Perspectives on the use of mesenchymal stem cells in vascularized composite allotransplantation

Jan A. Plock1,2,*, Jonas T. Schneider1, Mario G. Solari1, Xin Xiao Zheng1,3 and Vijay S. Gorantla1*

1 Department of Plastic Surgery, University of Pittsburgh Medical Center, Pittsburgh, PA, USA
2 Division of Plastic and Hand Surgery, University Hospital Zurich, Zurich, Switzerland
3 Research Center for Translational Medicine, Shanghai East Hospital, Tongji University, Shanghai, China

INTRODUCTION

Reconstructive transplantation has emerged as clinical reality over the past decade. Long-term graft acceptance has been feasible in extremity and facial vascularized composite allotransplantation (VCA) under standard immunosuppression. Minimizing overall burden of lifelong immunosuppression is key to wider application of these non-life saving grafts. Allograft tolerance is the holy grail of many cell-based immunomodulatory strategies. Recent protocols using mesenchymal stem cells from bone marrow and adipose tissue offer promise and potential in VCA. This article provides an overview of the experimental basis, the scientific background and clinical applications of stem cell-based therapies in the field of reconstructive allotransplantation.

Keywords: adipose stem cells, cell-based therapy, immunomodulation, composite tissue allotransplantation, tolerance

Like solid organ transplants, VCA requires long-term multidrug immunosuppression to prevent graft rejection mediated predominantly by the highly immunogenic skin component in these allografts. Medication toxicity could result in metabolic, infectious, or neoplastic complications. VCA is inherently different from solid organ transplantation in its non-life saving, yet life-enhancing impact on recipients. Further, unlike solid organs, clinical success is dictated not only by graft acceptance and survival, but also by nerve regeneration, which determines ultimate functional outcomes. These characteristics of VCA, drive the debate focused on the risks of lifelong immunosuppression mandated for graft survival balanced against the benefits of functional and quality of life outcomes. Thus, implementation of cellular therapies that integrate the concepts of immune regulation for graft acceptance with those of nerve regeneration could optimize the outcomes of these reconstructive modalities and minimize overall burden of immunosuppression. Such strategies could expand clinical feasibility and realize routine applicability of reconstructive transplantation.

Mesenchymal stem cells (MSCs) are pluripotent cells that are present in multiple tissues, including bone marrow (BM), adipose tissue, skin, muscle, blood, and placenta and can be isolated and expanded ex vivo. MSCs are capable of differentiation in vitro along multiple mesenchymal lineages such as osteocytes, chondrocytes, myocytes, adipocytes, and Schwann cells (SC) thereby emerging as a promising tool for tissue engineering and cell therapy. Current literature on MSCs points to a wide range of immunological functions and interactions with other cell types (4, 5). Recent data support findings that MSCs mediate their actions through multiple mechanisms including paracrine effects. Various groups have shown some key differences between adipose-derived MSCs (AD-MSCs) and bone marrow derived MSCs (BM-MSCs) in vitro and in vivo (6–8). In particular, it has been demonstrated that MSCs have the capacity to suppress T cell activation and proliferation (9). Compared to BM, adipose tissue is a rich source of MSCs with up to 10-fold higher yield of MSCs (10). More recent publications demonstrate that AD-MSCs might also have higher immunomodulatory and immunosuppressive potential in vitro as compared to BM-MSCs (11–20). The ease of procurement of large volumes of AD-MSCs through techniques such as liposuction is an important benefit because of expeditious approach and minimal morbidity. Depending on time considerations for cell expansion, the overall duration of cell retrieval to cell infusion could be significantly shortened for AD-MSCs as compared to MSCs from other sources.

There is a growing complement of first in human studies addressing potential of MSC based cell therapies in autoimmune diseases, facilitation of hematopoietic stem cell engraftment in...
BM transplantation and in solid organ transplantation (4, 21, 22). Recent experimental and clinical studies highlight their potential for immunomodulation, tolerance induction, and prophylaxis and treatment of graft versus host disease (GVHD) (23–25). In VCA, the efficacy and effectiveness as well as mechanisms and outcomes of such therapies may be affected by the antigenicity of the skin component (26), as or by the differential tissue composition of these grafts.

**IMMUNOLOGICAL FUNCTION OF MSCs**

The effects of MSCs on innate and adaptive immunity have been reported in the literature (4, 5). MSCs modulate the innate function of monocytes, macrophages, natural killer (NK) cells, and dendritic cells (DCs). They are capable of modifying the maturation of DC, thereby inhibiting their antigen-presenting function and inducing the generation of tolerogenic DCs. This results in downregulated MHC II and chemokine expression (27–30). MSCs show intermediate expression of MHC I and do not express MHC II on their surface, which reduces their antigenicity. However, the intracellular MHC II can become relevant when NK lyse transplanted MSCs (31, 32). In addition, MSCs also affect innate immunity through HLA-G expression leading to inhibition of NK cells and reduction of IFN-γ expression (33, 34). English et al. (35) have shown that MSCs can directly induce regulatory T cell (Treg) generation. These Tregs play a significant role in the development of tolerance.

MSCs PROMOTE NERVE REGENERATION

The functional advantages of VCA over prosthetic fitting rely not only on motor recovery but also sensory recovery following regeneration of peripheral nerves and reintegration of neuronal pathways into the premotor cortex of the brain. MSCs have shown promise in improving clinical and electrophysiological outcomes in animal models of peripheral nerve injury (37–39). In comparison to local delivery, some groups have demonstrated significantly accelerated functional neuronal recovery following systemic administration of MSCs (40). The postulated mechanisms for such outcomes could include direct and paracrine effects (38, 41).

MSC USE IN ALLOTRANSPLANTATION

Bartholomew et al. (9) first described prolongation of skin graft survival after allotransplantation in primates with a single application of expanded BM-MSCs at day 0. Sbano et al. (42) also reported similar results with cultured BM-MSC administered on day 0 and 3 in a rat model. Skin graft survival was prolonged, although grafts were rejected without supplementary immunosuppression.

![Figure 1](link)
Solari et al. (43) demonstrated long-term islet allograft survival in rats using syngeneic MSCs. In this study, repetitive application of syngeneic BM-MSCs was superior to allogeneic BM-MSCs. However, long-term tolerance could only be achieved in 40%, and cells were expanded up to P8. Their inherent immunomodulatory function has promoted use of MSCs in BM transplantation, solid organ transplantation, and treatment of GVHD (30). BM-MSCs have been used in experimental animal heart, liver, kidney, and islet transplantation (14, 30, 43–49). Immunomodulation was related to the suppression of alloreactive effector lymphocytes (50) through expression of a variety of cytokines (51–53) (Figure 1) and generation of CD4/CD25/FoxP3 Tregs that achieved long-term tolerance in animal studies via adaptive immune mechanisms (31, 50). Data on AD-MSCs in solid organ transplantation is scarce; however sources for MSCs other than BM have been supported/investigated (54). In addition, studies have confirmed the multifaceted properties of MSCs including their potential as part of induction therapy (55), in GVHD (56), tolerance induction (55, 57), and facilitating engraftment of BM transplants (58, 59). Several clinical trials have demonstrated their immunosuppressive function that could have potential in autoimmune disease (32, 60, 61). Such properties could offer potential for MSCs in acute and chronic rejection after VCA. MSCs have been successfully used in pediatric patients as rescue therapy of GVHD and repetitive rejection of BM transplants (62).

CRITICAL ISSUES FOR MSC USE IN VCA

The success of organ transplantation and VCA is dependent on graft acceptance in the absence of GVHD. Various induction and maintenance regimens have been successful in controlling acute rejection. All these drugs have their role in VCA for specific immunosuppressive functions, but their unwarranted collateral effects on MSCs are less well investigated.

Several studies in solid organs have reported pre-transplant, peri-transplant, or post-transplant use of MSCs for immunomodulation (63–65). However, the negative effects of the depletion regimen that include irradiation, and polyclonal (antithymocyte globulin/serum), or monoclonal antibodies (e.g., alemtuzumab) on MSC recruitment, homing, and function remain to be clarified.

INTERACTION WITH IMMUNOSUPPRESSIVE DRUGS

Several immunosuppressive drugs significantly affect in vitro activity of MSCs. Hoogduijn et al. (66) investigated the in vitro effect of tacrolimus, rapamycin, and MPA on human MSCs and vice versa. Exposure of MSCs to high dosages of tacrolimus was toxic and reduced cell viability. While tacrolimus did not inhibit proliferation rates of MSCs, rapamycin, and MPA led to a dose-dependent reduction of proliferation and differentiation at therapeutic dosage levels. Unlike rapamycin, tacrolimus did not affect the osteogenic differentiation potential of MSCs (67). The effects of Cyclosporin A (CsA) on MSCs were insignificant. Some authors demonstrated a synergistic immunosuppressive effect of MSCs and MPA (68, 69). However, the immunomodulatory properties of MSCs were antagonized by rapamycin and tacrolimus. In vivo experiments support these findings of synergistic or opposing effects between MSC and pharmacological immunosuppression (68, 70). Hoogduijn’s et al (66) reported that MSCs inhibited the suppressive effect of tacrolimus and rapamycin on alloreactive lymphocytes but had no effect on MPA.

TIMING AND DOSAGE OF MSCs

Currently, there is no consensus on dosing and timing of administration of MSCs in cell-based VCA protocols. Experimental studies indicate that the dosage of MSCs showing beneficial results ranges from 5 × 10^6 to 5 × 10^7 cells/kg body weight and time point (Table 1). In rats, the total amount of BM-MSCs administered over time was 6–10 × 10^6 cells, whereas in pigs total amounts from 5 to 12.5 × 10^7. BM-MSCs were used. In a study utilizing AD-MSCs, as many as 24 × 10^6 cells/kg bodyweight were administered over three time points (58, 59, 71). MSCs were administered as early as day −30 to as late as +21 relative to transplantation or at different frequency in the interim (57–59). The time point was chosen with regards to the desired effect such as induction, immunomodulation, or support of BM engraftment.

HOMING OF MSCs/CHIMERISM

Eggenhofer et al. (72) recently reported a murine study reduced life span of intravenously delivered cultured MSCs due to entrapment in the lung capillaries or liver sinusoids. Others have shown distribution to other organs like kidney and spleen, BM, and peripheral blood (73). Kuo et al. (58) demonstrated recruitment and homing of MSCs to perivascular sites with long-term survival in VCA models. In addition peripheral blood chimerism after VCA with MSC-co-transplantation was demonstrated in pig and rat models (57–59). Indeed, the first-pass clearance of MSCs in lung and liver may be an obstacle for these cell therapies (74, 75). Several strategies including vasodilation, co-transplantation, and repetitive infusions have been suggested to increase cell passage. Freshly isolated MSCs have been shown to be superior to culture-expanded MSCs in terms of lower entrapment potential due to smaller size as well as better homing potential (76). They are found in high number in sites of trauma and ischemia after several days (77).

CULTURED MSCs

For therapeutic indications, MSCs need to be expanded in culture to achieve sufficient numbers for transplantation. Karp and Leng (78) reported that the culture medium and conditions are likely to influence the properties of MSCs as well as their morphology (79, 80). The number of passages inversely correlates with the number of surface antigens and homing potential (81–84). Cultured MSCs may be morphologically indistinguishable from fibroblasts and even show similar cell-surface markers, differentiation potential, and immunologic properties (80). Multiple cell passages not only affect proliferation and differentiation but also alter cell-surface antigens and cytokine production (85, 86). To our knowledge, there is no study to date on the immunomodulatory potential of long-term-cultured MSCs. In our view, short-term culture of MSCs (<P3) and freshly isolated MSCs must be given preference in cell therapy protocols until we gain more insights into the properties of expanded MSCs.

MSCs IN VASCULARIZED COMPOSITE ALLOTRANSPLANTATION

Only a few groups have advocated the use of BM-MSCs or AD-MSCs for immunomodulatory strategies in VCA (Table 1).
Table 1 | Overview of the currently available experimental literature on MSC based cellular therapy for immunomodulation in VCA.

| Reference       | Type of graft | Species | MSCs amount/type | MSC application | Induction regimen | Immunosuppression | Main outcome                      |
|-----------------|---------------|---------|------------------|----------------|------------------|-------------------|-----------------------------------|
| Pan et al. (59) | Hindlimb      | Rat     | $10^7$ BM-MSCs   | Day $-30$      | Irradiation, ALS, BMT | Rapamycin         | Chimerism in peripheral blood Tolerance (>100 days) Protection against GvHD |
|                 |               |         | (allogeneic)     |                |                  |                   |                                   |
| Kuo et al. (59) | Hindlimb      | Pig     | $10^7$ BM-MSCs   | Day $-1$, +3, +7, +14, +21 | Irradiation, BMT | Cyclosporin        | Perivascular MSC engraftment (graft) Chimerism in peripheral blood Tolerance (>200 days) Protection against GvHD Treg $\uparrow$ |
|                 |               |         | (allogeneic)     |                |                  |                   |                                   |
| Kuo et al. (57) | Hindlimb      | Rat     | $2 \times 10^8$ AD-MSCs | Day $+7, +14, +21$ | ALS               | Cyclosporin        | Tolerance (>120 days) Treg $\uparrow$ |
|                 |               |         | (allogeneic)     |                |                  |                   |                                   |
| Kuo et al. (88) | Face          | Pig     | $2.5 \times 10^7$ BM-MSCs | Day $-1$, +3, +7, +14, +21 | N/A               | Cyclosporin        | IL-10 $\uparrow$ Treg $\uparrow$ |
|                 |               |         | (allogeneic)     |                |                  |                   |                                   |
| Aksu et al. (71) | Skin flap     | Rat     | $2-3 \times 10^6$ (repetitive) BM-MSCs (syngeneic) | Days $0, +7, +14, and +21$ | Irradiation, BMT (repetitive) | Cyclosporin | Syngeneic MSCs limit toxicity of allogeneic BMT Prolongation of tolerance Enhanced mixed chimerism |

Despite growing enthusiasm, the basic experience is limited to few experimental studies with MSCs. Aksu et al. (71) reported that co-administration of host BM-MSCs with unmodified donor BM and immunosuppression (CsA + irradiation) enabled prolonged survival of full MHC mismatched rodent vascularized skin grafts with generation of mixed chimerism and absence of GvHD. Outcomes positively correlated with number of times the BM-MSCs were administered. Pan et al. (59) reported a rat hindlimb VCA model where limb transplants were performed a month after conditioning with total body irradiation, and anti-lymphocyte-serum followed by allogeneic BM-MSC and BM infusion. This resulted in stable chimerism, donor specific tolerance, and no GvHD. Allogeneic BM-MSC transplantation with or without co-transplantation of BM has been shown to be successful in prolongation (>200 days) of pig limb allograft survival after irradiation and CsA treatment (58, 87). Repetitive high dose BM-MSC treatment was also successful in prolonging survival in a pig hemi-facial transplantation model without conditioning therapy (88). The authors reported only mild rejection of the graft (Grade I–II), improved under CsA treatment. The positive effects of BM-MSCs on rejection grades were correlated to IL-10 upregulation and Treg induction. Despite the prolongation, all grafts succumbed within 90 days. In a different approach, Kuo et al. (57) administered three fold numbers of AD-MSCs under temporary immunosuppression in a rat hindlimb allotransplantation model. After cessation of immunosuppression, this regimen prolonged allograft survival significantly with stable tolerance in 89% for >150 days. Treg populations were significantly increased and elevated donor lymphoid cell counts (RT1n) resulted in stable peripheral blood chimerism until endpoint. The same group conducted a study in a swine hindlimb allotransplantation model using BM-MSCs, irradiation, and short-term CsA. They were able to demonstrate prolongation of the survival (>100 days in 67%) and an increase of the Treg population.

**CONCLUSION**

Taken together, emerging literature evidence highlights the potential promise of MSC based cellular therapies for immunomodulation and neurodegeneration in VCA. Extensive experimental and clinical studies in the areas of solid organ transplantation, hematopoietic stem cell co-transplantation and autoimmune disease underscore the relevance and impact of MSC based therapies in VCA. Traditionally, the chief obstacle hampering application of BM-MSCs has been the limited cell yield and requirement for donor cell expansion. The high cell yields of AD-MSCs from adipose sources obtained through easy, fairly non-invasive techniques have enabled expeditious cell processing, thus expanding the clinical feasibility of these therapies. These advantages, combined with the insights supporting the superior immunomodulatory potential of AD-MSCs versus BM-MSCs truly advocate adipose-based cellular therapies. The higher cell yields also facilitate repetitive infusion both systemically and locally in the graft. Freshly isolated AD-MSCs can overcome the loss of viability and entrapment during the first-pass phenomenon in the capillary systems of the lung. Importantly, MSCs mediate paracrine effects on remote tissues through specific cytokines, chemokines, and growth factors. It still remains to be defined if such paracrine effects mediate also tolerogenic or immunomodulatory effects in VCA or other
applications. Future protocols should carefully address the dosing and timing of MSC administration and the effects of conditioning regimens and maintenance immunosuppression on function of these cells. Most notably, the effect of MSC-based strategies on nervous regeneration, critical for functional outcomes in VCA offers these cells. Most notably, the effect of MSC-based strategies on regimens and maintenance immunosuppression on function of and timing of MSC administration and the effects of conditioning Plock et al. MSCs in vascularized composite allotransplantation

REFERENCES

1. Petruzzo P, Lanzetta M, Dubernard JM, Landin L, Cavadas P, Mar- greiter R, et al. The international registry on hand and composite tissue transplantation. Transplantation (2010) 90:1596–4. doi:10.1097/ TP0b013e3181f1472

2. Petruzzo P, Kanitakis J, Badet L, Pialat JF, Boutrov S, Charpulat R, et al. Long-term follow-up in composite tissue allotransplantation: in-depth study of five (hand and face) recipients. Am J Transplant (2011) 11:808–16. doi:10.1111/j.1600-6143.2011.03469.x

3. Petruzzo P, Testelin S, Kanitakis I, Badet L, Lengele B, Girbon JP, et al. First human face transplantation: 5 years outcomes. Transplantation (2012) 93:236–40. doi:10.1097/TP. 0b013e3182d4a6f6

4. Singer NG, Caplan AI. Mesenchymal stem cell mechanisms of inflammation. Annu Rev Pathol (2011) 6:457–78. doi:10.1146/annurev-pathol-011110-130230

5. English K. Mechanisms of mesenchymal stem cell immunomodulation. Immunol Cell Biol (2013) 91:19–26. doi:10.1038/icb.2012.56

6. Izadpanah R, Trygg C, Patel B, Kriedt C, Dufour J, Gimble JM, et al. Biologic properties of mesenchymal stem cells derived from bone marrow and adipose tissue. J Cell Biochem (2006) 99:1285–97. doi:10.1002/jcb.20094

7. Liu TX, Martinez M, Hutmacher DW, Hui JH, Lee EH, Lim B. Identification of common pathways mediating differentiation of bone marrow- and adipose tissue-derived human mesenchymal stem cells into three mesenchymal lineages. Stem Cells (2007) 25:750–60. doi:10.1634/stemcells.2006-0394

8. Noël D, Caton D, Roche S, Bony C, Lohmann S, Castella L, et al. Cell specific differences between human adipose-derived and mesenchymal-stromal cells despite similar differentiating potentialities. Exp Cell Res (2008) 314:1573–84. doi:10.1016/j. yexcr.2007.12.022

9. Bartholomew A, Surgeon C, Siatskas M, Ferrer K, McIntosh K, Patil S, et al. Mesenchymal stem cells suppress lymphocyte proliferation in vitro and prolong skin graft survival in vivo. Exp Hematol (2002) 30:42–8. doi:10.1016/S0301-472X(01)00769-X

10. De Ugarte DA, Morizono K, Elbar- bary A, Alfonso Z, Zuk PA, Zhu M, et al. Comparison of multi- lineage cells from human adipose tissue and bone marrow. Cells Tissues Organs (2003) 174:101–9. doi:10.1159/000071150

11. Mielie SM, Zwaginga JJ, Fibbe WE, Roelofs H. Adipose tissue-derived multipotent stromal cells have a higher immunomodulatory capacity than their bone marrow-derived counterparts. Stem Cells Transl Med (2013) 2:455–63. doi:10.5966/sctm.2012-0148

12. Cui L, Yin S, Liu W, Zhang W, Cao Y. Expanded adipose-derived stem cells suppress mixed lymphocyte reaction by secretion of prostaclinldin E2. Tissue Eng (2007) 13:1185–95. doi:10.1089/ten.2006.0315

13. Fang B, Song Y, Lin Q, Zhang Y, Cao Y, Zhao RG, et al. Human adipose tissue-derived mesenchymal stem cells as salvage therapy for treatment of severe refractory acute graft-vs.-host disease in two children. Pediatr Transplant (2013) 11:814–7. doi:10.1111/j.1399-3094. 2007.00780.x

14. Wan CD, Cheng R, Wang HB, Liu T. Immunomodulatory effects of mesenchymal stem cells derived from adipose tissues in a rat orthotopic liver transplantation model. Hepato- tobiliary Pancreat Dis Int (2008) 7:29–33.

15. Constantin G, Marconi S, Rossi B, Angiari S, Calderan L, Anguilieri E, et al. Adipose-derived mesenchymal stem cells ameliorate chronic experimental autoim- mune encephalomyelitis. Stem Cells (2009) 27:2624–35. doi: 10.1002/stem.194

16. Gonzalez MA, Gonzalez-Rey E, Rico L, Buscher D, Delgado M. Treatment of experimental arthritis by inducing immune tolerance with human adipose-derived mesenchymal stem cells. Arthritis Rheum (2009) 60:1006–19. doi:10.1002/art. 24405

17. Gonzalez-Rey E, Anderson P, Gonzalez MA, Rico L, Buscher D, Delgado M. Human adult stem cells derived from adipose tissue protect against experimental colitis and sepsis. Gut (2009) 58:929–39. doi:10.1136/gut.2008.168534

18. Cui L, Yin S, Liu W, Zhang W, Cao Y. Expanded adipose-derived stem cells suppress mixed lymphocyte reaction by secretion of prostaglandin E2. Tissue Eng (2007) 13:1185–95. doi:10.1089/ten.2006.0315

19. Fang B, Song Y, Lin Q, Zhang Y, Cao Y, Zhao RG, et al. Human adipose tissue-derived mesenchymal stem cells as salvage therapy for treatment of severe refractory acute graft-vs.-host disease in two children. Pediatr Transplant (2013) 11:814–7. doi:10.1111/j.1399-3094.2007.00780.x

20. Engela AU, Baan CC, Dor FJ, Weimann W, Hoogduijn MJ. On the mechanism of allogeneic mesenchymal stem cells as therapeutics. Bone Marrow Transplant (2011) 46:209–7. doi:10.1038/bmt.2010.87

21. Lee WP, Yaremchuk MJ, Pan YC, Randolph MA, Tan CM, Weiland AJ. Relative antigenicity of compo- nents of a vascularized limb allo- graft. Plast Reconstr Surg (1991) 87:401–11. doi:10.1097/00006534- 199103000-00001

22. Djouad F, Charbonnier LM, Bouffi C, Louis-Flence P, Bony C, Appa- rily F, et al. Mesenchymal stem cell transplantation of graft-versus-host disease. Front Immunol (2011) 3:241–8. doi:10.3389/fimmu.2011.00216

23. Parekkadan B, Milwad JM. Mesenchymal stem cells as therapeutics. Annu Rev Biomed Eng (2010) 12:87– 117. doi:10.1146/annurev-bioeng-070909-105309

24. Weng JY, Xu D, Geng SX, PengYW, Wang Z, Lu ZS, et al. Mesenchy- mal stem cell as salvage treatment for refractory chronic GVHD. Bone Marrow Transplant (2010) 45:1732– 40. doi:10.1038/bmt.2010.195

25. Bernardo ME, Ball LM, Cometa AM, Roelofs H, Zecca M, Avanzini MA, et al. Co-infusion of ex vivo expanded, parental MSCs prevents life-threatening acute GVHD, but does not reduce the risk of graft failure in pediatric patients undergoing allogeneic umbilical cord blood transplantation. Bone Marrow Transplant (2011) 46:209–7. doi:10.1038/bmt.2010.87

26. Lee WP, Yaremchuk MJ, Pan YC, Randolph MA, Tan CM, Weiland AJ. Relative antigenicity of compo- nents of a vascularized limb allo- graft. Plast Reconstr Surg (1991) 87:401–11. doi:10.1097/00006534-199103000-00001

27. Djouad F, Charbonnier LM, Bouffi C, Louis-Flence P, Bony C, Appa- rily F, et al. Mesenchymal stem cell transplantation of graft-versus-host disease. Front Immunol (2011) 3:241–8. doi:10.3389/fimmu.2011.00216

28. English K, Barry FP, Mahon BP. Murine mesenchymal stem cells suppress dendritic cell migration, maturation and antigen presenta- tion. Immunol Lett (2008) 115:50– 8. doi:10.1016/j.imlet.2007.10.002

29. Li M, Sun K, Wielniak LA, Murphy WJ. Immunomodulation and phar-macological strategies in the treat- ment of graft-versus-host disease. Expert Opin Pharmacother (2008) 9:2305–16. doi:10.1517/14656566.9.13.2305

ACKNOWLEDGMENTS

The authors have no financial interests. Jan A. Plock and Jonas T. Schneider are recipients of Swiss National Science foundation funding.
ONE (2011) 61:17899, doi:10.13171/ 
journal.pone.0017899 

Carried V, Garrido-Gomez J, 
Hernandez-Cortes P, Garzon I, 
Garcia-Garcia S, Saez-Moreano JA, et al. Combination of fibrin-agarose hydrogels and adipose-derived mesenchymal stem cells for peripheral nerve regeneration. J NeuroEng 
(2013) 10:026002. doi: 
10.1088/1741-2550/10/026022 

Marconi S, Castigione G, Turano E, 
Bissolotti G, Angiari S, Farinazzo A, et al. Human adipose-derived mesenchymal stem cells systemi-
cally injected promote peripheral nerve regeneration in the mouse model of sciatic crush. Tissue 
Eng Part A (2012) 18:1264–72. doi: 
10.1089/ten.TEA.2011.0491 

Chen CJ, Ou YC, Liao SL, Chen 
WV, Chen SY, Wu CW, et al. Trans-
plantation of bone marrow stro-
mal cells for peripheral nerve repair. Exp Neurol (2007) 204:443–53. doi: 
10.1016/j.expneurol.2006.12.004 

Shano P, Cuccia A, Mazzanti B, 
Urbani S, Giusti B, Lapini I, et al. Use of donor bone marrow mesenchymal stem cells for treat-
ment of skin allograft rejection in a preclinical model. Arch Der-
matol Res (2008) 300:115–24. doi: 
10.1007/s00004-007-0827-9 

Solarli MG, Srinivasan S, Boumaz 
A, Unadkat J, Harb G, Garcia-
Ocaña A, et al. Marginal mass 
islet transplantation with auto-
logous mesenchymal stem cells pro-
 motes long-term islet allograft sur-
 vival and sustained normoglycemia. 
J Autoimmun (2009) 32:116–24. doi: 
10.1016/j.jaut.2009.01.003 

Wu GD, Nolta JA, Jin YS, Barr 
ML, Yu H, Starnes VA, et al. Migration of mesenchymal stem cells to heart allografts during chronic rejection. Transplantation (2003) 75:679–85. doi: 
10.1097/01.TAT.0000048488.35010.95 

Inoue S, Popp FC, Koehl GE, Piso 
P, Schiltt HJ, Geissler EK, et al. Immunomodulatory effects of mesenchymal stem cells in a rat organ transplant model. Transplantation (2006) 81:1389–95. doi: 
10.1097/01.TAT.0000209912.06037.0b 

Zhou HP, Yi DH, Yu SQ, Sun GC, 
Cui Q, Zhu HJ, et al. Adminis-
tration of donor-derived mesenchy-
 mal stem cells can prolong the sur-
 vival of rat cardiac allograft. Trans-
plant Proc (2006) 38:3046–51. doi: 
10.1016/t transplantation.2006.10.002 

Itakura S, Asari S, Rawson J, Ito 
T, Tudorov I, Liu CP, et al. Mes-
enchymal stem cells facilitate the induction of mixed hematopoietic
 chimerism and islet allograft toler-
ance without GVHD in the rat. Am 
J Transplant (2007) 7:356–46. doi: 
10.1111/j.1600-6143.2006.01643.x 

Hong ZF, Huang XJ, Yin ZY, 
Zhao WX, Wang XM. Immunom-
suppressive function of bone mar-
row mesenchymal stem cells on acute rejection of liver allografts in rats. Transplant Proc (2009) 41:403– 
9. doi:10.1016/j.transproceed.2008. 
10.020 

Popp FC, Renner P, Eggenhofe 
R, Slowik P, Geissler EK, Piso P, et al. Mesenchymal stem cells as immunomodulators after liver transplantation. Liver 
Transpl (2009) 15:1192–8. doi: 
10.1002/lt.21862 

Crop MJ, Baan CC, Korevaar SS, Lje-
ermans JN, Weinma W, Hoogduijn 
MJ. Human adipose tissue-derived mesenchymal stem cells induce explosive T-cell proliferation. Stem Cells Dev (2010) 19:1843–53. doi: 
10.1089/scd.2009.0368 

Di Ianni M, Del Papa B, De Ioanni 
M, Moretti L, Bonicella E, Cecchi D, et al. Mesenchymal cells recruit and regulate T regulatory cells. Exp 
Hematol (2008) 36:309−18. doi: 
10.1016/j.exphem.2007.11.007 

Duffy MM, Ritter T, Ceredig R, 
Griffin MD. Mesenchymal stem cell effects on T-cell effector pathways. Stem Cell Res Ther (2011) 2:34. doi: 
10.1186/scrt75 

Casiraghi F, Azzolini N, Todeschi 
M, Cavintro RA, Cassis P, Solini S, et al. Localization of mesenchymal stromal cells dictates their immune or proinflammatory effects in kidney transplantation. Am J Transplant (2012) 12:2373−83. doi: 
10.1111/j.1660-4143.2012.04115.x 

De Girolamo L, Lucarelli E, Alessan-
dri G, Avanzini MA, Bernardo 
ME, Biagi E, et al. Mesenchymal stem/stromal cells: a new “cells as drugs” paradigm. Efficacy and crit-
ical aspects in cell therapy. Curr 
Pharm Des (2013) 19:2459−73. doi: 
10.2174/138161281381901005 

Tian J, Wu W, Xu X, Luo I, Zheng F, 
Messinger S, et al. Induction therapy with autologous mesenchymal stem cells in living-related kidney trans-
plants: a randomized controlled trial. JAMA (2012) 307:1169−77. doi: 
10.1001/jama.2012.316 

Le Blanc K, Frassoni F, Ball L, 
Urbani S, Giusti B, Lapini I, et al. 
Induction therapy with autologous mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host dis-
 ease: a phase II study. Lancet (2008) 371:579−86. doi:10.1016/S0140-6736(08)60690-X 

Figueroa FE, Carrion F, Villaneuva 
S, Khoury M. Mesenchymal stem cell treatment for autoimmune diseases: a critical review. Biol 
Res (2012) 45:269−77. doi:10.1590/ 
S0140-6736-2012000000008 

Lawitschka A, Ball L, Peters C. Nonpharmacologic treatment of chronic graft-versus-host disease in children and ado-
 lescents. Biol Blood Marrow Transplant (2012) 18:574−81. doi: 
10.1016/j.bbmt.2011.11.001 

Casiraghi F, Azzolini N, Cassis P, 
Imberti B, Mortig i M, Cugini D, et al. Pretransplant infusion of mesenchy-
 mal stem cells prolongs the survival of a semiallogeneic heart transplant through the generation of regulatory T cells. J Immunol (2008) 181:3933−46. 

Ge W, Jiang I, Baroja ML, Arp 
J, Zasko R, Liu W, et al. Infu-
sion of mesenchymal stem cells and rapamycin synergize to attenuate alloimmune responses and promote cardiac allograft tolerance. Am J 
Transplant (2009) 9:1760−72. doi: 
10.1111/j.1600-6143.2009.02271.x 

Oh Y, Lee RHI, Yu KM, Ko IH, Lee 
HJ, Ko AV, et al. Intravenous mesenchy-
 mal stem cells prevented rejection of allogeneic heart transplants by aborting the early inflammatory response. Mol Ther (2012) 20:2143− 
52. doi:10.1038/m.2012.165 

Hoogduijn MJ, Crop MJ, Kore-
vaar SS, Peeters AM, Eijken M, 
et. MSCs in vascularized composite allotransplantation. Frontiers in Immunology | Immunological Tolerance July 2013 | Volume 4 | Article 175 | 196

Frontiers in Immunology | Immunological Tolerance July 2013 | Volume 4 | Article 175 | 196

MSCs in vascularized composite allotransplantation
not migrate beyond the lungs after intravenous infusion. *Front Immunol* (2012) 3:297. doi:10.3389/fimmu.2012.00297

73. Gao J, Dennis JE, Muzic RF, Lundberg M, Caplan AI. The dynamic in vivo distribution of bone marrow-derived mesenchymal stem cells after infusion. *Cells Tissues Organs* (2001) 169:12–20. doi:10.1159/000047856

74. Schreper S, Deuse T, Reichen-spurner H, Fischbein MP, Robbins RC, Pelletier MP. Stem cell transplantation: the lung barrier. *Transplant Proc* (2007) 39:573–6. doi:10.1016/j.transproceed.2006.12.019

75. Fischer UM, Harting MT, Jimenez F, Monzon-Posasas WO, Xue H, Savitz SI, et al. Pulmonary pas-sage is a major obstacle for intra-venous stem cell delivery: the pul-monary first-pass effect. *Stem Cells Dev* (2009) 18:683–92. doi:10.1089/scd.2008.0253

76. Rombouts WJ, Ploemacher RE. Pri-mary murine MSC show highly effi-cient homing to the bone marrow but lose homing ability following culture. *Leukemia* (2003) 17:160–70. doi:10.1038/sj.leu.2402763

77. Schlösser S, Dennler G, Schweizer R, Eberli D, Stein JV, Enzmann V, et al. Paracrine effects of mesenchymal stem cells enhance vascular regen-eration in ischemic murine skin. *Microvasc Res* (2012) 83:267–75. doi:10.1016/j.mvr.2012.02.011

78. Karp JM, Leng Teo GS. Mesenchymal stem cell homing: the devil is in the details. *Cell Stem Cell* (2009) 4:206–16. doi:10.1016/j.stem.2009.02.001

79. Wagner W, Horn P, Castoldi M, Diehlmann A, Bork S, Saffrich R, et al. Replicative senescence of mesenchymal stem cells: a continua-tious and organized process. *PLoS ONE* (2008) 3:e2213. doi:10.1371/journal.pone.0002213

80. Hematti P. Mesenchymal stom-al cells and fibroblasts: a case of mistaken iden-tity? *Cytotherapy* (2012) 14: 536–21. doi:10.3109/14653249.2012.677722

81. Wynn RF, Hart CA, Corradi-Perini C, O’Neill L, Evans CA, Wrath JE, et al. A small proportion of mesenchymal stem cells strongly expresses functionally active CXCR4 receptor capable of promoting migration to bone marrow. *Blood* (2004) 104:2643–5. doi:10.1182/blood-2004-02-0526

82. Ruster B, Gottig S, Ludwig RJ, Bis-trian R, Muller S, Seifried E, et al. Mesenchymal stem cells display coordinated rolling and adhesion behavior on endothelial cells. *Blood* (2006) 108:3938–44. doi:10.1182/blood-2006-05-025098

83. Pinney DG, Prokop DJ. Concise review: mesenchy-mal stem/multipotent stromal cells: the state of transdiffer-entiation and modes of tissue regeneration in ischemic murine skin. *Stem Cells* (2007) 25:2896–902. doi:10.1634/stemcells.2007-0637

84. Sackstein R, Merzaban JS, Cain DW, Dagia NM, Spencer IA, Lin CP, et al. Ex vivo glycan engineering of CD44 programs human multipotent mesenchymal stromal cell trafficking to bone. *Nat Med* (2008) 14:181–7. doi:10.1038/nm1703

85. Vacanti V, Kong E, Suzuki G, Sato K, Canty JM, Lee T. Phenotypic changes of adult porcine mesenchy-mal stem cells induced by prolonged passaging in culture. *J Cell Physiol* (2005) 205:194–201. doi:10.1002/jcp.20376

86. Wagner W, Ho AD, Zenke M. Dif-ferent facets of aging in human mesenchymal stem cells. *Tissue Eng Part B Rev* (2010) 16:445–53. doi:10.1089/teng.TEB.2009.0825

87. Kuo YR, Chen CC, Shih HS, Goto S, Huang CW, Wang CT, et al. Prolongation of composite tissue allotransplant survival by treatment with bone marrow mesenchymal stem cells is correlated with T-cell regulation in a swine hind-limb model. *Plast Reconstr Surg* (2011) 127:569–79. doi:10.1097/PRS.0b013e318200a92c

88. Kuo YR, Chen CC, Goto S, Huang YT, Wang CT, Tsai CC, et al. Immunomodulatory effects of bone marrow-derived mesenchymal stem cells in a swine hemic-facial allotransplantation model. *PLoS ONE* (2012) 7:e35459. doi:10.1371/journal.pone.0035459

89. Schneeberger S, Gorantla VS, Bran-dacher G, Zeevi A, Demetris AJ, Lunz JG, et al. Upper-extremity transplantation using a cell-based protocol to mini-mize immunosuppression. *Ann Surg* (2013) 257:345–51. doi:10.1097/SLA.0b013e31826a90f6

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any com-mercial or financial relationships that could be construed as a potential con-flict of interest.

Received: 06 May 2013; paper pending publish-able: 27 May 2013; accepted: 18 June 2013; published online: 23 July 2013. Citation: Plock IA, Schneider JT, Solari MG, Zheng XQ and Gorantla VS (2013) Perspectives on the use of mesenchymal stem cells in vascularized composite allo-transplantation. Front. Immunol. 4:175. doi:10.3389/fimmu.2013.00175

This article was submitted to Frontiers in Immunological Tolerance, a specialty of Frontiers in Immunology. Copyright © 2013 Plock, Schneider, Solari, Zheng and Gorantla. This is an open-access article distributed under the terms of the Creative Commons Attribu-tion License, which permits use, distribu-tion and reproduction in other forums, provided the original authors and source are credited and subject to any copy-right right notices concerning any third-party graphics etc.