

Effects of mesenchymal stem cells to prevent adhesions for vascular reoperations: An experimental study

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ABSTRACT

Objectives: In the present study, we aimed to investigate whether mesenchymal stem cells (MSCs) were useful to reduce cutaneous adhesions, particularly for vascular reoperations.

Materials and methods: In this experimental study, 12 adult male Wistar Albino rats were used. In Group 1 (n=2, negative control group), no incision was performed. In Group 2 (n=5, positive control group), skin and subcutaneous tissues were incised. In Group 3 (n=5, MSC group), skin and subcutaneous tissues were incised and 3×10⁶ MSCs were applied. Macroscopic scoring of adhesion and histopathological scoring of tissue repair response were evaluated.

Results: Macroscopic view of the adhesion values ($P_{\text{adjusted}} < 0.0175$), histopathological evaluation values (histiocytic response, vascularization, and granulocytic response and total response) ($P_{\text{adjusted}} < 0.0175$), and collagen deposit values ($P_{\text{adjusted}} < 0.0175$) of Group 3 were significantly lower than Group 2.

Conclusion: Our study results suggest that the use of MSCs seems to be useful to prevent adhesion formation in cutaneous injuries and that MSCs promote wound healing without adhesions in the experimental setting.

Keywords: Adhesions, cutaneous, mesenchymal stem cells, rat, vascular reoperations.

After abdominal surgeries, fibrosis, and adhesions may occur in more than 90% of patients, leading to bowel dysfunction, infertility, chronic pelvic discomfort, and difficult redo surgery.^[1]

Postoperative adhesions are based on four main components: mesothelial cell loss,^[2] fibrin deposition,^[3] decreased fibrinolysis,^[4] and local inflammation.^[5] To reduce this adhesion, several methods have been used, such as to close only the regions over the large vessels, to use different synthetic and biological grafts, and to wash with dextran.^[6] Damaged tissue repair is completed in three stages. Tissue repair begins with hemostasis and, following the inflammatory period ending

within 24 to 48 hours, proliferative and maturation stages occur. During the inflammatory period, the infection shield is strengthened by neutrophil and macrophage migration, and the foundations of tissue repair are discarded at this stage which can be considered as an identical with angiogenesis.^[7]

Macrophages stimulate collagen production and angiogenesis by secretion of the transforming growth factor-beta (TGF- β), platelet-derived growth factor (PDGF), interleukin 1 (IL-1), platelet-activated factor (PAF), transforming growth factor alpha (TGF- α), tumor necrosis factor alpha (TNF- α), fibroblast growth factor (FGF), and epidermal growth factor (EGF), in addition to fibroblastic proliferation and

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differentiation.^[8] Following hemostasis, platelets and thrombus formation form the matrix tissue with the help of the cell transfer.^[9] Fibrinogen, fibronectin, platelet factor 4 (PF4), thromboxane A₂ (TxA₂), TGF- β , PDGF, amines, prostoglandines (PGs) that are discharged from platelet granules are the main components of angiogenesis and tissue repair.^[10]

Mesenchymal stem cells (MSCs) can act as anti-inflammatory, anti-proliferative, angiogenic, and immune modulator and inhibit the immune response in organ transplantations.^[11]

In the present study, we aimed to investigated whether MSC were useful to reduce cutaneous adhesions, particularly for vascular reoperations in a rat model.

MATERIALS AND METHODS

This experimental study was carried out at Kirikkale University Animal Research Laboratory. The study protocol was approved by the Kirikkale University Animal Researches Local Ethics Committee (No. 17/48, Date: 01/12/2017). All animals received human care in compliance with the principles of laboratory animal care developed by the National Academy of Sciences.

In Vivo rat model

In this experimental study, a total of 12 healthy adult male Wistar Albino rats weighing 300 to 350 g and aged >5 months were used. In Group 1 (n=2, negative Control group), no incision was performed to the cutaneous and subcutaneous tissues. In Group 2 (n=5, positive control group), skin and subcutaneous tissues were opened, contact of tissues with air and blood was maintained, and the skin was closed again after the contact. No further intervention was made. In Group 3 (n=5, MSC group), skin and subcutaneous tissues were opened, contact of tissues with air and blood was maintained. Subsequently, 3×10^6 MSCs were applied into the skin and subcutaneous tissues and the skin was closed.

Throughout the study, the animals were kept at the Animal Research Laboratory (Kirikkale University, Kirikkale, Turkey) under veterinary supervision. The rats were kept at a room temperature of $25 \pm 1.9^\circ\text{C}$ and humidity of $52 \pm 6\%$, and received a standard diet as well as water ad libitum. All animals were followed for 24 days. On Day 24, all animals were euthanized, and blood and tissue samples were obtained for investigation.

Isolation of MSCs

Cell isolation and culture: The MSCs were isolated from the subcutaneous flank adipose tissue of rats using the method of Karaca et al.^[12]

Cell characterization: The MSCs were characterized using immunofluorescence staining of CD13 and CD29 molecules using the method of Karaca et al.^[12] The flow cytometry analysis was performed against CD29, CD90, CD54, MHC Class I, CD45, CD106, and MHC Class II for characterization of MSCs. Flow cytometry analysis was performed.^[13]

Macroscopic scoring of the adhesions

Adhesions in the cutaneous tissues were evaluated macroscopically as 0: No adhesions, 1: Slim and easily separable adhesions, 2: Moderate adhesions with blunt dissection, and 3: Severe adhesions necessitating sharp dissection.

Histopathological scoring of tissue repair response

In the study, samples were fixed in 4% formalin solution. After two days, tissue samples were washed with the water. Before embedding in the paraffin, tissue samples were soaked in ethanol (60%, 70%, 80%, 90%, and 100%) and xylene for one hour. Cross sections were obtained and hematoxylin and eosin (H&E) staining was used for the histopathological examinations. All samples were examined and classified semi-quantitatively between 0 and 3 in terms of fibrosis, histiocytic response, vascularization, and granulocytic response (Table 1). Using the Masson's trichrome staining (MTS), collagen deposits were evaluated as 0 to 3 (Table 1).

The immunofluorescent antibody (IFA) imaging

The green fluorescent protein (GFP)-labeled stem cells were visualized and recorded on a fluorescent antibody microscope.

Caspase-3 measurements

Caspase-3 levels of the blood samples were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Elabscience, Wuhan, Hubei, China).

Statistical analysis

Statistical analysis was performed using the IBM SPSS version 20.0 (IBM Corp., Armonk, NY, USA). Descriptive data were expressed in median (min-max) or number and frequency. The Kruskal-Wallis analysis of variance and the Mann-Whitney U test with the Bonferroni adjustment were performed.

Table 1. Semi-quantitative classification for histopathological scoring

	Semi-quantitative classification			
	0	1	2	3
Fibrosis	No	Few fibroblasts	Fibroblastic proliferation and increased collagen	Fibrosis, collagen bundles
Histiocytic response	No	Rare macrophage	High amounts of histiocytes, rare multinucleated giant cells	Granuloma formation
Vascularization	No	Mild vasodilatation	Severe congestion	Hemorrhage + neovascularization
Granulocytic response	No	Low	Moderate	High

Table 2. Histopathological evaluation results of groups

	Group 1 (Negative control) (n=2)		Group 2 (Positive control) (n=5)		Group 3 (MSC group) (n=5)		*p
	Median	Min-Max	Median	Min-Max	Median	Min-Max	
Hematoxylin and eosin							
Fibrosis	0	0-0	2	2-3	1	0-2	0.024
Histiocytic response	0	0-0	3	3-3	1	0-2	0.008
Vascularization	0	0-0	2	2-3	1	0-1	0.009
Granulocytic response	0	0-0	3	2-3	1	0-2	0.013
Total response	0	0-0	10	9-11	2	2-7	0.008
Masson's trichrome							
Collagen deposit	0	0-0	3	2-3	1	0-2	0.016
Macroscopic view of the adhesion	0	0-0	3	2-3	0	0-2	0.016
Caspase 3 pg/mL	12.27	11.20-13.35	12.25	11.58-13.67	13.49	11.44-14.61	0.573

* p value indicates the results of Kruskal-Wallis analysis of variance.

A *p* value of <0.05 was considered statistically significant. When the Bonferroni adjustments were used, a *P*_{adjusted} value of <0.0175 was considered statistically significant.

RESULTS

According to the macroscopic examination, adhesion scores of Group 3 were significantly lower than Group 2 (*P*_{adjusted} <0.0175).

Histopathological evaluation results of all groups are shown on Table 1. Adhesions in the cutaneous tissues were evaluated macroscopically, and the difference among the groups was statistically significant (*p*<0.05) (Table 2). Pairwise comparisons which were performed to analyze the reason for the significant difference were performed (Table 3).

The fibrosis/adhesion severity and collagen deposit density were evaluated by H&E and MST staining,

Table 3. Pairwise comparisons

	Group 1-Group 2		Group 1-Group 3		Group 2-Group 3	
	z	<i>P</i> _{adjusted}	z	<i>P</i> _{adjusted}	z	<i>P</i> _{adjusted}
Hematoxylin and eosin						
Fibrosis	-2.049	0.040	-1.640	0.101	-2.132	0.033
Histiocytic response	-2.449	0.014	-1.296	0.195	-2.805	0.005
Vascularization	-2.160	0.031	-1.342	0.180	-2.739	0.006
Granulocytic response	-2.049	0.040	-1.673	0.094	-2.495	0.013
Total response	-1.991	0.047	-2.029	0.042	-2.660	0.008
Masson's trichrome						
Collagen deposit	-2.049	0.040	-1.296	0.195	-2.479	0.013
Macroscopic view	-2.049	0.040	-0.966	0.334	-2.495	0.013

**P*_{adjusted} value indicate the results of Mann-Whitney U test with Bonferroni adjustment; *P*_{adjusted} <0.0175 indicates statistical significance.

which were significantly increased in the positive control group, but not increased in the MSC group (Figure 1).

Using the H&E staining, fibrosis, histiocytic response, vascularization, and granulocytic response and total response were analyzed and a statistically significant difference was observed ($p < 0.05$) (Table 3). Histiocytic response, vascularization, and granulocytic response and total response values of Group 3 were significantly lower than Group 2 ($P_{\text{adjusted}} < 0.0175$).

Using the MTS, collagen deposit scores were evaluated and the difference among groups was statistically significant ($p < 0.05$) (Table 2). Pairwise comparison results are presented in Table 3. Collagen deposit scores of Group 3 (MSC group) were significantly lower than Group 2 ($P_{\text{adjusted}} < 0.0175$).

DISCUSSION

In case of insufficient or excessive healing processes, a non-healing wound or a hypertrophic scar, including functional loss may occur. It may also cause psychosocial effects.^[14] The stages of the physiological wound healing is hemostasis, inflammation, proliferation, and remodeling.^[15,16] During the proliferative phase, there are formation of granulation tissue, deposition of the collagen, reepithelization and wound contraction. If there is no full regeneration, repair damage occurs, such as scar formation.^[14,17-19]

Several different cell types including macrophages, fibroblasts, and contractile myofibroblasts participate in the proliferative phase of wound repair and play a critical role in regulating the size and quality of the scar.^[17-19]

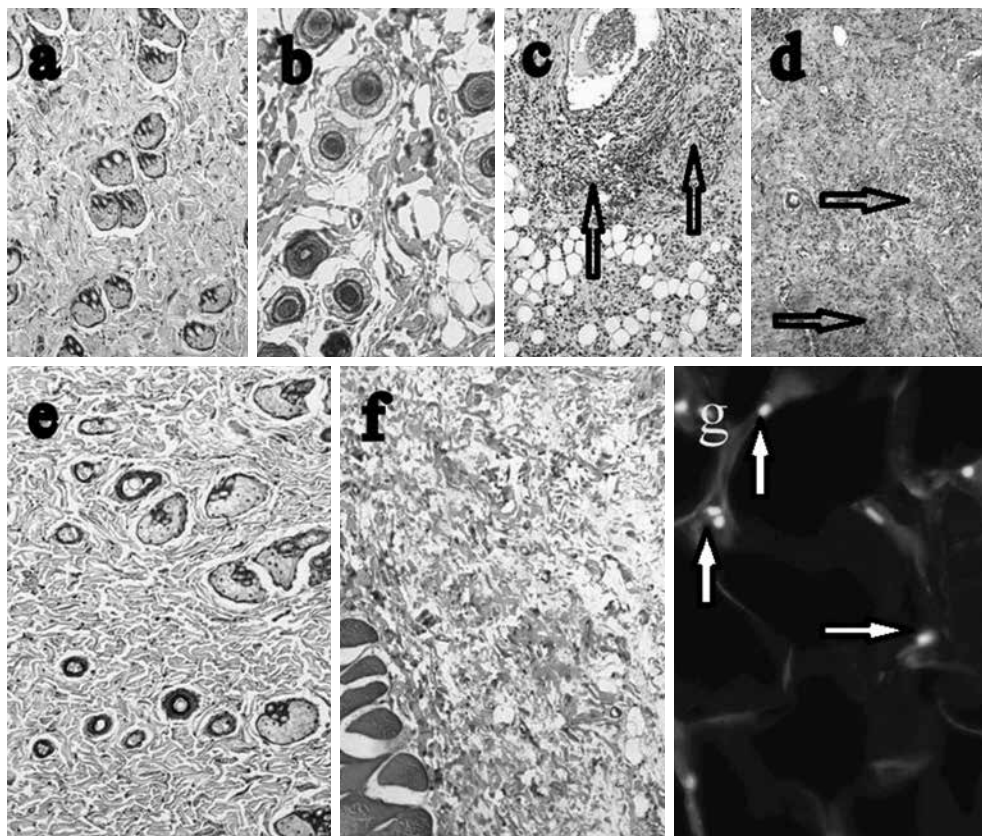


Figure 1. Representative examples of negative control cases (Group 1), non-stem cell cases (Group 2), and stem cell cases (Group 3). The stem cell evaluation was visually estimated by a single pathologist on H&E, MTS, and immunofluorescence stain sections in a semi-quantitative manner. Slides were scanned at $\times 20$ magnification (0.785 mm^2). Area representing negative control cases (Group 1) showing no inflammation (a, b), positive control cases (Group 2) (c, d) showing intense inflammation (arrows), and stem cell cases (Group 3) (d-f) showing minimal inflammation with immunofluorescent positive stem cells (Group 3) (g) (arrows) were observed.

H-E: Hematoxylin and eosin; MTS: Masson's trichrome staining.

The TGF- β is released by macrophages in the wound, and the ability of these cells to migrate and adhere to granulation tissue is responsible for the presence of TGF- β during recovery. The TGF- β and other cytokines cause the filling of the wound by mesenchymal cells.^[19] There is macrophage cell plasticity and these cells can produce different phenotype of fibroblast-like cells. Migration in other cell types is associated with the ability to obtain mesenchymal phenotype.^[18,20-22]

In the present study, we investigated the efficacy of MSCs to prevent cutaneous adhesions in a rat model for vascular reoperations. Macroscopic view of the adhesion scores, histopathological evaluation scores (histiocytic response, vascularization, and granulocytic response and total response), and collagen deposit scores of Group 3 were significantly lower than Group 2. Our results showed that the use of MSCs helped to prevent adhesion formation in cutaneous injuries.

Adult MSCs can differentiate into various cells and tissues, showing a critical role in wound repair and tissue regeneration. Due to their multipotency and immunosuppressive abilities, they are an attractive treatment tool for regenerative medicine and tissue engineering.^[23] The MSCs also contribute to the reconstruction of the skin in cutaneous wounds; however, there are still difficulties to overcome, before MSCs are widely used in the clinical setting.^[24]

Cardiovascular adhesions may cause an increased risk for inadvertent damages in the heart and large vessels and intraoperative bleeding.^[25] Medical or biomedical options to limit and control perivascular adhesions have to incorporate the recent advancements of pathogenesis and pathophysiology of adhesions.^[26] Transplanted MSCs may differentiate into various cell lines in wound areas and provide immunomodulatory effects.^[23] The MSCs can support *in vivo* wound healing by varying to the endothelial cells, myofibroblasts, and pericytes.^[27]

The induction of mechanical stress in the skin leads to the release of chemokines which are involved in various cytokines, particularly those recruit MSCs in the bloodstream.^[28] In addition, these chemokines increase the bone marrow stem cell mobility, thereby facilitating mobilization of MSCs into the peripheral blood and wound healing sites. The accumulation of MSCs in injured areas may transdifferentiate more than one skin component cell type and, thus, contribute to wound repair.^[27]

Physiological accumulation of sufficient MSCs can lead to more cell type differentiation. The result is better functional organization of injured tissue. The accumulation of circulating MSCs, usually given from the bone marrow stroma to specific tissue, may be one of the effective strategies for tissue regeneration.^[27]

In clinical trials, MSCs have been used for the successful treatment of chronic wounds and have been reported in skin wound healing, including the inflammatory, proliferation and remodeling phases. The MSC treatment via intradermal injection have been shown to significantly accelerate the wound closure.^[29-32]

The preclinical design in an animal model is the main limitation of the present study. Therefore, the true effect of MSCs should be investigated in further studies in human.

In conclusion, our study results suggest that the use of MSCs seems to be useful to prevent adhesion formation in cutaneous injuries and that promote wound healing without adhesions in the experimental setting. However, the use of MSCs to prevent adhesions in humans must be further investigated, particularly in revision cases.

Declaration of conflicting interests

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