observed.[48]

In a more recent study, Lin et al[49] took another approach and developed a mixed hydrogel system based on polypeptide copolymers for continuous local delivery of TAC together with a fast-degrading hydrogel to enhance TAC release rate. Release rate was first studied in vitro and showed no initial burst release and sustained TAC release that correlated with the degradation of the hydrogel. Subsequent in vivo investigations were done in male Lewis rats that received 1 mL TAC-loaded (10 mg/mL) mixed hydrogel subcutaneously. An observation period of 28 d showed that plasma concentrations of TAC were stable at around 10 ng/mL. In skin allotransplantation, a single administration of the mixed hydrogel could prevent rejection for at least 3 wk.[49] However, the depot persisted for >3 mo, which raised concerns among the authors about possible fibrosis formation at the injection site.[50]

To address this issue, the authors recently investigated a TAC-loaded hydrogel made of polyethylene glycol (PEG)-polyester (Figure 3).[50] Initial in vitro testing showed a complete degradation of the hydrogel over a 30-d period without initial burst release and a steady release of TAC. Afterward, in vivo investigation using a Brown Norway-to-Lewis rat skin allotransplantation model was done. One mL of hydrogel was loaded with TAC and injected subcutaneously near the allograft. The treated animals showed a significant prolongation of graft survival as compared with nontreated controls, with a median survival time of 19.5 d and 9 d, respectively. Two of 6 treated animals showed no signs of rejection with intact skin tissue until the endpoint at POD 30. However, 4 animals suffered from premature rejection with histological evidence of epidermolysis, hair loss, and necrosis of the epidermis. Both upper and deep dermis showed signs of severe inflammation. Systemic levels of TAC were between 25 and 42 ng/mL and thus higher than

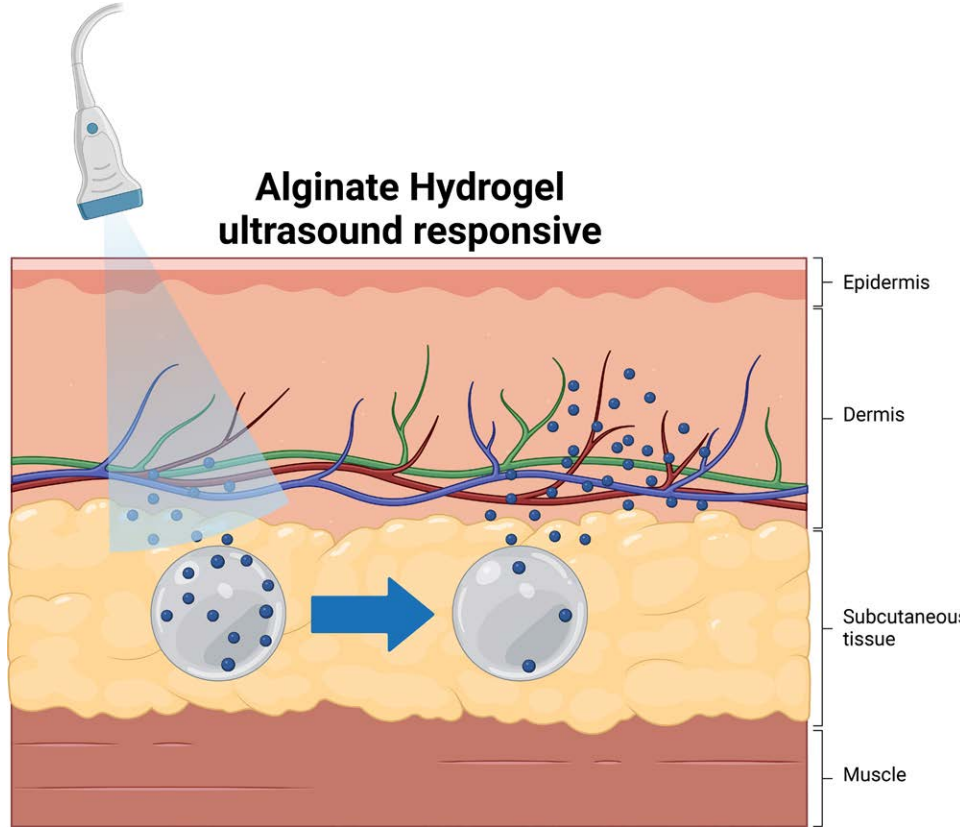

FIGURE 2. Mechanism of action of TAC-loaded ultrasound responsive hydrogel. While having continuous TAC release without stimulation in the therapeutic range, on-demand release of additional TAC can be achieved by ultrasound stimulation. Created with BioRender.com. TAC, tacrolimus.

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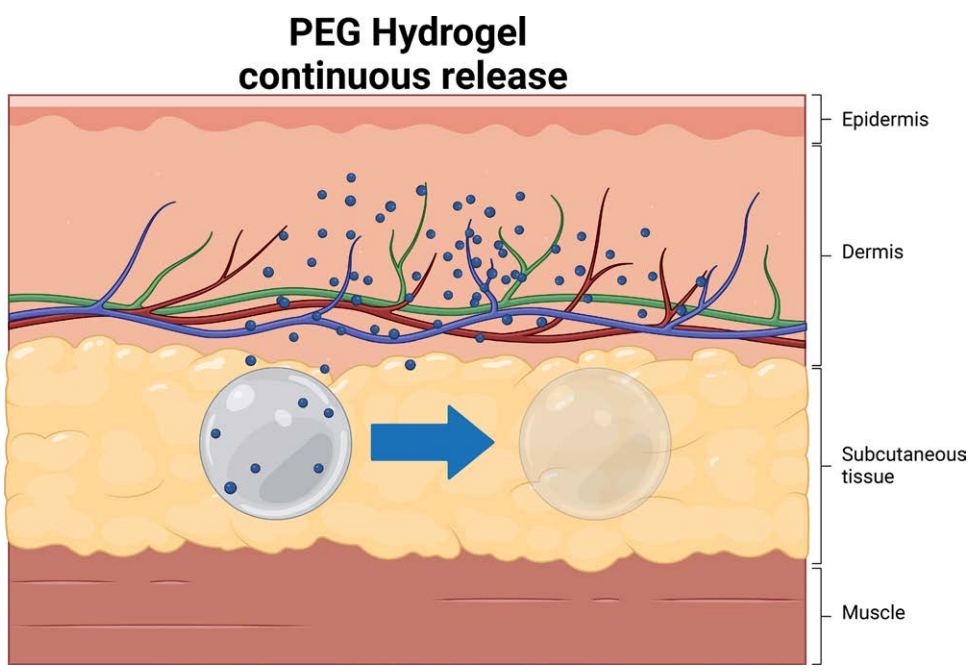

FIGURE 3. Mechanism of action of TAC-loaded PEG hydrogel. The subcutaneous implantation of the hydrogel leads to continuous TAC delivery directly into the dermal skin layer for local immunosuppression. Created with BioRender.com. PEG, polyethylene glycol; TAC, tacrolimus.

the usual therapeutic range of 5–20 ng/mL. Besides that, this DDS showed no burst release of TAC in vivo, and the hydrogel was degraded to a large extent during the time of observation.[50]

In summary, TGMS-TAC was able to effectively prolong graft survival in rat and porcine VCA models with low systemic levels, leading to lower TAC-associated side effects than systemic TAC. Ultrasound-responsive hydrogel has the particularity to increase drug release upon ultrasound stimulus while having a continuous release without external stimulus. Polypeptide copolymer-based hydrogels showed stable plasma concentrations of TAC but raised concerns about the potential for fibrosis at the injection site of the hydrogel. Addressing this issue, PEG-polyester hydrogel was mostly degraded at the end of observation time, significantly prolonged graft survival but resulted in supratherapeutic systemic levels of TAC. Overall, the discussed studies highlight the potential of hydrogel-based DDS in VCA, offering controlled and localized drug release effectively prolonging graft survival.

Nanoparticles

Another TAC-DDS is based on FDA-approved nanoparticles made from tyrosine-derived triblock copolymers, loaded in a water-soluble film.[57,58] Like TGMS, this biodegradable and bioresorbable polymer can self-assemble and encapsulate hydrophobic molecules such as TAC. It can be implanted subcutaneously for local immunosuppression.[58] Gama et al[51] investigated a topical formulation of the TAC-encapsulated nanoparticles in a VCA model with nonhuman primates. However, using this application form, graft survival could not be prolonged and acute rejection could not be prevented.[51] The group optimized the design of the DDS into subcutaneously implantable disks containing the TAC-loaded nanoparticles to provide long-term localized immunosuppression and graft survival (Figure 4).[58] They then investigated the effect of TAC-loaded implants on acute rejection in a nonhuman primate VCA model in combination with standard induction and triple immunosuppressive maintenance therapy.[52] Adult male cynomolgus monkeys underwent heterotopic, partial-face VCA. TAC-loaded circular implants with a diameter of 4 mm were placed subcutaneously along the graft suture line. In total 24 implants were used, totaling 3 cm^2, with the aim of providing therapeutic doses of TAC in a controlled manner over the first 7–10 d to prevent acute rejection. Further intramuscular TAC was supplemented when TAC levels dropped. Early acute rejection could be prevented in all animals. One recipient showed signs of rejection on POD 12 that was treated by intravenous administration of methylprednisolone. Tissue biopsy analysis showed no signs of rejection at POD 30 and 60. Systemic TAC levels reached values between 60 and 112 ng/mL at POD 3 and stabilized at around POD 30 at values of 20–40 ng/mL. The animals developed adverse effects such as diabetes.[52]

In brief, subcutaneously placed, nanoparticle-based DDS loaded with TAC have successfully prevented early rejection and prolonged graft survival in a nonhuman primate VCA model. However, systemic TAC concentrations reached high levels, leading to TAC-associated adverse effects.

Disks

The third TAC-DDS that has been investigated is a TAC-loaded biodegradable disk made of double-walled polymer microspheres using poly(lactic-co-glycolic acid) (PLGA) and poly(L-lactic acid) (Figure 5). Unadkat et al[53] investigated the impact of the TAC-loaded disks in a Brown Norway-to-Lewis rat hindlimb VCA model. The authors implanted one disk that contained 40 mg TAC either subcutaneously in the ipsilateral transplanted allograft or in

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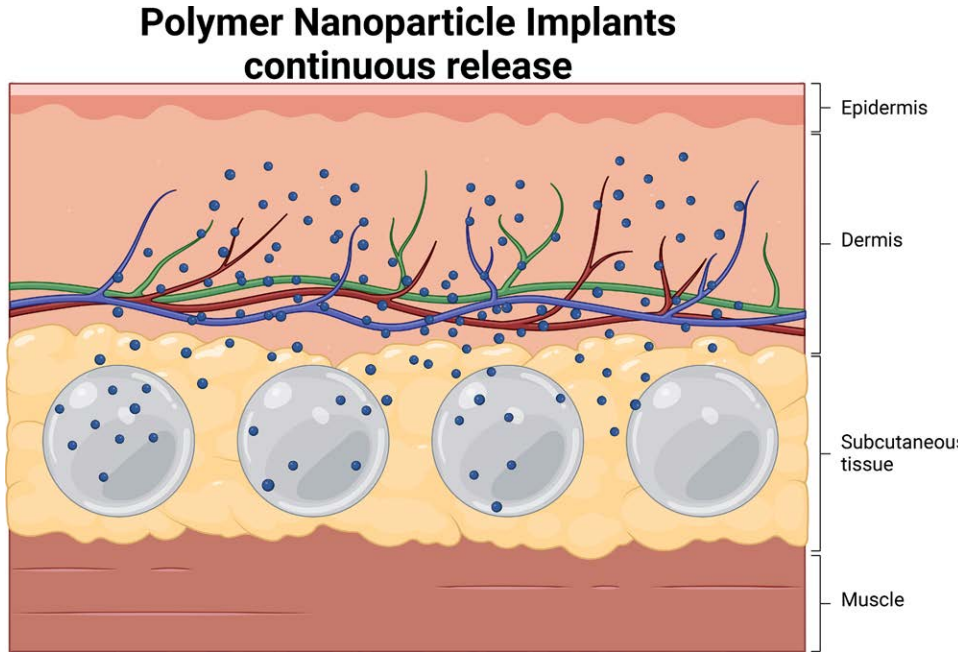

FIGURE 4. Mechanism of action of TAC-loaded polymer nanoparticle implants. The subcutaneous implants deliver TAC in a continuous manner directly into the dermal skin layer for local immunosuppression. Created with BioRender.com. TAC, tacrolimus.

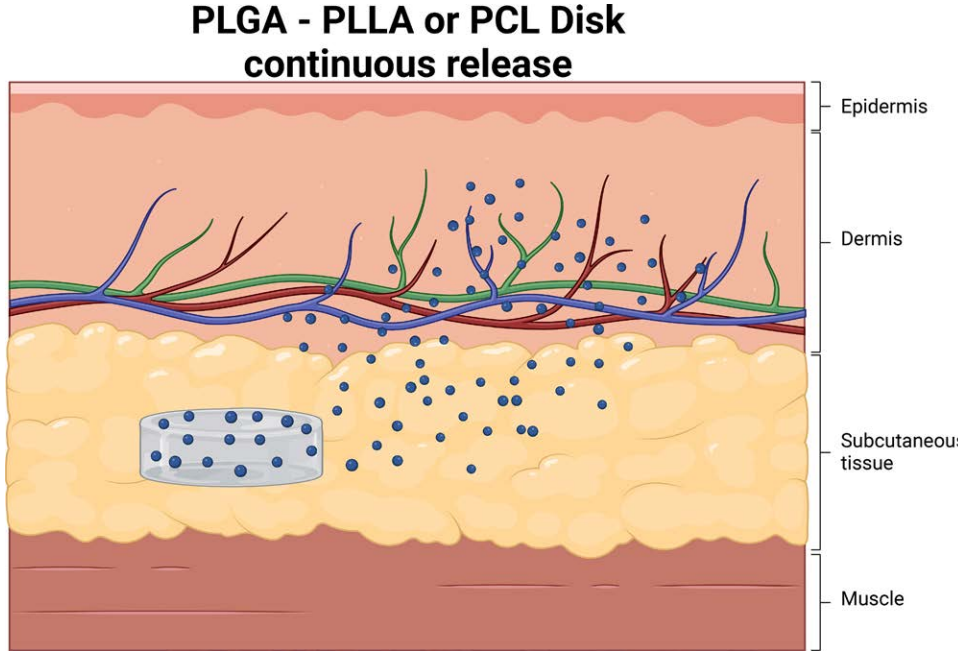

FIGURE 5. Mechanism of action of TAC-loaded PLGA-PLLA and PCL disks. The subcutaneous implantation of the disks leads to continuous TAC delivery directly into the dermal skin layer for local immunosuppression. Created with BioRender.com. PCL, polycaprolactone; PLGA, poly(lactic-co-glycolic acid); PLLA, poly(L-lactic acid); TAC, tacrolimus.

the contralateral nontransplanted limb and compared it to nontreated animals. The treated rats received antilymphocyte serum during transplantation. Animals that got the disk in the nontransplanted limb rejected the allograft after around 154 d. In contrast, a long-term 100% graft survival for >180 d was observed in the group where the disk was implanted in the allograft. Nontreated animals rejected their allograft acutely after 6 d. Burst release of TAC on POD 1 led to systemic blood concentration of 29–33 ng/mL in both the contralateral and the ipsilateral groups. In both groups, the implanted TAC-releasing disk subsequently maintained systemic TAC levels between 5 and 10 ng/mL from POD 11 to POD 142. After POD 142, TAC concentrations were <5 ng/mL. Local TAC concentrations in the TAC-disk draining lymph nodes were multifold higher as compared with systemic concentrations, 12 766.05 ± 566.6 ng (n = 2) per gram of lymphatic tissue for the group that had the disk implanted ipsilaterally and 921.45 ± 747.48 ng/g (n = 2; $P = 0.226$) for the second group. In lymph nodes without TAC-eluting

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disks, there were 17.75 ± 5.16 ng of TAC per gram and 29.75 ± 12.09 ng/g of lymphatic tissue, respectively ($P = 0.505$). TAC levels were also measured in muscle tissue where the TAC-releasing disk was implanted. The TAC concentration in the group that had the disk in the allograft was 139.8 ± 52.18 ng/g of muscle tissue versus 62.2 ± 11.03 ng/g in the group that had the disk in the contralateral limb (n = 2; $P = 0.228$). Adverse effects were not reported.[53]

Due to the initial burst release with high blood levels of TAC, the same group recently reported on a TAC-loaded biomaterial made of polycaprolactone (PCL) (Figure 5).[54] PCL is an FDA-approved material and is used in multiple implantable medical devices.[59] The advantages are lower initial burst and long-term systemic TAC exposure while also being placed locally in the allograft. The group investigated the effect of TAC-loaded disks in a Brown Norway-to-Lewis rat hindlimb VCA model. A disk loaded with 5 mg TAC was implanted either in the allograft or in the nontransplanted contralateral limb. The effects were compared with animals that got no treatment or daily systemic TAC administration (total of 45 mg TAC during 150 d for a 300 g rat). Single-implanted TAC-loaded disk achieved long-term graft survival for >200 d for the intragraft implanted disk group, as did the systemic TAC-treatment. Survival was significantly shorter in rats that had the disk implanted in the nontransplanted limb with rejection after 70 d. In comparison, untreated animals had a median graft survival of 8 d. Burst release was observed at POD 2 with TAC levels of 15 ± 7.6, 18.3 ± 6.6 ng/mL for intragraft disk and contralateral disk implanted animals, respectively. By POD 14, the values dropped to 8.5 ± 2.1 and 9.9 ± 5.3 ng/mL, respectively. For the animals that had the TAC-releasing disk implanted in the contralateral limbs, the systemic TAC levels quickly dropped and reached a steady state between 2 and 5 ng/mL until endpoint. The same systemic TAC levels were observed for the group that had the TAC-releasing disk implanted in the allograft until POD 105, after which TAC-levels dropped <2 ng/mL. Animals that were treated with systemic TAC had average blood concentrations of 5–15 ng/mL. Locoregional TAC concentrations in skin, muscle, and draining lymph node were significantly higher (concentration in lymph node > muscle > skin) in the animals with the intragraft implanted disk compared with the systemic TAC treated group, and the group that had the disk implanted in the nontransplanted limb. No signs of graft rejection were observed macroscopically and microscopically in the animals with ipsilateral implantation of the TAC-releasing PCL disk. The transplanted animals that received the TAC-eluting disk did not show significant changes in kidney function, which was similar to untreated animals. Moreover, no hyperglycemia or diabetes was observed. In contrast, animals that received systemic TAC showed signs of nephrotoxicity and had significantly higher blood glucose levels between POD 60 and 120.[54]

Essentially, investigation of subcutaneously implanted, TAC-releasing PLGA-disks resulted in prolonged graft survival of up to 180 d in a rat VCA model. TAC levels were locoregionally high and accumulation of TAC in lymph nodes near the graft could be observed. However, a distinct burst release of TAC was noted. To address this issue, a formulation using PCL disks was developed. Long-term graft survival for >200 d was achieved without signs of graft rejection nor adverse effects. Burst release using this formula could be attenuated.

DISCUSSION

Wider application of VCA in a clinical setting is mainly hampered by the high incidence of acute rejection episodes and the adverse effects that are associated with the currently used long-term immunosuppression therapy. As VCA generally is a life-enhancing but not life-saving procedure, the risk-to-benefit ratio of the transplantation itself and the postoperative immunosuppressive therapy must be carefully analyzed. TAC is one of the standard immunosuppressive agents used in SOT as well as in VCA. For systemic application, it has a narrow therapeutic range from 5 to 20 ng/mL, requiring close monitoring. Adverse effects such as nephro- and pancreatic toxicity, development of PTDM, opportunistic infections, and malignancies are associated with long-term TAC immunosuppression.

As compared with SOT, most types of VCA contain externalized skin, which enables macroscopic visual monitoring of graft rejection. The easily accessible skin pad also enables direct and local application of immunosuppressive agents. Local injection of free TAC was successful in a rodent model.[60] However, as these animals had only a single major histocompatibility complex mismatch, these results may not be applicable in a clinical setting due to a higher number of major histocompatibility complex mismatches in humans. DDS for local immunosuppression represent a chance for VCA as they could make great contribution to the reduction of risks and burdens that come along with systemic immunosuppression. Acute rejection is frequent in upper extremity transplant recipients. Nearly 88% of these patients experience at least 1 episode of acute rejection in the first posttransplant year.[16] Different therapeutic strategies are currently applied with intravenous steroids and adjustment of the immunosuppressive therapy being the most common to treat acute rejection.[7]

Investigations performed in the past decade have resulted in the promising development of TAC-delivering DDS based on biomaterials for local, intragraft immunosuppression. Various biomaterials have been used such as hydrogels (TGMS, mixed hydrogel, alginate, and PEG-polyester), nanoparticles, or disks (PLGA-poly(l-lactic acid) and PCL). The use of these DDS led to locoregionally high, but systemically low levels of TAC in rodent, porcine, and nonhuman primate models. Significantly longer graft survival could be achieved when compared with injection of free TAC directly into the graft. However, as shown in the studies of Dzhonova et al[46] (TGMS-TAC), Fries et al[47] (TGMS-TAC), Wu et al[50] (PEG-polyester), and Lellouch et al[52] (nanoparticle-based disks), signs of rejection were observed after a certain time despite local immunosuppression. Only in the study of Feturi et al,[54] using TAC-eluting PCL disks, apparently no acute graft rejection events were observed.

The underlying mechanism of local immunosuppression is thought to be based on the interactions of TAC with local secondary lymphoid organs. In particular, the draining lymph nodes of VCA grafts are key sites for priming of alloreactive T cells, which are predominantly responsible for T-cell–mediated acute rejection.[61,62] Investigations

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done by Unadkat et al[53] using their disk-based DDS have shown in a mixed lymphocyte reaction assay that there was significant hyporesponsiveness in T cells from draining lymph nodes of the treated allograft as compared with T cells from lymph nodes of the contralateral limb or the spleen. This observation may be explained by TACs pronounced lipophilic molecular structure[31] that leads to its accumulation in the local lymph nodes—more than in skin or muscle tissue—as Feturi et al[54] have confirmed in further investigations of the same TAC-DDS. Moreover, their study has shown that draining lymph nodes of animals treated with the TAC-DDS have a significantly higher concentration of TAC (>250 ng/g) when compared with systemic TAC treatment (<50 ng/g).[54] The high local concentrations of TAC lead to impairment of T-cell maturation and proliferation in the secondary lymphoid organs around the allograft. A further potential advantage of local DDS with regard to prevention of rejection is that they affect the formation of tertiary lymphoid structures in the skin, another place of T-cell proliferation and activation.[46]

Long-term follow-up that includes clinical as well as psychological care is paramount for the success of VCA.[63] In this context, monitoring of the immunosuppressive treatment plays an important role. The presented DDS have the potential to enhance patient compliance by reducing the intake of immunosuppressive medication. Repetitive injections of TGMS-TAC every 70 d in a rat model prolonged graft survival for >280 d.[46] The same DDS applied in a porcine model resulted in long-term survival from 56 to 93 d after a single injection.[47] The PCL disk achieved long-term survival for >200 d.[54] These results indicate that repetitive subcutaneous application of TGMS-TAC every 60–90 d, and possibly longer intervals for PCL disks, may result in long-term graft survival, spanning several years. Question about esthetic consequences at the site of DDS application may arise, but negative esthetic effects should be manageable as all the discussed DDS are made of biodegradable substances. One of them, the PEG-polyester hydrogel, has been optimized to be degraded in large parts after the release of TAC, preventing fibrosis around the depot site.[50]

The results of the summarized preclinical experiments are promising. The TGMS-TAC hydrogel and the nanoparticle-based implants were taken a step further and applied in porcine and nonhuman primates models, respectively, with encouraging results. However, further validation of biomaterial-based TAC-delivery systems in large-animal models is needed. The research focus should lay on the reduction of rejection incidence, increase of long-term graft survival and evaluation of toxicity outcomes. The goal should be to generate solid data to establish evidence of the advantages of local immunosuppression in VCA—especially low rates of side effects—in view of future clinical translation.

A combination of measures targeting the whole process of VCA from patient selection to graft preservation to postoperative care and immunosuppressive therapy may allow a wider clinical application of VCA in the future. Major advances have been and are being made in all these areas, something VCA can only benefit from. The first and at the same time most important step in a VCA process is patient selection.[11,64] If a patient's needs cannot be adequately met by existing therapy, such as prosthetic fitting, VCA may be a potential indication. Besides the medical indication, several points need to be considered. VCA postoperative care includes a prolonged rehabilitation program and complex medical regimen with regular medical follow-up appointments. Patient selection for VCA needs a careful and detailed analysis of the physical condition, medical comorbidities, psychologic status, and social factors.[9,11]

A key success factor for VCA is the ischemia time of the harvested graft. The goal is to reduce it to a minimum to reduce damage from ischemia/reperfusion injury that occurs from the activation of the innate immune system.[15] Accepted ischemia times for upper extremity vary from around 4–6 h.[15,65] The current standard of VCA preservation is static cold storage (SCS) in ice slurry, a preservation technique derived from SOT.[66] The aim is to preserve the graft by slowing down the metabolic processes by induction of hypothermia.[67] However, several limitations exist when using SCS in VCA. Among them are tissue damage after prolonged hypothermic preservation, difficulty in assessing graft viability before transplantation, and limited preservation time.[68] New approaches to graft preservation may prolong the tolerated time from graft harvesting to transplantation. Using common extracorporeal perfusion systems, porcine VCA grafts could be preserved for up to 24 h.[66] In an experimental study, human forearm grafts have been preserved for up to 24 h.[69] Rezaei et al[70] conducted a study where they compared the preservation outcome of human forearm grafts after SCS versus ex vivo normothermic perfusion. They could demonstrate that the use of ex vivo normothermic perfusion could overcome the limitations set by SCS by extending preservation time, enabling limb quality assessment, and allowing limb reconditioning before transplantation.[70] In summary, extracorporeal perfusion seems to be a promising strategy for VCA graft preservation.

Currently, the diagnosis of rejection is based on clinical presentation and microscopic findings from biopsies only. Additional, noninvasive strategies to help diagnose rejection and to determine the most adequate treatment would be helpful.[71] Several biomarkers, such as endothelial cell activation markers or metalloproteinase levels were found to correlate with VCA rejection.[71] In a case of face transplantation, Win et al[72] identified endothelial adhesion markers such as intercellular adhesion molecule 1 and vascular cell adhesion protein 1 to be associated with antibody-mediated rejection. Further investigation will certainly lead to the discovery of biomarkers that will enable the precise diagnosis of rejection types and lead to the development of rejection type-specific therapies.

Finally, a consensus should be reached for the whole medical and surgical procedure in VCA. A first proposal for such a consensus document has recently been published by Longo et al[63] for face transplantation. In such consensus articles, important points regarding the whole process in VCA should be discussed with the goal of creating a gold standard of practice and policy to promote the development of the field.

CONCLUSIONS

Localized immunosuppression using biomaterial-based DDS represents an encouraging approach to enhance graft survival and reduce toxic side effects of immunosuppressive drugs in VCA patients. Preclinical models using

TAC-releasing DDS have demonstrated high local immunosuppressive effects with reduced incidence of side effects due to low systemic TAC levels. However, to reduce acute rejection events in translational animal models or clinical reality, the use of additional low-dose systemic TAC treatment may be envisaged. Overall, using a combination of TAC-DDS with low systemic immunosuppression, patients may benefit through efficient graft immunosuppression and survival with negligible systemic side effects, resulting in better compliance and quality of life.

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