Premise and promise of mesenchymal stem cell-based therapies in clinical vascularized composite allotransplantation

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Abstract: PURPOSE OF REVIEW Over the past decade, clinical vascularized composite allotransplantation (VCA) has enabled functional and quality of life restoration in a wide range of indications secondary to devastating tissue loss. However, the spectre of toxicity and long-term complications of chronic immunosuppression has curtailed the momentum of VCA. This study summarizes the literature evidence behind successful mesenchymal stem cell (MSC)-based cell therapies highlighting their multipronged immunomodulatory, restorative and regenerative characteristics with special emphasis towards VCA applications. RECENT FINDINGS Experimental and clinical studies in solid organs and VCA have confirmed that MSCs facilitate immunosuppression-free allograft survival or tolerance, stimulate peripheral nerve regeneration, attenuate ischaemia-reperfusion injury, and improve tissue healing after surgery. It has been hypothesized that MSC-induced long-term operational tolerance in experimental VCA is mediated by induction of mixed donor-specific chimerism and regulatory T-cell mechanisms. All these characteristics of MSCs could thus help expand the scope and clinical feasibility of VCA. SUMMARY Cellular therapies, especially those focusing on MSCs, are emerging in solid organ transplantation including VCA. Although some clinical trials have begun to assess the effects of MSCs in solid organ transplantation, much scientific domain remains uncharted, especially for VCA.

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Premise and promise of mesenchymal stem cell-based therapies in clinical vascularized composite allotransplantation

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Purpose of review
Over the past decade, clinical vascularized composite allotransplantation (VCA) has enabled functional and quality of life restoration in a wide range of indications secondary to devastating tissue loss. However, the spectre of toxicity and long-term complications of chronic immunosuppression has curtailed the momentum of VCA. This study summarizes the literature evidence behind successful mesenchymal stem cell (MSC)-based cell therapies highlighting their multipronged immunomodulatory, restorative and regenerative characteristics with special emphasis towards VCA applications.

Recent findings
Experimental and clinical studies in solid organs and VCA have confirmed that MSCs facilitate immunosuppression-free allograft survival or tolerance, stimulate peripheral nerve regeneration, attenuate ischaemia-reperfusion injury, and improve tissue healing after surgery. It has been hypothesized that MSC-induced long-term operational tolerance in experimental VCA is mediated by induction of mixed donor-specific chimerism and regulatory T-cell mechanisms. All these characteristics of MSCs could thus help expand the scope and clinical feasibility of VCA.

Summary
Cellular therapies, especially those focusing on MSCs, are emerging in solid organ transplantation including VCA. Although some clinical trials have begun to assess the effects of MSCs in solid organ transplantation, much scientific domain remains uncharted, especially for VCA.

Keywords
hand and face transplantation, immunomodulation, mixed chimerism, regulatory T cells, tolerance

INTRODUCTION

Until recently, few reconstructive options were available for patients with disfiguring and functional devastating tissue defects secondary to trauma, oncological resection or congenital malformation. Over the past 2 decades, the technical, immunologic and functional feasibility of vascularized composite allotransplantation (VCA) as an alternative restorative option has been established in such indications. Overall, intermediate and long-term graft and patient outcomes have been encouraging for extremity and facial VCA with improved quality of life [1,2]. The prospect of allograft dependency on chronic, lifelong drug immunosuppression, with the risk of infectious, metabolic or neoplastic complications remains a significant hurdle for clinical advancement of VCA [3]. Development of safe and effective protocols consistent with immunosuppression-free graft survival is an immediate priority in nonlife saving transplants such as VCA.

Unlike other solid organs, optimal functional recovery is a prerequisite in VCA, which relies on timely and proper sensory and motor nerve regeneration and reintegration for overall success. Without it, a VCA would be deemed a failure, increasing the risk/benefit ratio of long-term immunosuppression and questioning the ethical equipoise of VCA. Also unlike solid organs, VCA are extraneous grafts requiring matching for size, skin colour, tone and anatomical congruity. These requirements limit the

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KEY POINTS

- Clinical vascularized composite allotransplantation is an expanding therapeutic option for functional restoration after devastating tissue loss, however, hampered by the need for long-term immunosuppression.
- Cellular therapies including mesenchymal stem cells show promise in establishing long-term operational allograft tolerance and obviate to the hurdles of drug-immunosuppression.
- Mesenchymal stem cells show additional beneficial effects (peripheral nerve regeneration, attenuation of I/R injury, facilitation of tissue healing after surgery) that additionally expand clinical feasibility of VCA.

available donor-pool and may require donor grafts to be shared over long distances, with increased cold ischaemia times and possible risks of worse ischaemia-reperfusion (I/R) injury. All these constraints jeopardize the life-enhancing benefits of VCA procedures. Research efforts striving to overcome these obstacles have included the use of cellular therapies such as those incorporating mesenchymal stem cells (MSCs), given their multifaceted effects ranging from immunomodulation and tissue healing to neuroregeneration and mitigation of I/R injury [4,5▪▪,6].

Herein we provide a brief overview of the premise and potential of MSC therapy in addressing different therapeutic goals inherent to VCA, focusing on their broad-based beneficial effects and recently emerging outcomes of experimental studies and clinical trials in solid organ transplantation (SOT) and VCA.

MESENCHYMAL STEM CELLS

A broad selection of different cell types could play a relevant role in cell-based therapy for VCA. Regulatory T cells (T_{regs}) [7], dendritic cells [8,9], MSCs, whole bone marrow (WBM) and other cells have been evaluated in SOT and VCA. MSCs are undifferentiated, multipotent, self-renewing cells, widespread throughout the body, possessing angiogenic, immunomodulatory, pro-neurogenic and antiapoptotic functionality and capable of mesenchymal tissue differentiation, for example bone, muscle, cartilage, endothelium and fat [10,11]. Together, these characteristics have relevance and impact in VCA. MSCs have been isolated from various tissues including, bone marrow mesenchymal stromal cells (BM-MSCs) and subcutaneous fat tissue (adipose-derived stem cells; ASCs). The latter source is appealing due to ease of procurement, low morbidity and high cell yields. ASCs may also have superior immunomodulatory potency [5▪▪], as compared with bone marrow or other sources of MSCs [5▪▪,6,12,13].

POTENTIAL OF MESENCHYMAL STEM CELL THERAPIES IN CLINICAL VASCULARIZED COMPOSITE ALLOTRANSPLANTATION

Donor-cell chimerism

A multitude of experimental reports in small and large animals have demonstrated prolonged allograft survival in VCA either after single or repetitive MSC administration [14▪▪,15,16,17▪,18▪,19–24]. MSCs seem to favour the establishment of mixed donor-cell chimerism in VCA recipients [25–27]. Cetrulo *et al.* found that hematopoietic cell infusions induced donor-specific tolerance to myocutaneous flap VCA in a large animal model with persistent donor-cell chimerism over weeks in a manner similar to VCA transplantation in established chimeras [28]. This is of important translational value for reconstructive transplantation, as VCA donors are usually brain dead and all tissues have to be procured simultaneously. Although some authors successfully achieved long-term tolerance after single infusion of WBM even without persistent peripheral donor-cell chimerism [25], other reports suggest a positive effect of preestablished and stable donor-specific chimerism on composite allograft survival across major histocompatibility mismatches [16]. Thus, although donor-cell chimerism is regarded as key step for long-term tolerance, it is not yet clear if sustained chimerism is actually required for graft survival. Interestingly, in a recent report allografts survived after loss of donor-specific chimerism and were not rejected after reconstitution with host hematopoietic cells [29].

Modulation of immune response

The immunomodulatory and anti-inflammatory properties of MSCs hold promise as a therapeutic approach for induction of allograft tolerance, even though it is not clear yet if MSCs alone are able to induce a solid long-term transplant tolerance or if cellular therapies have to be supported at least in part by other cell types and/or drug-based immunosuppressive regimens. As far as it concerns VCA, there is only a small body of evidence for the modulatory effects of MSCs. Recent reports point out to a beneficial immunomodulatory effect of both BM-MSCs and ASCs on the survival rate of rat hind limbs

while coupled to a short initial course of calcineurin inhibitors [17▪,18▪].

There are several putative mechanisms by which MSCs can exert their effect to dampen the immune response to alloantigens, all summarized recently by Kim *et al.* (reviewed in [30]).

One mechanism under intensive investigation is the MSC-mediated T_{reg} recruitment and expansion in both peripheral blood and allograft [17▪,22,31]. In contrast, however, Jiang *et al.* found that T_{reg} depletion in transplant recipients did not compromise BM-MSC ability to suppress allograft rejection in murine heart transplant recipients [32].

Most of MSC's immunosuppressive activity is mediated through paracrine signalling, with the MSC secretome playing a pivotal role and acting on a variety of pathways including interaction with T and B lymphocytes, as well as inhibition of macrophages, monocytes and natural killer cells among others.

Soluble factors involved are for example indoleamine 2,3-dioxygenase, transforming growth factor-β, nitric oxide, tumour necrosis factor-inducible gene 6 protein and prostaglandins. Moreover, cells of the immune system can shift from a pro-inflammatory to an anti-inflammatory phenotype under MSC influence.

Other than dampening the recipient's imm une reaction against allografts, through their suppressive function MSCs offer the opportunity to attenuate graft-versus-host disease (GvHD) as well (reviewed in [33]), especially when given in conjunction with hematopoietic stem cell (HSC) or WBM, where the risk of GvHD is increased.

Syngeneic, allogeneic and xenogeneic mesenchymal stromal cells

It is important to distinguish between allogeneic, syngeneic or even xenogeneic MSC sources. Donor-derived (allogeneic) cells need to be procured at the time of transplantation along with the graft and either frozen down, further processed or placed in culture and expanded for later administration. Autologous (syngeneic) cells are readily available from the host, can be freshly procured any time and repetitively administered. Chen *et al.* reported that a single injection of syngeneic ASCs along with antilymphocyte serum and a short course of cyclosporine A achieved rodent limb VCA survival in 66% recipients [18▪]. In comparison, studies using allogeneic MSCs reported a lower long-term survivor rate [17▪,20], suggesting that even if MSCs are immunoprivileged (due to absence of MHC Class II markers), autologous cells could have a higher survival rate and better impact on the outcome. One group recently used (xenogeneic) human ASCs (hASC) to induce skin allograft tolerance in mice [24]. hASC monotherapy accomplished only slight improvements in graft survival. In contrast, combination of hASCs with murine WBM achieved 100% long-term survival, suggesting rejection of the xenogeneic ASCs. We recently reported that BM-MSCs and ASCs are not substantially different in efficacy in a rat osteomyocutaneous VCA model with a short course of tacrolimus under an antilympho cyte serum preconditioning regimen [17▪]. Both regimens successfully promoted immunosuppression-free long-term survival in around 50% of the recipients with transient peripheral chimerism and increased T_{reg} levels. The in-vitro immunomodulatory function of ASCs was superior to that of BM-MSCs.

Drug–mesenchymal stem cell interactions

Recent literature evidence reaffirms that an induction regimen or early short-course immunosuppression is probably essential for the success of tolerance strategies [34,35]. Eggenhofer *et al.* reported accelerate rejection and worse outcomes after MSC therapy without concomitant immunosuppression [36]. Allogeneic mouse islets were prolonged after MSCs in combination with CTLA-4-Ig co-stimulatory blockade but not with isolated MSC therapy [37▪]. Lee *et al.* reported that in the absence of preconditioning, hASCs achieved only a discrete prolongation of murine allogeneic skin grafts survival injection of conditioned medium prolonged graft survival and reduced inflammatory tissue cytokine levels, suggesting a paracrine mechanism [34]. In another study, repetitive administration of omental rat ASCs over 3 days without adjunct immunosuppression delayed skin graft rejection without long-term tolerance, despite increased T_{reg} levels in skin specimens [35]. Similarly, Larocca *et al.* found that allogeneic ASCs can prolong skin graft survival and recruit T_{regs} into draining lymph nodes. Of interest, in that study, allogeneic donor-matched ASCs were more efficient than host-matched syngeneic or third-party ASCs [38]. Nevertheless, the toxic effect of concomitant conditioning and maintenance drugs on MSC viability and function should not be underestimated as supported by our recent in-vitro findings [39]. Singh *et al.* reported that sirolimus (rapamycin) is superior to tacrolimus in preserving the T_{reg} phenotype and FoxP3 expression [40]. Given the in-vivo impact and relevance of such toxicity, the timing, dosing and frequency of cellular therapies may need to be optimized and tailored in the context of such induction regimens in order to avoid collateral effects.

Supportive use of different cell types

The presence of active vascularized bone marrow in certain VCA may benefit allograft survival [41]. It remains unclear if systemic administration of a similar amount of WBM would achieve similar effects or if the donor-specific bone marrow niche is a requirement that fosters the development of robust and stable donor-cell chimerism. T_{reg} induction and recruitment may be important for maintenance of peripheral tolerance and avoidance of rejection. A recent report by Obermajer *et al.* suggests direct T-cell conversion from Th1-T cells to T_{regs} by MSC influence as a mechanism of T_{reg} induction [42]. A 2013 study revealed that T_{reg} injection (at lower doses than with WBM therapy) along with vascularized bone marrow transplantation achieved 90% graft acceptance and sustained peripheral donor-cell chimerism without need for cytoreduction [7]. In a canine VCA model, Mathes *et al.* found that intragraft T_{reg} levels were similar in long-term VCA recipients regardless of bone marrow infusion. This was confirmed *in vitro* by increased suppressor function on alloantigen-stimulated T-cell proliferation by T_{regs} derived from long-term acceptors [14▪▪]. An interesting potential approach could be the use of purified MSCs along with HSCs, which have been shown to enhance engraftment and reconstitution [43]. Early clinical protocols combining ASCs and HSCs for tolerance induction in kidney transplantation show promising results [5▪▪]. However, further experimental studies are needed to elucidate if this is a valid option for VCA.

Therapy of acute and chronic rejection

Studies investigating the potential ability of auto logous, allogeneic, fresh or frozen MSCs for attenuation and therapy of rejection in allotransplantation are scant. The characteristics of MSC in promoting T_{reg} recruitment, anti-inflammatory function and paracrine secretion of anti-inflammatory factors may all benefit their role in prevention or manage ment of acute or chronic rejection. However, without substantive research, these purported advantages remain speculative in VCA.

Routes of administration

The mode of MSC administration in VCA is of relevance as most cells get entrapped in lungs and filtering organs such as liver and spleen after intravenous injection [44]. Albeit the simplicity and ease of intravenous injection, barring potential embolic events, it is still under debate if MSCs need to home and engraft locally into the target tissue to exert their therapeutic effect or if they can exert their immunomodulatory effects from distance in a paracrine fashion. According to a recent report early, high-dose, intravenous MSC administration should be preferred [32]. Alternative approaches represent the direct intraarterial injection to improve target cell-load [45], local scaffold-assisted delivery to favour cell viability and proliferation at the target site and diminish spreading of cells to have high loco-regional effects [46] or cell encapsulation to allow for systemic administration but with improved cell survival through a protective 'cell coating' [47,48]. Despite all these approaches, the ideal therapeutic dosage of MSCs for a given application remains unknown.

Nerve regeneration, protection from ischaemia-reperfusion injury, wound and bone healing

The versatility of MSC therapy potentially extends beyond tolerance induction and modulation of immune responses after VCA. There are many other supplementary benefits of MSCs of relevance to VCA outcomes. Most notable is peripheral nerve regeneration, which is critical in VCA, where unlike in SOT, overall functional restoration relies on motor and sensory recovery in the graft. Multiple studies confirm that bone marrow and ASCs facilitate functional recovery after peripheral nerve injury [49–51]. Intravenous MSCs home to injured sciatic nerves and improve functional recovery after transection [51]. ASCs have been shown to exert their beneficial effects through paracrine neurotrophic and angiogenic effects, a mechanism that is still debated [49]. MSC therapy aiming at enhancing nerve regeneration could necessitate repetitive treatments due to the slow nature of the regeneration process. I/R injury is linked with acute and chronic rejection and worse long-term outcomes in transplantation [52]. The ability of MSCs in mitigating reperfusion injury has been attributed to paracrine anti-inflammatory effects [53], with reduction in I/R injury in skin flaps [54,55] and in renal I/R injury [56,57]. In the latter study, MSCs but not conditioned medium were able to ameliorate I/R injured kidneys, which is in line with own findings where BM-MSCs but not conditioned medium were anti-inflammatory on activated endothelium in a critically ischaemic skin flap [53]. Effective mitigation of I/R injury after cold ischaemia could promote VCA allocation over larger geographical distances, decreasing ischaemia-related time constraints and expanding VCA donor-pool. MSCs also enhance bone healing, which is of utmost relevance for (bony) face and extremity transplantation [58]. BM-MSCs have been shown to improve healing of

critical calvarial defects after systemic infusion despite a low local cell engraftment rate [59]. Finally, the antiscarring, antiapoptotic and angiogenic properties of MSCs could all potentially contribute to improved wound healing and overall outcomes.

Mesenchymal stem cell therapies for clinical solid organ transplantation and bone-marrow transplantation

First clinical trials have begun evaluating the use of MSCs for induction of allograft tolerance in SOT, especially in kidney transplantation [5▪▪,60–63]. MSC administration prior and after kidney transplantation seems to be safe and efficacious in reducing the dose of maintenance drugs [60,62]. In a very recent case series, pre- and one post-transpl ant administration of autologous MSCs expanded peripheral T_{regs} and decreased T-cell proliferation retaining graft function in kidney transplant patients [63]. An interesting approach by Vanikar *et al*. included combined ASC and HSC therapy prior to transplantation under a nonmyeloablative conditioning regimen for immunomodulation in kidney transplantation showing a 94.7% 5-year survival compared with 84% in the control group [5▪▪]. Other than tolerance induction, cellular therapy of GvHD is another aspect under clinical investigation [64,65]. In cases of steroid-refractory GvHD in HSC-transplanted patients, repetitive third-party BM-MSC infusions over 3 weeks achieved more than 70% responders with cessation of GvHD symptoms [66], which is in line with a very recent report [64]. Fortunately, human freeze-thawed BM-MSCs have recently been found to retain their immunosuppressive activity against GvHD, potentially allowing for third party off-the-shelf cell products for therapy of such conditions [67].

The Pittsburgh Protocol is the first cell therapy protocol used in upper extremity transplantation incorporating WBM that has facilitated monotherapy maintenance of VCA in compliant patients [13]. Other groups have administered WBM in clinical VCA as part of a multidrug immunosuppression protocol [6]. However, combination cellular therapies of WBM, HSCs and MSCs remain unexplored. A combined approach could bring promising benefits in VCA as well, underscored by recent evidence suggesting improved engraftment of HSC co-administered with MSCs [68]. Experimental data for other cell products such as ASCs in VCA are also scarce.

CONCLUSION

Proof of principle has been established for cellular therapies using MSCs in both SOT and VCA. Clinical trials have investigated MSC therapy for renal transplantation. Although there are clinical reports of WBM infusion after hand and face transplants [6,69], there are no reported clinical attempts incorporating isolated or enriched MSCs in VCA. Multiple studies in the literature reinforce the promise and potential for MSCs in prolonging allograft survival and other aspects (promoting nerve regeneration, protecting from I/R injury) that could improve overall graft outcomes after VCA. Many questions do persist, such as the mechanisms underlying tolerance or graft acceptance, optimizing the conditioning regimen in the context of induction immunosuppression, the dosing, timing, route and frequency of cell administration and the use of other cell types such as ASCs in conjunction with MSCs to improve synergistic, complementary or additive efficacy after VCA.

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Conflicts of interest

There are no conflicts of interest.

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