Tropical Journal of Pharmaceutical Research January 2022; 21 (1): 73-78
ISSN: 1596-5996 (print); 1596-9827 (electronic)
© Pharmacotherapy Group, Faculty of Pharmacy, University of Benin, Benin City, 300001 Nigeria.

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v21i1.12

Original Research Article

Effect of bone marrow mesenchymal stem cells on tissue reconstruction and angiogenesis following fat transplantation

Yao Shi[1]*, Li Shi[2]
[1]Department of Breast Surgery, The First Hospital of China Medical University, Shenyang, Liaoning 110001, [2]Department of Orthopaedic Surgery, The First Affiliate Hospital of Liaoning University of Traditional Chinese Medicine, Shenyang, Liaoning 110847, PR China

For correspondence:* ***Email:* *b7pcqx@163.com*

Sent for review: 1 October 2021 *Revised accepted: 7 January 2022*

Abstract

Purpose: *To investigate the effect of bone marrow mesenchymal stem cells (BMSCs) on tissue reconstruction and microvascular formation after fat transplantation.*
Methods: *Adipose tissue from male BALB/C nude mice were used to extract and culture BMSCs. Eighty mice were assigned to control and study groups. Control mice were injected with 0.3 g adipose tissue in normal saline, while the study group was injected with 0.3 g adipose tissue containing 5×10^5 mouse BMSCs. Micro-vessel count was determined by CD31 antibody staining, while VEGF protein expression was assayed by Western blotting.*
Results: *At each time point, the micro-vessel count of adipose tissue was significantly higher in the study group than in controls ($p < 0.05$). At 12 weeks, VEGF protein in adipose tissue of mice was significantly higher in the study mice than in control mice ($p < 0.05$).*
Conclusion: *BMSCs enhance adipose tissue reconstruction and induce adipose tissue microvascular formation through up-regulation of the protein expression of VEGF.*

Keywords: *Bone marrow mesenchymal stem cells, Fat transplant, Tissue reconstruction, Angiogenesis*

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0) and the Budapest Open Access Initiative (http://www.budapestopenaccessinitiative.org/read), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Tissue transplantation aimed at improving or restoring physiological function or appearance is a popular method used for treating soft tissue defects caused by trauma, congenital malformation and surgical excision [1]. Autologous fat tissue is an ideal soft tissue filling material with the advantages of high biocompatibility and immuno-compatibility. Besides, fat tissue can be easily obtained through fat aspiration, a simple process which does not cause appreciable lesions at the donor site. However, due to liquefaction and necrosis after ischemia and hypoxia, autologous fat is easily absorbed, and it may also form cysts and fibrosis, resulting in low survival, high degree of absorption, and poor long-term effect [2,3].

Angiogenesis is crucial in fat transplantation since improved microvascular formation in tissues with fat transplantation is key to

© 2022 The authors. This work is licensed under the Creative Commons Attribution 4.0 International License

enhancing success of the transplantation [4]. Bone marrow-derived mesenchymal stem cells (BMSCs) are important in that they can differentiate into multiple types of cells across the dermal layer and participate in various biological activities of cells [5]. Recently, studies have revealed that under certain conditions, BMSCs participate in the formation of blood vessels by differentiating into fat cells and secreting some cytokines, but not much is known about their effect on transplantation [6].

In the present study, 80 healthy male BALB/C nude mice served as animal model for investigating the effect of BMSCs on tissue reconstruction and microvascular formation in fat transplantation, as well as the associated mechanism.

EXPERIMENTAL

Animals

Eighty healthy male BALB/C nude mice [license number: SYXK (Anhui) 2016-002)], with mean body weight of 23 ± 2 g and mean age of 8 ± 1 weeks, were provided by Maanshan Fengyuan Pharmaceutical Co. Ltd. Each mouse was raised in a single cage at an average temperature of 23 ± 2 ℃ under 12-h light/12-h dark cycle, and allowed *ad libitum* access to feed and water. This research received approval from the Animal Ethical Committee of The First Hospital of China Medical University approval no. 20200981), and performed according to "Principles of Laboratory Animal Care" (NIH publication no. 85-23, revised 1985), [7].

Collection of fat tissues

Fifteen mice were selected at random and sacrificed via cervical dislocation. Mouse subcutaneous fat was excised under aseptic conditions, washed with aseptic saline, and weighed. Each fat weighed 0.3 g, and it was stored in a separate EP tube.

Extraction and culture of BMSCs

Five BALB/C nude mice were randomly selected and sacrificed via cervical dislocation. The mice were fixed in 75 % ethanol for 5 min, and the tibia and femur were removed under aseptic conditions and immersed in PBS buffer. The bone marrow was rinsed with low-sugar cell culture medium (LG-DMEM) until the diaphysis was white and slightly transparent. The cell suspension was collected in a 15-ml centrifuge tube and centrifuged at 100 rpm for 5 min. The cells were re-suspended in LG-DMEM culture medium, and cell count was adjusted to a density of 5×10^6/ mL. The cells were inoculated into 6-well plates and cultured in a 5 % CO_2 incubator at 37 ℃. Then, P3 generation cells at logarithmic growth phase were used for subsequent experiments.

Grouping and establishment of tissue reconstruction model for fat transplantation

The remaining 60 mice were randomly divided into control group and study group. The mice were anesthetized with intraperitoneal injection of 150 µL of 13 % barbitone sodium. Under aseptic conditions, the right back of each mouse was disinfected. A longitudinal incision of about 0.3 cm was made on the right back of each mouse with ophthalmic scissors. The control group was injected with 0.3 g fat tissue in normal saline, while the study group was injected with 0.3 g fat tissue containing 5×10^5 BMSCs from mouse.

Treatments and evaluation of parameters

Fat block volume was measured. Animal sacrifice was done via cervical dislocation at 4, 8 and 2 weeks after implantation of the fat block. Under aseptic conditions, the skin was cut open with ophthalmic scissors, and the transplanted fat tissue block was taken out and photographed. The wet weight of the fat block was obtained using a microscale, while the volume of fat block was measured using quantitative water method.

Hematoxylin and eosin (H&E) staining

The fat blocks were subjected to fixation in 4 % paraformaldehyde, dehydration in gradient alcohol, clearing, wax-embedding, and slicing into 3-µm thick slices which were dried at 40 ℃ and stained with conventional H & E. The pathological morphology of fat tissues of mice in each group was examined and recorded.

Counting and monitoring of micro-vessels

The fat blocks were fixed in 4 % paraformaldehyde, dehydrated in gradient ethanol, cleared, wax-embedded and cut into 3-µm slices. The slices were dried at 60 ℃ and dewaxed, followed by tissue antigen retrieval using high-temperature and high-pressure valve. Then, CD31 antibody was added and incubated overnight, followed by color development using DAB chromogenic agent, hematoxylin staining, neutral gum sealing, and micro-vessel count under a microscope. In this process, blood vessel was indicated as a single endothelial cell or endothelial cell clusters present as yellow or brown granules.

Evaluation of quality of fat survival

The fat blocks were processed into 3-µm thick slices as described earlier for micro-vessels. The slices were dried at 40℃ and subjected to conventional H & E staining. Inflammatory cell infiltration, fibrosis and vesicles were evaluated. The degree of each parameter was divided into 6 grades i.e., from 0 to 5, where 0 meant *absent*, and 5 meant *large*. The scores for inflammatory cell infiltration, fibrosis and vesicles were calculated.

Western blot assay for protein expression of VEGF

Fat tissues in the two groups of mice were weighed, lysed, ground and centrifuged. The supernatant was subjected to protein measurement with BCA method. Then, the total protein was resolved with SDS-polyacrylamide gel electrophoresis and transferred to PVDF membrane which was subjected to incubation with VEGF 1° antibody for 12 h at 4 °C. Subsequently, the membrane was incubated with HRP-linked 2° antibody at room temperature for 2 h. The blots were subjected to ECL and imaged. Levels of VEGF protein were determined in relation to that of beta-actin which served as internal control.

Statistical analysis

The SPSS 20.0 software package was used for statistical analysis. Fat block volume, inflammation score, fibrosis score, vesicle score, micro-vessel count, VEGF protein and other measurement data were in line with normal distribution, and are presented as mean ± SD. Comparison of same index at various times was done with appropriate ANOVA. Difference between the two groups at each time point was compared with independent sample *t*-test, while LSD *t*-test was used to compare differences at various time points in each group. Values of $p < 0.05$ were taken as indicative of statistically significant differences.

RESULTS

Fat block volume

The fat block volumes of the two groups were reduced time-dependently. At each time point, fat block volume was significantly higher in the study group than in control mice. These data are presented in Table 1.

Table 1: Comparison of fat block volume between the two groups at each time point (mean ± SD)

Group	4 weeks (mL)	8 weeks (mL)	12 weeks (mL)
Control	0.35±0.05	0.20±0.02[a]	0.08±0.01[ab]
Study	0.41±0.04[c]	0.31±0.05[ac]	0.18±0.02[abc]
t	4.190	9.135	20.000
P	<0.001	<0.001	<0.001

[a]$P < 0.05$, vs same group at 3 weeks; [b]$p < 0.05$, vs same group at 8 weeks; [c]$p < 0.05$, vs control group at the same time point

Inflammatory, fibrotic and vesicular scores

At 12 weeks, inflammatory, fibrotic and vesicular scores in the study group were markedly lower than the corresponding scores in the control group ($p < 0.05$; Table 2).

Table 2: Comparison of inflammation scores, fibrosis scores and vesicle scores

Group	Inflammation score	Fibrosis score	Vesicle score
Control	2.15±0.11	2.89±0.24	3.19±0.69
Study	1.45±0.08[a]	1.84±0.12[a]	1.95±0.28[a]
t	23.015	17.500	7.447
P-value	<0.001	<0.001	<0.001

[a]$P < 0.05$, vs control group

Morphological features of fat blocks

In the control group, the size of adipose tissue was different from that in the study group; there were more vacuolar defects, and the morphology and cell membranes of adipocytes was blurred. In the study group, the morphology of adipocytes was intact and uniformly arranged; there were obvious angiogenesis, and the number of blood vessels was large. Moreover, the blood vessels were relatively uniformly distributed. These results are shown in Figure 1.

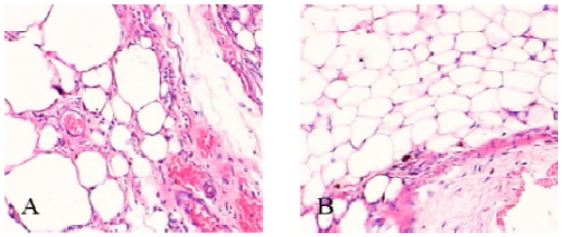

Figure 1: Photomicrographs showing image analysis of fat blocks in the two groups at 12 weeks. A: Fat tissue in control mice; B: fat tissue in study mice (H & E, x100)

Microvascular count of fat tissue

The micro-vessel counts in adipose tissue in the two groups increased with time. At the same time

point, the micro-vessel count in adipose tissue in the study group was markedly higher than control mice value ($p < 0.05$; Table 3).

Table 3: Comparison of microvascular count of fat tissue between the 2 groups at each time point (mean ± SD)

Group	4 weeks	8 weeks	12 weeks
Control	14.56±1.25	16.35±1.46[a]	37.89±2.56[ab]
Study	19.86±0.86[c]	28.79±2.56[ac]	20.46±2.16[abc]
t	15.621	18.877	23.271
P-value	<0.001	<0.001	<0.001

[a]$P < 0.05$, vs same group at 3 weeks; [b]$p < 0.05$, vs same group at 8 weeks; [c]$p < 0.05$, vs control group at the same time point

Protein expression of VEGF in fat tissues

At 12 weeks, VEGF protein in adipose tissue of mice was significantly up-regulated in the study group, relative to control, as shown in Figure 2.

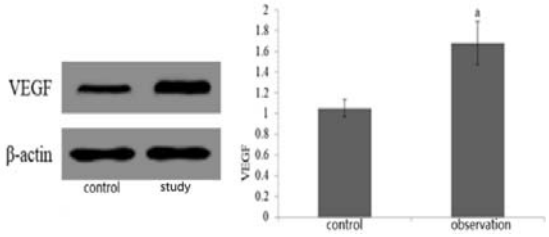

Figure 2: VEGF protein expressions in fat tissues of the two groups

DISCUSSION

Fat transplantation has been applied for more than one hundred years, but due to the high degree of absorption of transplanted fat tissue, the treatment effect of fat transplantation is unstable, and the procedure is rarely used [7]. However, with rapid developments in liposuction technology, fat transplantation, especially autologous fat transplantation, has started to attract increased attention. Fat transplantation is often used in the treatment of congenital malformation, skin depression, atrophy and wrinkles. It is also used for facial rejuvenation, breast augmentation and other cosmetic purposes. Fat transplantation is beneficial in treating vocal cord injury, chronic ulcers and radiation damage, and it results in significant improvements. Autologous fat tissue is convenient for sampling. It is rich in tissue sources, and it has good histocompatibility. However, due to the high energy consumption of fat cells and poor resistance to ischemia and hypoxia, the degree of survival of fat tissue after transplantation is low (about 30 to 60 %) [8,9]. Therefore, studies related to fat transplantation are currently focused on how to speed up blood supply to the transplanted fat tissue and shorten the ischemic period [10].

Mesenchymal stem cells (MSCS) are endogenous cells of the mesoderm which are widely found in bone marrow, umbilical cord, peripheral blood vessels, adipose tissue, amniotic fluid and synovium. The BMSCs have become one of the most studied stem cells in regenerative medicine and tissue engineering, due to their unique characteristics of adherent growth and multi-differentiation potential: they can differentiate into fat, bone, muscle, cartilage and other tissues [11,12]. Previous studies have shown that BMSCs can be transformed into mature adipocytes which can be used as seed cells for the repair of tissue defects and atrophy [13]. In this study, the fat volume of mice was markedly raised in the study group, relative to the control group. Inflammation score, fibrosis score and vesicle score were significantly reduced in study mice, when compared to control mice. Moreover, adipose tissue size was different in the two groups. There were higher level of vacuolar defects in control mice, and the morphology and cell membrane of adipocytes were blurred. In the study group, the morphology of adipocytes was intact and uniformly arranged; there was obvious angiogenesis, and there were a large number of blood vessels which were relatively uniformly distributed. These results suggest that BMSCs can improve adipocyte viability and long-term survival. Recent studies have found that BMSCs enhanced hematopoietic function by decomposing some cytokines [14]. Demir *et al* [15] found that BMSCs could be transformed into vascular smooth muscle cells (VSMCs) under certain conditions, to participate in vascular remodeling of tissues. It was found in this study that the micro-vessel counts in adipose tissue in the two groups increased with time. At the same time point, the micro-vessel count of adipose tissue in the study group was significantly higher than that in the control group. These results indicate that BMSCs promoted microvascular formation and mitigated hypoxia and ischemia in adipose tissue after fat transplantation.

Vascular endothelial growth factor (VEGF) which acts on vascular endothelial cells, has strong enhancing effects on division, chemotaxis, regeneration and repair of vascular endothelial cells. It also induces the formation of primitive vascular network and plays an important regulatory role in vascular formation and regeneration [16,17]. Kobozev *et al* [18] reported that BMSCs induced neovascularization through VEGF and other vascular growth-promoting

factors. In the present study, it was found that VEGF protein level in adipose tissue of mice was markedly up-regulated in the study mice, relative to control mice. These results suggest that BMSCs induced microvascular formation in adipose tissue after transplantation by regulating the expression of VEGF protein, thereby enhancing the degree of success of fat transplantation.

CONCLUSION

This study has demonstrated that BMSCs promotes tissue reconstruction after fat transplantation, induce microvascular formation in fat tissue, and improves long-term survival of fat cells after transplantation. These effects are mediated through up-regulation of the protein expression of VEGF.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was performed by the authors named in this manuscript, and all liabilities pertaining to claims relating to the content of this manuscript will be borne by the authors. Yao Shi designed the study, supervised the data collection, and analyzed the data. Yao Shi interpreted the data and prepared the manuscript for publication. Yao Shi and Li Shi supervised the data collection, analyzed the data and reviewed the draft of the manuscript.

Open Access

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0) and the Budapest Open Access Initiative (http://www.budapestopenaccessinitiative.org/read), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

REFERENCES

1. *Ramalingam R, Worgall S, Rafii S, Crystal RG. Downregulation of CXCR4 gene expression in primary human endothelial cells following infection with E1(-)E4(+) adenovirus gene transfer vectors. Mol Ther 2000; 2(4): 381-386.*
2. *Kang D, Fu S, Luan J. Which Fat Processing Can Achieve Optimal Transplantation in Patients With Insufficient Fat Resource? Ann Plast Surg 2019; 83(4): 459-463.*
3. *Tomc J, Kolosa K, Zegura B, Kamensek U, Breznik B, Turnsek TL, Filipic M. Adipose tissue stem cell-derived hepatic progenies as an in vitro model for genotoxicity testing. Arch Toxicol 2018; 92(5): 1893-1903.*
4. *Calvo-Gallego JL, Dominguez J, Gomez Cia T, Ruiz-Moya A, Gomez Ciriza G, Martinez-Reina J. Comparison of the viscoelastic properties of human abdominal and breast adipose tissue and its incidence on breast reconstruction surgery. A pilot study. Clin Biomech (Bristol, Avon) 2020; 71: 37-44.*
5. *Gad ES, Salama AAA, El-Shafie MF, Arafa HMM, Abdelsalam RM, Khattab M. The Anti-fibrotic and Anti-inflammatory Potential of Bone Marrow-Derived Mesenchymal Stem Cells and Nintedanib in Bleomycin-Induced Lung Fibrosis in Rats. Inflammation 2020; 43(1): 123-134.*
6. *Yang R, Wang X, Liu S, Zhang W, Wang P, Liu X, Ren Y, Tan X, Chi B. Bioinspired poly (γ-glutamic acid) hydrogels for enhanced chondrogenesis of bone marrow-derived mesenchymal stem cells. Int J Biol Macromol 2020; 142: 332-344.*
7. *World Health Organization. Principles of laboratory animal care. WHO Chron 1985; 39: 51-56.*
8. *Wang Y, Zhou S, Yang R, Zou Q, Zhang K, Tian Q, Zhao W, Zong L, Fu Q. Bioengineered bladder patches constructed from multilayered adipose-derived stem cell sheets for bladder regeneration. Acta Biomater 2019; 85: 131-141.*
9. *Lv X, Feng C, Liu Y, Peng X, Chen S, Xiao D, Wang H, Li Z, Xu Y, Lu M. A smart bi-layered scaffold supporting keratinocytes and muscle cells in micro/nano-scale for urethral reconstruction. Theranostics 2018; 8(11): 3153-3163.*
10. *Gilbert-Honick J, Ginn B, Zhang Y, Salehi S, Wagner KR, Mao HQ, Grayson WL. Adipose-derived Stem/Stromal Cells on Electrospun Fibrin Microfiber Bundles Enable Moderate Muscle Reconstruction in a Volumetric Muscle Loss Model. Cell Transplant 2018; 27(11): 1644-1656.*
11. *Xu T, Yu X, Yang Q, Liu X, Fang J, Dai X. Autologous Micro-Fragmented Adipose Tissue as Stem Cell-Based Natural Scaffold for Cartilage Defect Repair. Cell Transplant 2019; 28(12): 1709-1720.*
12. *Murata M, Terakura S, Wake A, Miyao K, Ikegame K, Uchida N, Kataoka K, Miyamoto T, Onizuka M, Eto T, et al. Off-the-shelf bone marrow-derived mesenchymal stem cell treatment for acute graft-versus-host disease: real-world evidence. Bone Marrow Transplant 2021 May 11.*
13. *Liu Y, Li P, Qiao C, Wu T, Sun X, Wen M, Zhang W. Chitosan Hydrogel Enhances the Therapeutic Efficacy of Bone Marrow-Derived Mesenchymal Stem Cells for Myocardial Infarction by Alleviating Vascular Endothelial*

Cell Pyroptosis. J Cardiovasc Pharmacol 2020; 75(1): 75-83.

14. *Wang M, Zhao X, Qiu R, Gong Z, Huang F, Yu W, Shen B, Sha X, Dong H, Huang J, et al. Lymph node metastasis-derived gastric cancer cells educate bone marrow-derived mesenchymal stem cells via YAP signaling activation by exosomal Wnt5a. Oncogene 2021; 40(12): 2296-2308.*
15. *Vasicek J, Kovac M, Balazi A, Kulikova B, Tomkova M, Olexikova L, Curlej J, Bauer M, Schnabl S, Hilgarth M, et al. Combined approach for characterization and quality assessment of rabbit bone marrow-derived mesenchymal stem cells intended for gene banking. N Biotechnol 2020; 54: 1-12.*
16. *Demir S, Erturk A, Gunal YD, Ozmen I, Zengin M, Yildiz D, Karaoz E, Karahan S, Senel E. Contribution of Bone Marrow-Derived Mesenchymal Stem Cells to Healing of Pulmonary Contusion-Created Rats. J Surg Res 2021; 261: 205-214.*
17. *Hu XM, Yang W, Du LX, Cui WQ, Mi WL, Mao-Ying QL, Chu YX, Wang YQ. Vascular Endothelial Growth Factor A Signaling Promotes Spinal Central Sensitization and Pain-related Behaviors in Female Rats with Bone Cancer. Anesthesiol 2019; 131(5): 1125-1147.*
18. *Lima-Ojeda JM, Castren E. Brain-Derived Neurotrophic Factor and Vascular Endothelial Growth Factor: "Siamese Twins" in Antidepressant Action. Biol Psychiatry 2019; 86(2): 81-83.*
19. *Kresnoadi U, Lunardhi LC, Agustono B. Propolis extract and bovine bone graft combination in the expression of VEGF and FGF2 on the preservation of post extraction socket. J Indian Prosthodont Soc 2020; 20(4): 417-423.*