The Role of Adipose-Derived Stem Cell Angiogenesis in Breast Reconstruction with Fat Grafting: Literature Review

Abstract

Fat grafting has been widely used in breast reconstruction surgery by plastic reconstructive surgeons for various diseases in the last few decades. Fat grafting had the advantages of being biocompatible, easy access, cost-effective, minimal complications, and minimal injury to the donor sites. However, with the development of liposuction methods and various survival fat volume prediction tools, although autologous fat grafting technology has been gradually improved over a century, the fat graft loss rate remains the biggest problem ranging from 20 to 90% at 1 year later. In our review, we will review the angiogenesis process that occurs in the stromal vascular fraction in fat grafting breast reconstruction.

Keywords: angiogenesis, stromal vascular fraction, fat grafting, breast reconstruction

Introduction

Adipose-Derived Stem Cells (ASCs) are localized in the Stromal Vascular Fraction (SVF) of subcutaneous adipose tissue, which has a heterogeneous collection of mesenchymal cells [2].SVF is obtained by separating lipoaspiration enzymatically or mechanically. SVF consists of the non-adipocyte fat fraction and consists of stromal cells such as fibroblasts and other mesenchymal stromal cells as well as vascular cells (endothelial and smooth muscle). Stromal cells produce and maintain the extracellular matrix that attaches and supports cells in the SVF. Recently, intraoperative mechanical isolation procedures have been developed to produce tissue-derived stromal vascular fraction (tSVF), which makes the clinical application of tSVF more feasible than enzymatic isolated SVF (cellular SVF) [3].

Characteristics of Adipose-Derived Stem Cells

ASC has the ability of self-regeneration, asymmetric division, and pluripotency. Further differentiation of SVF into various cellular populations has been reported by Li et

al., based on CD31/CD34 and CD146 status: mature endothelial cells (CD31+/CD34−), endothelial stem cells (CD31+/CD34+), ASCs (CD31−/ CD34+) and pericytes (CD146+/CD31−/CD34−) [4]. Cellular markers of ASCs and their subpopulations, coupled with their location adjacent to blood vessels, contribute to their characteristics as described previously. The components of SVF are summarized in **Figure. 1** [5]. Additionally, ASC does not express Major Histocompatibility Complex-II and thus has potential immunomodulatory functions that benefit post-transplantation recipient sites [2,4].

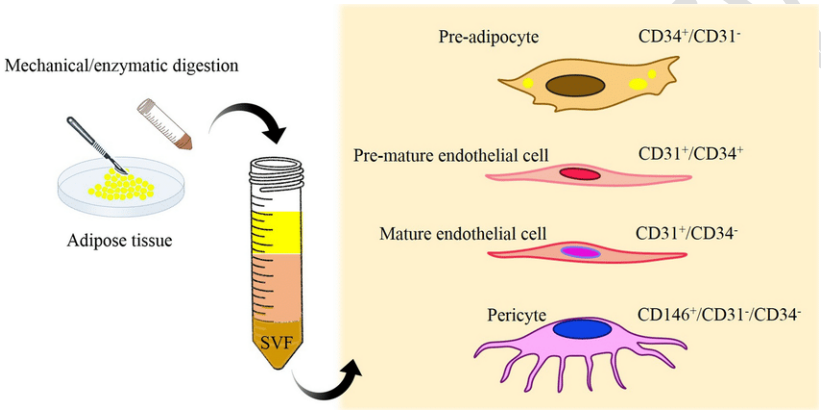

Figure. 1. The cellular components of the stromal vascular fraction (SVF). Four abundant cell populations with different surface markers have been characterized within SVF

Fat Grafting in Breast Reconstruction

Fat grafting has been widely used in breast reconstruction surgery by plastic reconstructive surgeons for various diseases in the last few decades. Fat grafting had the advantages of being biocompatible, easy access, cost-effective, minimal complications, and minimal injury to the donor sites [1]. ASCs originate from the stromal components of adipose tissue, and have the potential to proliferate extensively and differentiate into various cell lineages such as adipogenic, osteogenic, myogenic, chondrogenic, and vascular endothelial cell types [6]. In addition, the easy retrieval of ASCs, low donor site morbidity and the fact that adipose tissue aspirates are thought to contain much higher concentrations of stem cells compared with bone marrow, make these cells an attractive tool in improving Long-term survival of fat grafting. Preclinical studies also show that ASCs can contribute to adipose tissue regeneration and maintain graft viability by promoting angiogenesis [6].

Additionally, two concerns have emerged regarding the application of stem cell-based techniques in breast reconstruction surgery. The possibility of graft contamination during extracorporeal stem cell enrichment procedures requires special attention in the sterilization process [4,6]. Another controversial issue is the alleged cross-talk between stem cells and breast carcinogenesis [6]. It has been suggested that stem cells can induce molecular cascades and changes in the breast microenvironment that can promote de novo carcinogenesis, proliferation of residual cancer cells and metastasis [4,6]. Available data from preclinical and clinical studies are conflicting, although results from preclinical models support the theory, clinical studies have not reported an increased risk of recurrence [4]. The number of stem cells transplanted is also believed to play an important role in the observed mismatch. However, important cutoff values cannot be determined with the existing evidence [6].

Currently, advances in the technical aspects of autologous fat grafting have limited the incidence of complications. As noted by Groen et al., the total complication rate was lower (8.4%, 95% CI 7.6–9.1) compared to other breast reconstruction procedures [4].

Adipose-derived Stem Cells Angiogenesis in Fat Grafting

Angiogenic Properties of ASCs

Adipose-derived mesenchymal stem/stromal cells can induce angiogenesis **(Figure. 2),** and they exert their pro-angiogenic effects mainly through paracrine secretion [7]. The hASC secretome is rich in several growth factors and cytokines, many of which are known to be pro-angiogenic. These include VEGF, fibroblast growth factor 2 (FGF-2, also known as basic fibroblast growth factor), platelet-derived growth factor (PDGF), transforming growth factor β (TGF-β) and hepatocyte growth factor (HGF) [7]. The abundance of these growth factors makes ASCs attractive because they can influence many different pathways and mechanisms of angiogenesis.

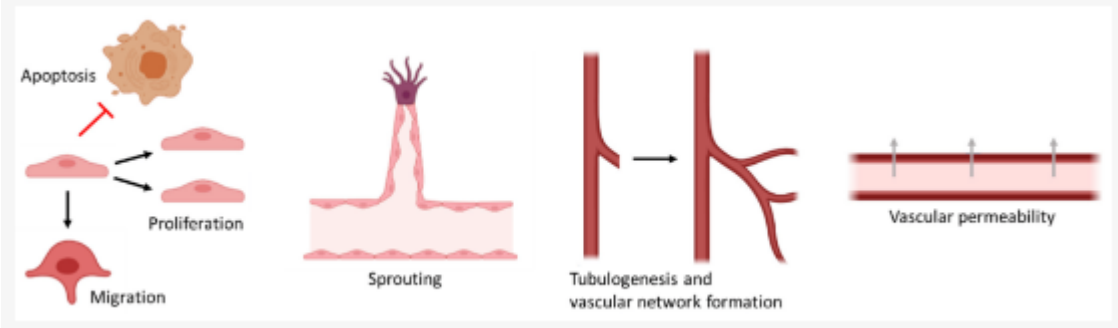

Figure. 2. Mechanisms of angiogenesis induced by adipose-derived mesenchymal stem/stromal cells (ASCs). ASCs promote endothelial cell (EC) proliferation and migration while inhibiting apoptosis. Proliferating and migrating ECs form tubular structures, and this initial vascular network is further organized by vessel sprouting, i.e., formation of new vessels from existing ones in response to angiogenic stimuli. In mature blood vessels, ASCs regulate vascular permeability.

ASCs express β-1 integrin (CD29) which participates in angiogenesis and CD44 hyaluronate and osteopontin receptors, which are essential in the development of the extracellular matrix and pathological processes such as neoplasia [1].In vitro and in vivo studies show that ASCs are often found lining the outside of microvessels, and they assume a stabilizing role by differentiating into pericytes and by expressing common pericyte surface proteins such as the smooth muscle late pericyte marker α-actin (α-SMA), neuronal/glial antigen 2 (NG2) or platelet-derived growth factor receptor β (PDGFRβ) [7]. IGF-1 upregulates α-SMA in ASCs, indicating that it can induce ASC differentiation toward pericyte-like cells **(Figure. 3)**.

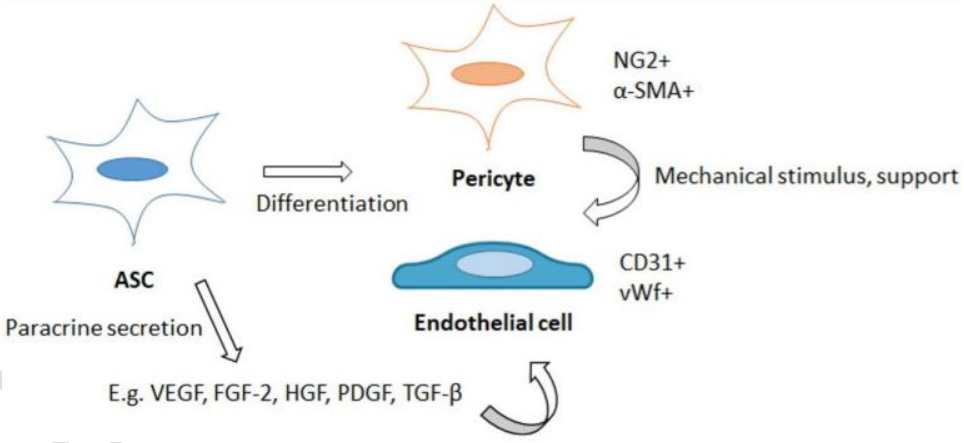

Figure. 3. ASCs stimulate angiogenesis via paracrine secretion of growth factors, which regulate endothelial cells, and by differentiating into ECs expressing, e.g., CD31 and vWf, or by developing pericyte characteristics, including expression of NG2 and α-SMA. Pericytes provide an additional mechanical stimulus and support for ECs to stabilize developing vascular structures.

In other study, EPCs from human peripheral blood or HUVECs were combined with ASCs in a fibrin matrix and implanted on the chorioallantois membrane (CAM) of fertilized chicken eggs [8]. The results showed that ASC induced the migration of blood

vessels into fibrin structures either independently or when administered together with EPC or HUVEC. Capillary-like structures and significantly more perfused blood vessels were observed in ASC-HUVEC implants compared with ASC-EPC implants. When EPCs or HUVECs were transplanted alone, no blood vessels or only a few blood vessels were observed. Based on these results, it appears that the origin of ECs influences vascularization potential, with mature ECs serving as a more potent alternative to EPCs or ECFCs. On the other hand, no significant formation of vascular structures occurred after EC alone transplantation when implanted subcutaneously using scaffold materials, while ASC induced angiogenesis even when transplanted alone. It has been suggested that the scaffold matrix is not required for vasculogenesis after co-transplantation because cells delivered in media induce similar CD31-positive functional vasculature compared with cells transplanted in collagen-fibronectin gel [9]. It is possible that ASCs provide sufficient support for ECs to facilitate angiogenesis. The role of ASCs as a perivascular cell type after co-transplantation has been demonstrated in several studies

Conclusion

Adipose-derived mesenchymal stem/stromal cells have been studied extensively in the last two decades for their ability to modulate the immune system and participate in tissue regeneration. Because these cells can be easily harvested from adipose tissue, their low donor site morbidity makes them a better choice than bone marrow-derived mesenchymal stem cells. Basic research in co-culture with endothelial cells has demonstrated pro-angiogenic effects of ASC through paracrine secretion or through direct cell contact where ASC supports the formation of tube-like structures. Supplementation of IGF-1 in the culture medium promotes the expression of growth factors in ECs and ASCs that are important for angiogenesis via the PI3K/AKT signaling pathway. In addition, PDGFRβ activation further promotes vascular tissue formation in vitro, whereas activin A, secreted by ASCs, inhibits vascular tissue formation. ASCs can differentiate into endothelial cells, especially under three-dimensional culture conditions. FGF-2 and activation of the PI3K/AKT signaling pathway are critical in ASC endothelial differentiation. Additionally, ASCs can participate in the stabilization of microvessels by differentiating into pericytes.

References

1. Anna Trzyna and Agnieszka Banaś-Ząbczyk. Adipose-Derived Stem Cells Secretome and Its Potential Application in “Stem Cell-Free Therapy”. Biomolecules. 2021 Jun; 11(6): 878. Published online 2021 Jun 13. doi: 10.3390/biom11060878.

2. Pietro Gentile, Donato Casella, Enza Palma, Claudio Calabrese. Engineered Fat Graft Enhanced with Adipose-Derived Stromal Vascular Fraction Cells for Regenerative Medicine: Clinical, Histological and Instrumental Evaluation in Breast Reconstruction. J Clin Med. 2019 Apr; 8(4): 504. Published online 2019 Apr 12. doi: 10.3390/jcm8040504.

3. Linda Vriend, Joris A. van Dongen, Anouk Pijpe, Marianne K., Nieuwenhuis, Sandra J. M. Jongen, Martin C. Harmsen, Paul P. M. van Zuijlen, Berend van der Lei. Stromal vascular fraction-enriched fat grafting as treatment of adherent scars: study design of a non-randomized early phase trial. Trials. 2022; 23: 575. Published online 2022 Jul 19. doi: 10.1186/s13063-022-06514-3.

4. Shaun S Tan, Zhi Yang Ng, Weiqing Zhan, Warren Rozen. Role of Adipose-derived Stem Cells in Fat Grafting and Reconstructive Surgery. J Cutan Aesthet Surg. 2016 Jul-Sep; 9(3): 152–156. doi: 10.4103/0974-2077.191672.

5. Saber Khazaei, Ghazal Keshavarz, Azam Bozorgi, Mozafar Khazaei. Adipose tissue-derived stem cells: a comparative review on isolation, culture, and differentiation methods. Cell and Tissue Banking 23(1), March 2022. DOI: 10.1007/s10561-021-09905-z.

6. Anastasios Tsekouras, Dimitrios Mantas, Diamantis I. Tsilimigras, Ioannis Ntanasis-Stathopoulos, Michael Kontos, George C. Zografos. Adipose-derived stem cells for breast reconstruction after breast surgery – preliminary results. Case Reports Plast Surg Hand Surg. 2017; 4(1): 35–41. Published online 2017 Apr 19. doi: 10.1080/23320885.2017.1316201.

7. Swarna Rautiainen , Timo Laaksonen , Raili Koivuniemi. Angiogenic Effects and Crosstalk of Adipose-Derived Mesenchymal Stem/Stromal Cells and Their

Extracellular Vesicles with Endothelial Cells. Int J Mol Sci. 2021 Oct 8;22(19):10890. doi: 10.3390/ijms221910890.

8. Strassburg, S.; Nienhueser, H.; Stark, G.B.; Finkenzeller, G.; Torio-Padron, N. Co-culture of adipose-derived stem cells and endothelial cells in fibrin induces angiogenesis and vasculogenesis in a chorioallantoic membrane model. *J. Tissue Eng. Regen. Med.* 2016, *10*, 496–506.

9. Traktuev, D.O.; Prater, D.N.; Merfeld-Clauss, S.; Sanjeevaiah, A.R.; Saadatzadeh, M.R.; Murphy, M.; Johnstone, B.H.; Ingram, D.A.; March, K.L. Robust functional vascular network formation in vivo by cooperation of adipose progenitor and endothelial cells. *Circ. Res.* 2009, *104*, 1410–1420.