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***Experimental Study** / Deneysel Çalışma*

The effect of basic fibroblast growth factor and adipose tissue-derived mesenchymal stem cells on wound healing, epithelization and angiogenesis in a tracheal resection and end-to-end anastomosis rat model

Sıçan trakeal rezeksiyon ve uç uca anastomoz modelinde basic fibroblast büyüme faktörü ve yağ dokusundan elde edilen mezenkimal kök hücrelerin yara iyileşmesi, epitelizasyon ve anjiyogenez üzerine etkisi

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Background: This experimental study aims to investigate whether basic fibroblast growth factor, adipose tissue-derived from mesenchymal stem cells, or a combination of both, has an effect on wound healing, epithelization and angiogenesis in a tracheal resection and end-to-end anastomosis rat model.

Methods: During the first phase of the study, mesenchymal stem cells were isolated by the primary explant culture technique from the abdominal adipose tissue of rats. When the cells became confluent, they were passaged and characterized by using immunofluorescence staining technique. The cells were cryopreserved for an *in vivo* application. The rats were divided into four groups, including: the basic fibroblast growth factor (group 1), the mesenchymal stem cells (group 2), the mesenchymal stem cells and basic fibroblast growth factor (group 3) and control group (group 4). The rats were sacrified at day 60 and the anastomosis was evaluated macroscopically for granulation tissue formation, the stenosis and presence of tracheocutaneous fistula formation, and also microscopically for stenosis, epithelium regeneration, inflammation, collagen formation and neovascularization.

Results: The inflammation was significantly lower in the study groups (p=0.004, p=0.014, p=0.004), whereas the collagen formation and epithelial regeneration were significantly higher in the study groups, compared to the control group (p=0.015, p=0.022, p=0.026 and p=0.002, p=0.001, p=0.002).

Conclusion: Both basic fibroblast growth factor and adipose tissue-derived mesenchymal stem cells increased epithelial regeneration and connective tissue organization in this rat model. They may be used as an adjuvant therapy to surgical resection in patients undergoing tracheal resection.

Key words: Adipose tissue; angiogenesis; epithelization; immunofluorescence staining; mesenchymal stem cell; tracheal stenosis; wound healing.

Amaç: Bu deneysel çalışmada temel fibroblast büyüme faktörü, yağ dokusundan elde edilen mezenkimal kök hücre veya her ikisinin kombinasyonunun sıçan trakeal rezeksiyon ve uç uca anastomoz modelinde yara iyileşmesi, epitelizasyon ve anjiyogenez üzerine etkisi araştırıldı.

Çalışma planı: Çalışmanın ilk fazında mezenkimal kök hücreler, sıçan abdominal yağ dokusundan primer eksplant kültür tekniği ile izole edildi. Hücreler kültür kabının yüzeyini tamamen kapladıktan sonra pasajlandı ve immünfloresan boyama tekniği kullanılarak karakterize edildi. Bu hücreler *in vivo* uygulamada kullanılmak üzere dondurulduu. Sıçanlar dört gruba ayrıldı: temel fibroblast büyüme faktörü (grup 1), mezenkimal kök hücre (grup 2), mezenkimal kök hücre ve temel fibroblast büyüme faktörü (grup 3) ve kontrol grubu (grup 4). Sıçanlar 60. günde sakrifiye edildi ve anastomoz granülasyon doku oluşumu, darlık ve trakeokütanöz fistül oluşum varlığı makroskopik olarak; darlık, epitel rejenerasyonu, enflamasyon, kolajen oluşumu ve neovaskülarizasyon mikroskobik olarak değerlendirildi.

Bulgular: Enflamasyon çalışma gruplarında anlamlı düzeyde daha düşük iken (p=0.004, p=0.014, p=0.004), kolajen oluşumu ve epitel rejenerasyonu kontrol grubuna kıyasla çalışma gruplarında anlamlı düzeyde daha yüksekti (p=0.015, p=0.022, p=0.026 ve p=0.002, p=0.001, p=0.002).

Sonuç: Hem temel fibroblast büyüme faktörü hem de yağ dokusundan elde edilen mezenkimal kök hücreler, bu sıçan modelinde epitel rejenerasyonunu ve bağ doku oluşumunu artırdı. Bu hücreler trakeal rezeksiyon yapılan hastalarda cerrahi rezeksiyona adjuvan tedavi olarak kullanılabilir.

Anahtar sözcükler: Yağ dokusu; anjiyogenez; epitelizasyon; immünfloresan boyama; mezenkimal kök hücre; trakeal darlık; yara iyileşmesi.

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Tracheal stenosis may evolve after blunt trauma, an inhalation burn, bronchoplastic intervention, or a tracheotomy and intubation. The preferred treatment is performed with a prompt excision of the stenosis and surgical repair if the patient can tolerate it.[1] It is well known that after surgical interventions on the trachea and bronchus, better healing can be achieved by increased epithelization and angiogenesis.

The multilineage potential of adult mesenchymal stem cells (MSCs) from the bone marrow has been extensively characterized. Adipose tissue is another source of MSCs that can be differentiated into bone, cartilage, adipose tissue, muscle, and endothelial cells.[2-5] Therefore, adipose tissue-derived MSCs have great potential for being used in novel cellular therapies for the repair of damaged tissues. Experimental studies have demonstrated the healing effect of the basic fibroblast growth factor (bFGF) on tracheal defects.[6] In addition, it has also increased epithelial regeneration and early revascularization on tracheal autotransplantation models.[7-9]

This experimental study was designed to investigate whether the bFGF, MSCs derived from adipose tissue, or a combination of the two have any effect on wound healing by applying them to the anastomosis site in rate models that underwent a tracheal resection or an end-to-end anastomosis. We also investigated whether these two agents decrease the complications at the anastomosis site by increasing epithelial regeneration and neovascularization.

MATERIALS AND METHODS

Twenty-eight male Wistar albino rats (250-300 grams; 4-6 months old) were used in this study, and they all received humane care in compliance with the European Convention on Animal Care. The study protocol was approved by the Animal Ethics Committee of Kırıkkale University (Protocol number: 10/32). The rats were housed in and operated on in the animal laboratory and were divided into four groups as follows: group 1 (the bFGF group), group 2, (the MSC group), group 3 (the MSC and bFGF group), and group 4 (the control group). Each group contained seven animals.

Chemicals and reagents

Dulbecco's Modified Eagle's Medium/Ham's F12 (DMEM/F12) at a ratio of 1:1, fetal bovine serum (FBS) that had been previously tested for mycoplasma and endotoxins, trypsin/ethylenediaminetetraacetic acid (EDTA) [0.05% trypsin and 0.02% EDTA weight per volume (w/v)], and phosphate buffered saline (PBS) (Biochrom AG, Berlin, Germany) along with penicillin-streptomycin powder (10,000 units/ml penicillin and 10 mg/ml streptomycin), dimethylsulfoxide (DMSO), and the bFGF (Sigma-Aldrich, St Louis, MO, USA) were used in this study. In addition, immunocytochemicals (primary antibody, blocking serum, and secondary antibody) (Santa Cruz Biotechnology, Inc., Dallas, TX, USA) and various anesthetics (Richter Pharma AG, Wels, Austria) as well as cell culture dishes and other plastics (Greiner Bio-One GmbH, Frickenhausen, Germany) were also employed.

Isolation, culturing, and characterization of adipose tissue-derived MSC

Cell isolation and culture

In this study, the MSCs were isolated from the perigonadal and subcutaneous flank adipose tissue of the rats. After anesthesia with xylazine (10 mg/kg) and ketamine (50 mg/kg), this tissue was collected under sterile conditions. No enzymatic digestion methods were used during the cell isolation process. The tissue fragments were then directly plated in 6-well culture dishes and transferred into a transport medium (DMEM/F12 containing 10% FBS and 0.4% penicillin-streptomycin) in a petri dish where they were minced into 4-5 mm thick fragments and washed. Next, the tissue fragments were incubated in a primary medium (DMEM/F12 containing 20% FBS and 0.2% penicillin-streptomycin) in a 6-well culture dish under standard culture conditions [a humidified atmosphere of 95% air and 5% carbon dioxide (CO_2) at 37 °C]. The culture medium was replaced every day to avoid possible differentiative effects on the various cytokines originating from the mesenchymal cells. When the cells became confluent, they were passaged using the standard trypsinization method. The culture medium was then removed, and the attached cells were harvested using the trypsin/EDTA solution. Afterwards, the cell suspension was transferred into a centrifuge tube, and centrifuged at 800 rpm for five minutes to pellet the cells. The supernatant was then removed carefully, and the cells were incubated in DMEM/F12 containing 10% FBS and 0.2% penicillin-streptomycin. Following this, the MSCs were passaged three times and cryopreserved for *in vivo* applications. To do this, the cells were detached from the surface of the culture dish using the trypsin/EDTA solution, and the cell suspension was immediately transferred into a freezing medium containing 50% FBS, 40% DMEM/F12, and 10% DMSO in a cryogenic tube. The cryovials were then stored at -80 ºC for a period of 48 hours and the frozen vials were transferred to a liquid nitrogen container at -196 ºC for long-term storage. Next, the standardization of cell concentration for the animal

application, and the cells harvested from the three 6-well culture dishes were collected in one cryogenic tube. We then examined them for cell morphology and growth under an Olympus IX70 inverted microscope (Olympus Optical Co., Ltd., Shinjuku, Tokyo, Japan).

Cell characterization

The MSCs were characterized by using immunofluorescence staining against the CD13 molecule. To accomplish this, the cells grown on the culture dish were washed in the PBS and placed in methanol for five minutes at -10 ºC. After the fixation, the methanol was removed and desiccated. For blocking, the cells were incubated for 20 minutes with a blocking serum (normal goat serum). After this, the serum was removed, and the cells were washed three times in the PBS. They were then incubated for one hour with a primary antibody for the CD13 molecule. After five minutes of washing in the PBS, the cells were incubated for 45 minutes with the secondary antibody (donkey anti-goat Ig-TR; Santa Cruz Biotechnology, Inc.) and washed three times in the PBS. After washing them, the cells were mounted with a mounting medium and visualized under an Olympus BH2-RFL-T_3 100W high pressure mercury burner and reflected light fluorescence microscope attachment (Olympus Optical Co., Ltd., Shinjuku, Tokyo, Japan). All the procedures were performed at room temperature.

Surgery

The rats were anesthetized with 50 mg/kg of ketamine hydrochloride plus 10 mg/kg of xylazine, which was administered intramuscularly under sterile conditions. A vertical skin incision was then made over the trachea, and the two cartilage rings were removed. Reconstruction was achieved with 8/0 polypropylene sutures, and the trachea was aspirated thoroughly before the ligation of the sutures. After this was completed, the muscles and skin were closed sequentially with 3/0 silk sutures following the application of the bFGF and MSCs to the anastomosis site in the study groups, whereas the control group received nothing before the closure.

Preparation of basic fibroblast growth factor and mesenchymal stem cell for *in vivo* application

In the bFGF group, the stock bFGF solution was prepared (25 µg bFGF/ml PBS) immediately before it was used, and 2.5 µg/ml of it was soaked in absorbable gelatin sponge (0.2x10 mm) and applied to the anastomosis site.

In the MSC group, the cryopreserved cells which had been collected in one cryogenic tube were applied to the anastomosis site, and the cryovials, which had been stored at -196 ºC, were placed in a water bath at 37 ºC. The cells were then allowed to thaw immediately before the application. To remove the freezing medium, the cells were centrifuged at 800 rpm for five minutes. The pellet was then resuspended in the DMEM/F12 without serum and centrifuged at 800 rpm once again for five minutes. This was followd by the removal of the supernatant, and an absorbable gelatin sponge was soaked in the cell pellet and applied to the anastomosis site.

In the MSC and bFGF group, the cells were applied with 50 µl of the bFGF (1.25 µg/ml of bFGF) solution in an absorbable gelatin sponge. The MSCs and bFGF were then prepared and applied to the anastomosis site as previously described. In the control group, the anastomosis sites were left untreated.

Autopsy

The autopsies were performed on day 60 under general anesthesia. The trachea was totally dissected and evaluated by an examiner who was blinded to the groups for granulation tissue formation, stenosis, and tracheocutaneous fistula formation on the tracheal anastomosis site. Patients with a macroscopic presence of all of these parameters were graded as 1 and those without this presence were graded as 0.

Microscopy

The sections of the anastomosis site, and the nearby trachea were taken and placed in 10% buffered formalin. After the tissues were embedded in paraffin, slices of 5 microns in thickness were prepared and stained with hemotoxylin-eosin (H-E), and a histopathological analysis was performed by a pathologist blinded to the groups. The degree of epithelial regeneration, inflammation, vascularization, and collagen organization was then evaluated and scored. The method described by Mayer et al.[10] was used for the epithelial regeneration, with a grade of 0 representing no epithelium with single, non-confluent epithelial cells, a grade of 1 signifying a confluent, single-layered, non-ciliated epithelium, a grade of 2 denoting a confluent, multi-layered, non-ciliated epithelium, and a grade of 3 indicating a normal, mucociliary epithelium. In addition, a vascularization grade of 1 was given for less than 15 vessels on 10x magnification and a grade of 2 was applied for more than 15 vessels at the same magnification. Furthermore, inflammation and collagen organization were graded as mild (grade 1), moderate (grade 2), and severe (grade 3).

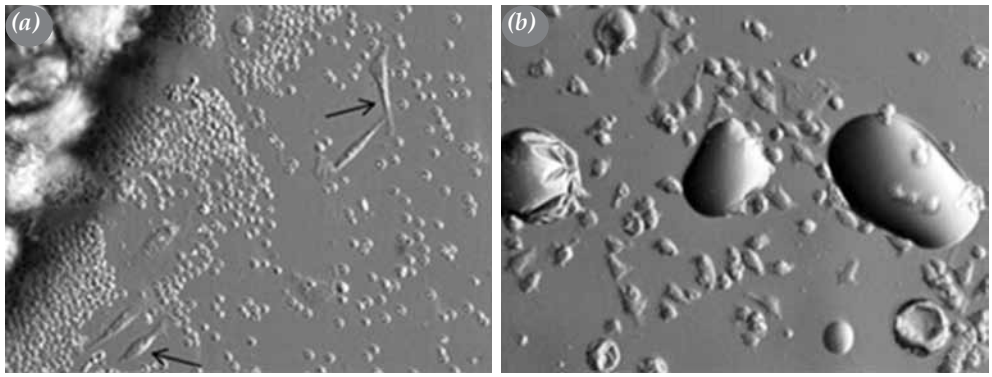

Figure 1. The morphological appearance of the cells derived from adipose tissue. **(a)** The cells at 36 hours of culture. **(b)** The cells after seven days of culture (H-E x 20).

The tracheal lumen area was measured using a 10x10 mm^2 grid with a Leica DMLS binocular microscope (Leica Microsystems GmbH, Wetzlar, Germany. The ratio of the cut surface of the luminal area at the anastomosis site to the nearby tracheal area was calculated, and the percentage of narrowing was termed the luminal area ratio.

Statistical analysis

Data analysis was performed with the SPSS version 11.5 for Windows (SPSS Inc., Chicago, IL, USA). The differences between the groups regarding macroscopic stenosis, the microscopic luminal area ratio, inflammation, collagen organization, and neovascularization were evaluated by the Kruscal-Wallis test with a Bonferroni adjustment. When the p value from the Kruscal-Wallis test was statistically significant, the Mann-Whitney U multiple comparison test was used to determine which group was responsible for the difference. A *p* value of less than 0.05 was considered to be statistically significant for all data, and a two-tailed comparison was also performed. The results showed that no statistical comparison was necessary for the epithelial regeneration.

RESULTS

Cell isolation and culture

After 36 hours of culture, the emergence of fibroblastic cell morphologies among the erythrocytes was identified (Figure 1a), and after seven days, the number of cells increased (Figure 1b). Large spreading cells with a branched shape (Figure 2a), rounded cells (Figure 2b), and fibroblastic cell morphology (Figure 2c) were also observed.

After 10-12 days, the cells became confluent and displayed the same morphology (Figure 3a). When this occurred, they were passaged three times and stored for *in vivo* applications. This cell morphology was maintained through repeated subcultures under non-stimulating conditions (Figure 3b). Furthermore, the passaged cells also showed a high capacity for proliferation; therefore, huge numbers of cells could be obtained in a short period of time. This meant that cells could be applied to the anastomosis site shortly after the isolation procedure.

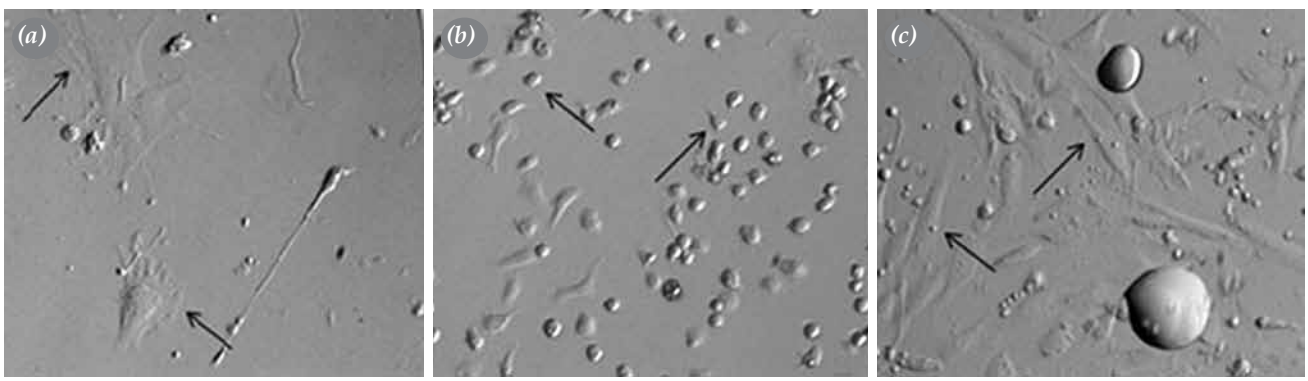

Figure 2. The morphological appearance of the cells derived from adipose tissue. **(a)** Spreading cells with a branched shape, **(b)** rounded cells, and **(c)** fibroblastic morphology (H-E x 20).

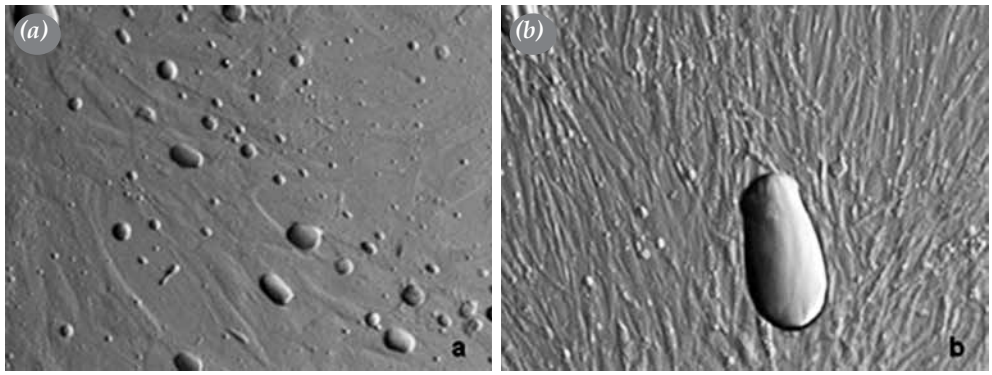

Figure 3. The morphological appearance of confluent cells after **(a)** 10-12 days of culture and **(b)** after the second passage (H-E x 20).

Cell characterization

The results of the immunofluorescence staining showed that these cultured cells were positive for CD13 (Figures 4a). A flow cytometry analysis was also conducted to check the homogeneity of the MSCs, and the results showed that these cells were positive for CD29, CD90, CD54, and MHC class I but negative for CD45, CD106, and MHC II. The homogeneity rate for the MSCs was as low as 98.68% (Figure 4b).

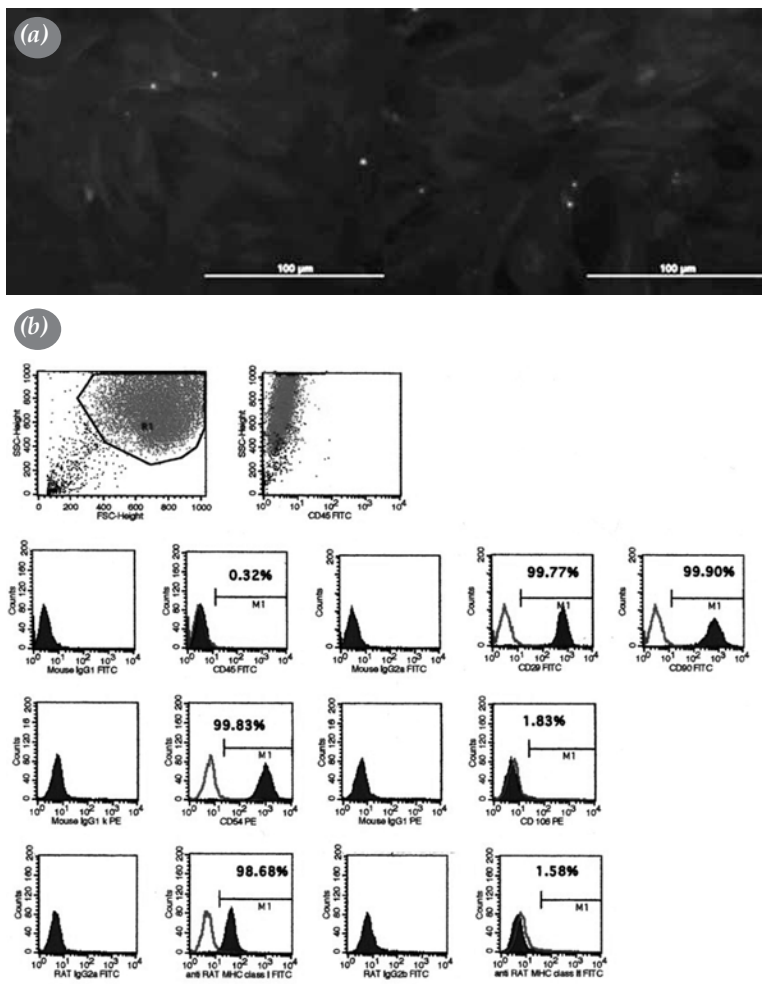

Figure 4. (a) The morphological appearance of CD13 + cells after the second passage (H-E x 20) along with **(b)** the results of the flow cytometry analysis.

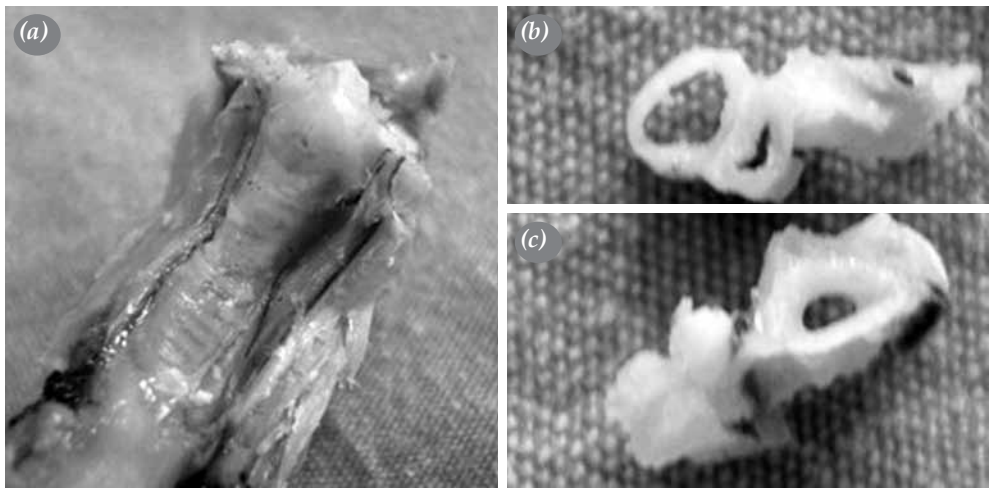

Figure 5. Tracheal stenosis in the control group **(a)** Extraluminal view. **(b)** Cut surface view, normal appearance of the trachea, **(c)** stenosis at the anastomosis site.

Macroscopic observation

Stridor was observed in all of the rats starting from the "awake" period which lasted 48 hours. Between the postoperative 10^{th} and 14^{th} days, one rat each from groups 1, 3, and 4 died. Macroscopically, in the control group, tracheal stenosis occurred statistically significantly more often than in the study groups (p=0.009) (Figure 5), but this was because no obvious stenosis was detected in the three study groups (Figure 6). Additionally, no granulation tissue formation or tracheocutaneous fistulas were detected in any of the groups.

Microscopic observation

When the luminal surface areas were compared microscopically, there was a significant difference

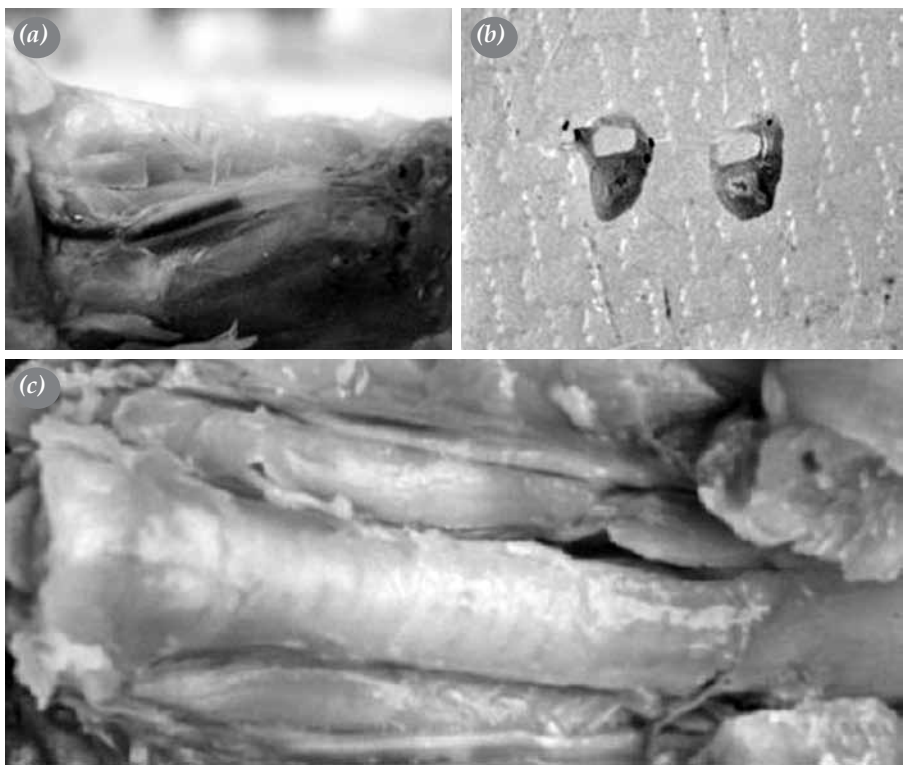

Figure 6. The appearance of the trachea in the mesenchymal stem cell group showing no obvious stenosis. **(a and c)** Extraluminal view. **(b)** Intraluminal view.

Table 1. The luminal area ratios of the groups

	Group 1	Group 2	Group 3	Group 4
	%	%	%	%
1st Rat	22	19	15	42
2nd Rat	15	2	13	31
3rd Rat	12	24	12	29
4th Rat	6	24	3	31
5th Rat	6	2	9	57
6th Rat	16	25	3	56
7th Rat	Died	4	Died	Died

between the control group and study groups. In the control group, the narrowing of the luminal surface area was higher than in groups 1, 2, and 3 (p=0.002, p=0.001, p=0.002, respectively). Furthermore, the luminal area ratios were between 6-22% in group 1, 2-25% in group 2, and 3-15% in group 3, but this increased to 31-57% in the control group (Table 1). There was also a statistically significant difference between the groups when the degree of inflammation was evaluated, with it being significantly lower in the three study groups (p=0.004, p=0.014, p=0.004, respectively). The collagen formation was statistically significantly higher in the study groups as well (p=0.015, p=0.022, p=0.026, respectively), and the epithelial regeneration was also statistically significantly higher in the study groups (p=0.002, p=0.001, p=0.002, respectively). Fisher's exact test was used to compare the bFGF group and the control group, and the difference was significantly close in terms of neovascularization (p=0.061) (Figure 9). When the loss of the rats in this group was taken into consideration, this difference was statistically remarkable. The scores of the microscopic and macroscopic examinations of all of the groups are shown in Table 2.

DISCUSSION

Airway ischemia is a major problem after tracheal reconstruction or bronchoplastic procedures. A delay in the healing process may cause complications such as dehiscence and stenosis. In order to avoid the strictures following surgery as a result of the formation of fibrosis caused by ischemia, agents like the bFGF and hyaluronic acid (HA) have been shown to decrease the degree of stenosis and inflammatory reactions. These agents may overcome the airway ischemia by augmenting revascularization and wound healing.[9-13] In addition, the bFGF and HA have been shown to increase the proliferation of chondrocytes in

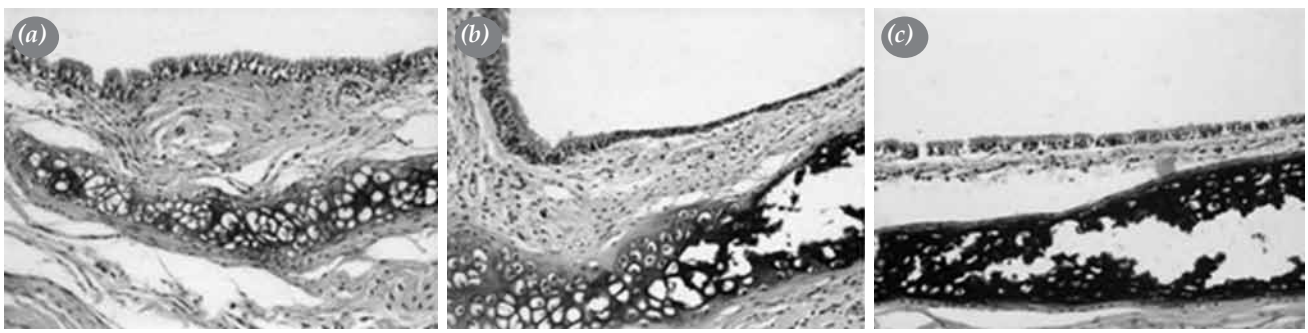

Figure 7. Increased collagen formation and widening in the subepithelial space in **(a)** the basic fibroblast growth factor group, **(b)** the basic fibroblast growth factor + mesenchymal stem cell group, and **(c)** the control group (H-E x 200).

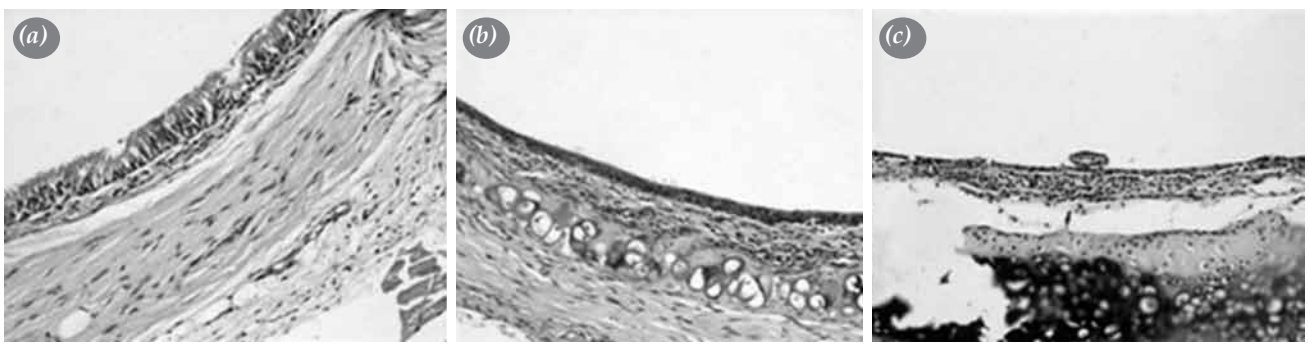

Figure 8. The epithelial regeneration in the study groups. **(a)** the normal pseudostratified respiratory epithelium (H-E x 200), **(b)** the regenerative cubic epithelium (H-E x 100), and **(c)** the regenerative single-layered epithelium (H-E x 100).

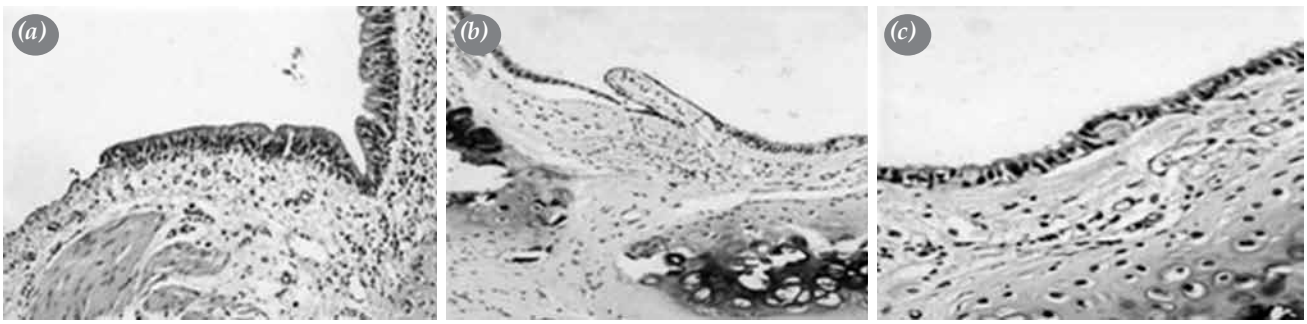

Figure 9. Increased vascular proliferation seen in **(a)** the basic fibroblast growth factor group (H-E x 200), **(b)** the basic fibroblast growth factor + mesenchymal stem cell group (H-E x 100), and **(c)** the control group (H-E x 200).

surgically created tracheal defects.[6] Sung and Won[9] also showed that revascularization and epithelial regeneration were enhanced by the bFGF in a tracheal autograft model.

The bFGF is effective with regard to the function and development of large numbers of cell, tissue, and organ systems, and it has been defined as being primarily mitotic for fibroblastic cells. Clearly, the bFGF plays an extremely significant role in tissue regeneration with its well-characterized functions of endothelial cell proliferation, migration, and stimulation of neovascularization.[14] In addition, Kobayashi et al.[15] reported that fibroblasts have a positive effect on tracheal epithelial regeneration *in vitro*, but it still remains unclear whether this occurred because of a direct effect or an increase in vascularization.

In experimental studies, the application doses ranged 10 ng - 2.5 µg in the various methods that were used.[9,10,16,17] Olech et al.[16] determined that an application of 10 ng of the bFGF on rabbit tracheas combined with inadequate dosage was responsible for the failure of healing process. However, successful results were reported by Mayer et al.[10] with a six-day application of 24 ng of the bFGF. Furthermore, Albes et al.[17] applied 2.5 µg of the bFGF in fibrin glue with success. Additionally, Igai et al.[18] preferred to use a topical application of the bFGF that was embedded in a gelatin sponge on the tracheal cartilage defects, and they observed that a large portion of the defect was filled within 12 months. Methods involving continuous release and increased doses of the bFGF have also been suggested as the source of successful results.[10,17] Additionally, Sung and Won[9] recommended the infusion of a single dose for 14 days because they assumed that the natural neovascularization process lasted two weeks. In our study, 2.5 µg/ml of the bFGF was applied in a single dose, and an absorbable gelatin sponge was used to carry the MSCs and bFGF to the anastomosis site. We suggest that the application of bFGF via an absorbable gelatin sponge is a more appropriate method as it maintains and releases the solution in a slow fashion. We also hypothesize that

Table 2. The microscopic and macroscopic examination scores of the groups

Variables	Group 1	Group 2	Group 3	Group 4	p^a
Macroscopic					
Tracheal stenosis	10.08	11.57	10.08	20.50[a]	0.009
Microscopic					
Epithelial regeneration	16.00[b]	16.00[c]	16.00[d]	3.50	<0.001
Degree of inflammation	11.00[e]	9.36[f]	11.00[g]	21.25	0.010
Collagen formation	17.33[h]	13.29[i]	15.67[j]	5.67	0.014
Neovascularization	16.33[k] (p=0.061)	15.14	12.17	8.00	0.09

a: The Kruskal-Wallis test with a Bonferroni adjustment was used. The values are given as mean rank, and the difference between group 4 and other groups was statistically significant (p=0.009). b: The difference between group 1 and other groups was statistically significant (p=0.002). c: The difference between group 2 and other groups was statistically significant (p=0.001). d: The difference between group 3 and other groups was statistically significant (p= 0.002). e: The difference between group 1 and other groups was statistically significant (p=0.004). f: The difference between group 2 and other groups was statistically significant (p=0.014). g: The difference between group 3 and other groups was statistically significant (p= 0.004). h: The difference between group 1 and other groups was statistically significant (p=0.015). i: The difference between group 2 and other groups was statistically significant (p=0.022). j: The difference between group 3 and other groups was statistically significant (p= 0.026). k: The difference was significantly close in terms of neovascularization (p=0.061) when Fisher's exact test was applied.

higher doses of bFGF might cause harm by over enhancing the growth of fibroblasts at the anastomosis site, thus causing an inevitable stenosis. Continuous application seems to be inappropriate due to the clinical difficulty and risk of infection.

Cellular therapy methods are gradually gaining importance for the repair of alveolar and upper respiratory tract injuries. There are endogenous adult stem cells in the alveolar epithelial tissue and conducting airways. In cases involving trauma or a pathological condition, these cells proliferate, and MSCs advancing from the bone marrow via blood circulation play a role in the repair of the injury.[19] However, inadequate numbers of cells from the bone marrow plus the slow dividing rate of the endogen-derived progenitor cells are important limitations for the recovery period. Thus, alternative treatment methods have emerged, for example cellular treatment in which the cells are used either alone, as in our study, or on various three-dimensional (3D) materials that are biocompatible or biologically degradable. In fact, both methods are sometimes utilized together with growth factors. Mesenchymal stem cells may also be obtained from the adipose tissue, peripheral blood, cord blood, amniotic fluid, placenta, and even from dental pulp, and they are characterized by a capacity for self-regeneration, viability over long periods of time, and their ability to transform into different cell types.[11,20] They also have a role in the regeneration and repair of injured tissues and organs in healthy individuals.[21,22] These stem cells can demonstrate different characteristics depending on the microenvironment in which they exist. Intercellular matrix components in the tissue and interactions with other cells play a part in the differentiation of MSCs as well.[23]

In this study, we evaluated MSCs as a contributory factor in the recovery of injured tissue through two basic points: their contribution to the differentiation of self cells in the tissue and tissue recovery as the result of fusion and their contribution to the accelerated recovery period via angiogenesis.[2,24]

The bFGF is also known to affect the revascularization process,[9] and it has also been shown to have a part in the longer viability of the cultures. This last aspect of the bFGH played a key role in our study. Although MSCs have the ability to migrate to injured areas and interact with cells there, this is not a simple process when the external application of the cells is taken into consideration because the transplanted cells may have difficulty in adapting to the injured area. However, in cases in which MSCs have been applied together with the bFGF, as in our study, we believe the evidence shows that the cells adapt to this process in a safer manner while also contributing to tissue recovery by dividing. In addition, growth factors excrected by MSCs also aid in the process. However, this is a point which should be investigated in the future.

Another advantage of our study was that the MSCs were isolated directly from the adipose tissue through the culture method without being subjected to any enzymatic procedures. Hence, cell loss that could have occurred due to proteolytic enzymes, such as collagenase that was used during the procedure, was minimized, and a sufficient cell count could be achieved in a relatively short time. Additionally, another advantage of adipose tissue is its availability since it may be easily obtained from the fat tissue of the individual who needs cellular therapy and autologously applied again after cell isolation has been performed. This is of great importance in terms of completely eliminating the immune response risk. Thus, this study was important because it served as the first venture into cellular therapy.

The major disadvantage with our study revolved around the difficulty we had in anastomosing the ends of the rat trachea because of their small diameters. This led to the inevitable deaths in the groups and may have affected the results of this study. We are currently planning a larger animal study for resection and reconstruction and also have a plan to apply growth factors intratracheally via an inhaler.

Conclusion

The results of this study showed that in a tracheal resection and an end-to-end anastomosis performed on rat models, the bFGF and MSCs increased epithelial regeneration and connective tissue organization while decreasing stenosis at the anastomosis side. This suggests that these two components may be used as an adjunct to surgical resection in patients undergoing a tracheal resection.

Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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