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The use of adipose-derived stem cells as sheets for wound healing

Meghan M. McLaughlin[1] and Kacey G. Marra[1-3,*]

[1]Department of Plastic Surgery; University of Pittsburgh; Pittsburgh, PA USA; [2]Department of Bioengineering; University of Pittsburgh; Pittsburgh, PA USA; [3]McGowan Institute for Regenerative Medicine; University of Pittsburgh; Pittsburgh, PA USA

Cellular therapies have shown immense promise in the treatment of nonhealing wounds. Cell sheets are an emerging strategy in tissue engineering, and these cell sheets are promising as a delivery method of mesenchymal stem cells to the wound bed. Cell sheet technology utilizes temperature dependent polymers to allow for lifting of cultured cells and extracellular matrix without the use of digestive enzymes. While mesenchymal stem cells (MSCs) have shown success in cell sheets for myocardial repair, examination of cell sheets in the field of wound healing has been limited. We previously developed a novel cell sheet composed of human adipose-derived stem cells (ASCs). Both single and triple layer cell sheets were examined in a full-thickness murine wound model. The treatment cell sheets were compared with untreated controls and analyzed at timepoints of 7, 14, 18 and 21 d. The ASC cell sheets showed increased healing at 7, 14 and 18 d, and this effect was increased in the triple layer cell sheet group. Future development of these cell sheets will focus on increasing angiogenesis in the wound bed, utilizing multiple cell types, and examining allogeneic cell sheets. Here we review our experiment, expand upon our future directions and discuss the potential of an off-the-shelf cell sheet. In the field of wound healing, such a cell sheet is both clinically and scientifically exciting.

Keywords: cell sheet, mesenchymal stem cell, adipose-derived stem cell, bone marrow-derived stem cell, wound healing, angiogenesis

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*Correspondence to: Kacey G. Marra;
Email: marrak@upmc.edu

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There is a pressing clinical need for novel methods to treat chronic and nonhealing wounds. Tissue engineering has developed cellular therapies to address this gap in clinical care. Specifically, stem cell therapy has demonstrated the ability to increase vascularization of the wound bed and accelerate healing. However, the challenge of cell survival remains. Cell sheets are a new development in regenerative medicine and show great promise in the area of wound healing. While past therapies have administered cells via injection or scaffold, cell sheets prevent the cell loss often associated with these methods.[1] Cell sheets allow for the delivery of cultured cells and their deposited extracellular matrix (ECM) without the damaging trypsinization of the cells.[2] These sheets include not only the ECM, but also ECM deposited proteins and maintained cellular junctions.[3]

Cell sheets are currently being investigated in many areas of tissue engineering. This technology has been implemented in corneal reconstruction,[4] treatment of esophageal ulceration,[5] periodonty,[6] cardiac repair[7-9] and wound healing,[10,11] our focus here. Both monolayer and multilayer cell sheets have shown success in an in vivo setting.[7,12]

Cell sheets utilize poly (N-isopropylacrylamide) (PIPAAm), a temperature responsive polymer. PIPAAm is used to coat culture dishes, and at temperatures above 32° (normal cell culture conditions), the culture surface is hydrophobic. This allows for normal adhesion and culture of cells. However, at temperatures below 32°, the surface becomes hydrophilic and cells spontaneously detach as a monolayer.[1,12] The cell sheets containing both cells and ECM can then be harvested and utilized in a number of applications.

While research has examined numerous cell types in the context of cell sheet

engineering, we have focused on the use of mesenchymal stem cells (MSCs). MSCs are adult stem cells that are most often isolated from bone marrow or adipose tissue. These cells are plentiful and multipotent. MSCs have been shown to differentiate into osteocytes, adipocytes, chondrocytes and myocytes,[13] as well as neurons and cardiomyocytes.[14]

There have been a limited number of studies that have examined cell sheets composed of MSCs. In vitro studies have shown that MSCs remain undifferentiated in culture, maintaining their stem cell properties.[15] Bone marrow-derived mesenchymal stem cell sheets have also been shown to remain viable and retain their multipotentiality when induced to differentiate. Furthermore, these cell sheets have been shown to deposit increased collagen over time during culture.[16]

MSC sheets have also been examined in an in vivo setting. Adipose-derived MSCs have been shown to repair scarred myocardium in rats. These cell sheets exhibited increased release of angiogenic factors when compared with a fibroblast control.[14] The use of MSC sheets in a hind limb ischemia model has also demonstrated enhanced angiogenesis.[17] Recently, investigators found that bone marrow-derived stem cell sheets were able to increase paracrine effects and activate signaling pathways, leading to increased cardiac function following heart failure in a rat model.[18]

For a number of years MSCs have played an important role in tissue engineering in the field of wound healing. As cited above, MSCs have the ability to differentiate into multiple lineages and have the capacity to secrete many growth factors associated with angiogenesis.[19] Both bone marrow-derived and adipose-derived stem cells have shown increased angiogenesis in wound healing when injected or delivered via a scaffold.[20] Furthermore, these cells have shown increased healing in both burn models and full-thickness wound models.[13] However, injection provides inefficient delivery of cells and scaffolds are accompanied by the risk of infection. The cell sheet model offers a unique delivery method that can best take advantage of the angiogenic properties of these MSCs.

Recently we investigated a multi-layer cell sheet comprised of human adipose-derived stem cells (ASCs) in the context of wound healing.[21] Cell sheets were composed of human adipose-derived stem cells that were isolated from human lipoaspirate in a protocol previously established in our laboratory.[22] These cells were cultured on temperature-responsive cell culture surfaces as described above (Thermo Scientific Nunc Upcell Surface). When applicable, these sheets were transferred using a fibrin patty and stacked to form a multi-layered cell sheet.

The in vivo portion of the study included three groups; a single-layer ASC cell sheet, a triple layer ASC cell sheet and an untreated control. Two 12-mm, full-thickness wounds were created on the backs of athymic nude mice. Cell sheets were applied directly to the wound bed at the time of healing. All wounds were splinted to prevent contraction. Wounds were photographed at 0, 7, 14, 18 and 21 d and harvested at each of the above time points for further histological analysis.

Examination of the cell sheets in vitro demonstrated that the ASCs retained their undifferentiated properties. qPCR analysis revealed no difference in FABP4, PPAR-γ and CD34+ expression between the cell sheets and cultured ASCs. However, both the cell sheets and the cultured ASCs showed significant differences from mature adipocytes. Importantly, immunohistochemistry was performed to stain for human lamin A/C. Positive staining was seen at all time points in harvested wounds, confirming the presence of ASCs in the wound bed until time of healing.

Examination of the wound area during the in vivo portion of the study revealed that the treatment groups showed significantly increased healing at days 7, 10, 14 and 18. This healing was increased in the triple layer ASC cell sheet group compared with the single layer ASC cell sheet group at 7, 10 and 14 d. These results are very promising; however, there was no difference between groups at 21 d. Future studies will focus on decreasing overall time to closure. This study suggests that the ASC cell sheet has the ability to recruit wound healing factors and increase proliferation and migration compared with the untreated control. This study has demonstrated the utility of an adipose derived stem cell sheet in full-thickness wound healing. Future studies will aim to improve upon the vasculogenesis in vivo and to optimize the ASC cell sheet as a wound healing strategy.

In the investigation of new wound healing strategies, increasing angiogenesis and vasculogenesis is key. Vascularization plays a fundamental role in wound healing and therefore is an important parameter for novel therapies.[11] While the results of our study showed a trend toward increased vascular density in the cell sheet treated groups, CD31 staining revealed that there was no significant difference in the vessel density between our treatment groups and control group. Moving forward, increasing the vascular density of the wound bed could accelerate overall time to healing and could also markedly improve the matrix organization of the wound. ASCs have great potential in their ability to release angiogenic factors, and perhaps the complex pathway leading to neovascularization requires more than one cell type. Increased vascularity has been seen in models using endothelial cells and fibroblasts. The ideal therapy may include a cell sheet comprised of multiple cell types.

It is important to note that ASCs were still detected in the wound bed at the time of full healing (21 d). A labeling method that would allow for cell detection in vivo could identify the fate of the cell sheet. While ASCs remained in the wound bed throughout the study, the proportion of remaining cells is unknown. Bone marrow-derived stem cell sheets have been shown to increase initial retention of cells compared with an injection of stem cells. However, overall survival of the cells has not been shown to increase.[18] Factors such as initial retention and survivability of the stem cells should be monitored in the ASC cell sheets to see if similar patterns are observed. Cell tracking could also be used to note any cellular migration or differentiation to better understand the behavior of these cell sheets in vivo.

Finally, there are a number of limits to the athymic mouse model. Since the skin of this model contracts, the wound bed must be splinted open to better mimic human healing. While this splitting prevents contraction, the skin of athymic

mice remains very different from human skin. It is necessary to begin studies in a small model, but once success has been shown in the athymic model it is important to validate results in a larger animal, such as a porcine model, that can better mimic the healing process of human skin.

Mesenchymal stem cell sheets, specifically those fabricated from bone marrow-derived and adipose-derived stem cells, show great promise as a tissue engineering technique in the scope of wound healing. Through increased delivery of growth factors and enhanced cell survival, stem cell sheets may be able to increase angiogenesis in the wound bed and could have wide clinical implications in chronic and acute wound healing.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Elloumi-Hannachi I, Yamato M, Okano T. Cell sheet engineering: a unique nanotechnology for scaffold-free tissue reconstruction with clinical applications in regenerative medicine. J Intern Med 2010; 267:54-70; PMID:20059644; http://dx.doi.org/10.1111/j.1365-2796.2009.02185.x
2. Yamada N, Okano T, Sakai H, Karikusa F, Sawasaki Y, Sakurai Y. Thermo-responsive polymeric surfaces; control of attachment and detachment of cultured cells. Makromol Chem, Rapid Commun 1990; 11:571-6; http://dx.doi.org/10.1002/marc.1990.030111109
3. Yang J, Yamato M, Shimizu T, Sekine H, Ohashi K, Kanzaki M, et al. Reconstruction of functional tissues with cell sheet engineering. Biomaterials 2007; 28:5033-43; PMID:17761277; http://dx.doi.org/10.1016/j.biomaterials.2007.07.052
4. Nishida K, Yamato M, Hayashida Y, Watanabe K, Maeda N, Watanabe H, et al. Functional bioengineered corneal epithelial sheet grafts from corneal stem cells expanded ex vivo on a temperature-responsive cell culture surface. Transplantation 2004; 77:379-85; PMID:14966411; http://dx.doi.org/10.1097/01.TP.0000110320.45678.30
5. Takagi R, Yamato M, Kanai N, Murakami D, Kondo M, Ishii T, et al. Cell sheet technology for regeneration of esophageal mucosa. World J Gastroenterol 2012; 18:5145-50; PMID:23066307
6. Hasegawa M, Yamato M, Kikuchi A, Okano T, Ishikawa I. Human periodontal ligament cell sheets can regenerate periodontal ligament tissue in an athymic rat model. Tissue Eng 2005; 11:469-78; PMID:15869425; http://dx.doi.org/10.1089/ten.2005.11.469
7. Sekiya N, Matsumiya G, Miyagawa S, Saito A, Shimizu T, Okano T, et al. Layered implantation of myoblast sheets attenuates adverse cardiac remodeling of the infarcted heart. J Thorac Cardiovasc Surg 2009; 138:985-93; PMID:19769884; http://dx.doi.org/10.1016/j.jtcvs.2009.02.004
8. Matsuura K, Honda A, Nagai T, Fukushima N, Iwanaga K, Tokunaga M, et al. Transplantation of cardiac progenitor cells ameliorates cardiac dysfunction after myocardial infarction in mice. J Clin Invest 2009; 119:2204-17; PMID:19620770
9. Masuda S, Shimizu T, Yamato M, Okano T. Cell sheet engineering for heart tissue repair. Adv Drug Deliv Rev 2008; 60:277-85; PMID:18006178; http://dx.doi.org/10.1016/j.addr.2007.08.031
10. Ng KW, Hutmacher DW. Reduced contraction of skin equivalent engineered using cell sheets cultured in 3D matrices. Biomaterials 2006; 27:4591-8; PMID:16720038; http://dx.doi.org/10.1016/j.biomaterials.2006.04.020
11. Hendrickx B, Verdonck K, Van den Berge S, Dickens S, Eriksson E, Vranckx JJ, et al. Integration of blood outgrowth endothelial cells in dermal fibroblast sheets promotes full thickness wound healing. Stem Cells 2010; 28:1165-77; PMID:20506500
12. Okano T, Yamada N, Okuhara M, Sakai H, Sakurai Y. Mechanism of cell detachment from temperature-modulated, hydrophilic-hydrophobic polymer surfaces. Biomaterials 1995; 16:297-303; PMID:7772669; http://dx.doi.org/10.1016/0142-9612(95)93257-E
13. Cherubino M, Rubin JP, Miljkovic N, Kelmendi-Doko A, Marra KG. Adipose-derived stem cells for wound healing applications. Ann Plast Surg 2011; 66:210-5; PMID:21200308; http://dx.doi.org/10.1097/SAP.0b013e3181e6d06c
14. Miyahara Y, Nagaya N, Kataoka M, Yanagawa B, Tanaka K, Hao H, et al. Monolayered mesenchymal stem cells repair scarred myocardium after myocardial infarction. Nat Med 2006; 12:459-65; PMID:16582917; http://dx.doi.org/10.1038/nm1391
15. Nash ME, Fan X, Carroll WM, Gorelov AV, Barry FP, Shaw G, et al. Thermoresponsive substrates used for the expansion of human mesenchymal stem cells and the preservation of immunophenotype. Stem Cell Rev 2013; 9:148-57; PMID:23354660; http://dx.doi.org/10.1007/s12015-013-9428-5
16. See EY, Toh SL, Goh JC. Multilineage potential of bone-marrow-derived mesenchymal stem cell cell sheets: implications for tissue engineering. Tissue Eng Part A 2010; 16:1421-31; PMID:19951089; http://dx.doi.org/10.1089/ten.tea.2009.0501
17. Ishii M, Shibata R, Numaguchi Y, Kito T, Suzuki H, Shimizu K, et al. Enhanced angiogenesis by transplantation of mesenchymal stem cell sheet created by a novel magnetic tissue engineering method. Arterioscler Thromb Vasc Biol 2011; 31:2210-5; PMID:21757660; http://dx.doi.org/10.1161/ATVBAHA.111.231100
18. Narita T, Shintani Y, Ikebe C, Kaneko M, Campbell NG, Coppen SR, et al. The use of scaffold-free cell sheet technique to refine mesenchymal stromal cell-based therapy for heart failure. Mol Ther 2013; 21:860-7; PMID:23358187; http://dx.doi.org/10.1038/mt.2013.9
19. Gharaee-Kermani M, Phan SH. Role of cytokines and cytokine therapy in wound healing and fibrotic diseases. Curr Pharm Des 2001; 7:1083-103; PMID:11472255; http://dx.doi.org/10.2174/1381612013397573
20. Branski LK, Gauglitz GG, Herndon DN, Jeschke MG. A review of gene and stem cell therapy in cutaneous wound healing. Burns 2009; 35:171-80; PMID:18603379; http://dx.doi.org/10.1016/j.burns.2008.03.009
21. Lin YC, Grahovac T, Oh SJ, Ieraci M, Rubin JP, Marra KG. Evaluation of a multi-layer adipose-derived stem cell sheet in a full-thickness wound healing model. Acta Biomater 2013; 9:5243-50; PMID:23022891; http://dx.doi.org/10.1016/j.actbio.2012.09.028
22. Lin YC, Brayfield CA, Gerlach JC, Rubin JP, Marra KG. Peptide modification of polyethersulfone surfaces to improve adipose-derived stem cell adhesion. Acta Biomater 2009; 5:1416-24; PMID:19129013; http://dx.doi.org/10.1016/j.actbio.2008.11.031