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Experimental Research

The effect of mesenchymal stem cells lyophilisate femoral artery of rat anastomosis: A histopathological and histomorphometric study

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ABSTRACT

Background and objective: Mesenchymal Stem Cells (MSCs) are well known for their tissue regeneration enhancing effect and their contribution to immune regulation. However, their contribution to the healing process of femoral artery anastomosis, especially to endothelialization, has not been studied sufficiently in the clinic. This study was carried out to evaluate the effects of MSC-lyophilisate from the human umbilical cord on anastomosis experimental study in rats histopathologically.

Method: After intraperitoneal anesthesia was applied to the rats, the femoral artery was exposed with a 2 cm incision in the right femoral region. After the artery was cut in the experiment and sham groups, femoral artery end-to-end anastomosis was performed using the primary suture technique. MSC-lyophilisate was poured in powder form onto the anastomosed outer surface of the vessel in the treatment group and saline solution was poured to the sham group. No intervention was made to the control group. The data analysis was performed with IBM SPSS Statistics 25.

Results: In the experiment group, flattening of the inner elastic lamina, morphological changes and vacuolization in the muscle fibers, inflammation in the adventitia and significant vascular wall thickening were observed in the femoral arteries of the rats after the intervention. According to the histopathological scoring results, tissue samples belonging to sham and experimental groups showed marked pathological findings such as endothelial damage, flattened areas where the folded structure in the inner elastic lamina disappeared, muscle fiber degeneration and inflammation in the adventitia.

Conclusion: Human umbilical cord-origin MSC-lyophilisate application holds an important place in femoral artery surgery. We evaluate that it will be meaningful to determine the MSC-lyophilisate dose for hemostasis without creating thrombus after anastomosis. MSC-lyophilisate will be used to provide hemostasis in areas with local bleeding in the future. In addition, it is recommended to make plans for an in-depth examination of possible problems and cases in future studies.

1. Introduction

Femoral artery anastomosis plays a key role in a free flap and replantation surgery [1]. The diameter mismatch between the vessels poses a problem in bringing them together, and the risk of thrombosis is high. Turbulence caused by sudden diameter changes triggers platelet aggregation [2]. Leakage may occur at anastomotic sites following femoral artery repair. To prevent this, pressure is applied to the anastomosis area or stitches are applied blindly. While trying to control blood leakage, the vascular endothelium is damaged. As a result, tunica media cannot be adequately blooded and endothelial nutrition is impaired [3].

Mesenchymal stem cells (Mesenchymal Stem Cells-MSCs) has a strong synthesis capability. MSCs are self-renewal and can express CD73, CD90 and CD105 biomarkers on their surface (they cannot express biomarkers such as HLA-DR, CD45, CD14 and CD34, CD14). In addition, they can differentiate into bone, fat, and cartilage [4–6]. MSCs, which are obtained from adipose tissue, are the cells with similar properties and potency compared to those synthesized from bone marrow [7–9]. Growth factors (VEGF) required for angiogenesis and adipogenesis are secreted by these cells and they are extremely ideal for adipose tissue regeneration [10]. In this way, its role in preserving the

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regenerated tissue volume is realized.

Bone marrow is rich in hematopoietic stem cell content in the isolation of MSC. However, the difficulties in the collection procedure and the need for general anesthesia have restricted the use of bone marrow in both clinical and research studies [6]. Perinatal sources such as the placenta, umbilical cord and amniotic fluid also have great importance for MSC [11,12]. Considering that placental MSCs is an allogenic source, it has advantages such as being painless, easily lyophilized (MSC-lyophilisate), accessible and free of charge compared to bone marrow.

Other factors that make MSC-lyophilisate preferable including anti-inflammatory factors, chemokines, cytokines, growth factors, and Extracellular Vesicles (EVs) [13]. MSCs, compared to MSC-lyophilisate, have been found to cause tumors [14,15] and accumulation of cell and cell-related by-products in the microvasculature [16] toxic effect). In addition, it has been found to have a therapeutic effect [17,18] varying according to the microenvironment. In this respect, MSC-lyophilisate is preferred because of its therapeutic effect, non-toxicity and easy production, safer in intravenous administration.

MSC secretomes were used in experimental studies and observed to have benefits. Based on the studies in which secretomes were effective, it was assumed that MSC-L might also be effective. Unfortunately, the literature about MSC-L lacks any study similar to our study. The use of stem cells, which have a unique effect in tissue regeneration, and the easy access to these cells from the placenta seem promising for arterial anastomosis. In this study, it was aimed to evaluate the thrombus effect of lyophilized mesenchymal stem cell on artery anastomosis histopathologically.

2. Material and method

2.1. Research design and laboratory rat selection

This study, carried out with 27 Wistar albino male rats weighing approximately 250 g, is an experimental design. The rats were obtained from Department of Experimental Animals, Adıyaman University Faculty of Medicine. 27 rats were divided into 3 groups as Control, Sham and Experiment Group with 9 rats in each group. Each rat was placed in separate cages. Light and dark cycles were applied for 12 h each. All rats were fed as much as desired. Histopathological evaluation was performed in the laboratories of Adıyaman University Faculty of Medicine, Department of Histology and Embryology.

2.2. Procedure

In our study, intraperitoneal anesthesia was applied to all rats divided into 3 groups during the procedure. Accordingly, Ketamine 90 mg/kg and Xylazine 10 mg/kg were injected. The rats were positioned in a supine position to operate on the right femoral region. All surgical procedures were performed by a single surgeon. The femoral artery was exposed with a 2 cm incision in the right femoral region. A skin incision was performed on the rats in group 1 (control group), then the skin was closed again by checking the robustness of the right femoral artery structure. In Group 2, the femoral artery was totally cut after fixation with clamps (using an approximator) and sutured again with prolene 9-0, then 1.5 cc saline solution was poured over the vessel and the skin was closed primary. The experiment group rats of femoral artery were cut after fixation with clamps and sutured again with 9-0 prolene. And then an average of 1.5 million MSC-lyophilisate was poured in powder form onto the anastomosed outer surface of the vessel (4-suture technique was applied with a 90-degree angle). To make histopathological and biochemical evaluation, blood and tissue samples were taken at the end of the 7th day following the procedure, and then the rats were sacrificed [19].

2.3. Cell collection and lyophilization process

After obtaining the necessary legal permissions, MSCs were obtained from Tissue Typing Laboratories of Istanbul Acıbadem University (human umbilical cord was used as a source to obtain MSC). Stem cells were multiplied by incubating in 75 cm^2 flasks in serum-free Dulbecco's Modified Eagle's Medium (DMEM) culture medium at 37 °C and 5% Cardiondioxide (CO2) atmosphere. When the cells reached 70–80% confluence on the flask surface, the number of cells was increased by passing the passage with the collagenase type II enzyme.

While the cells were transferred to the vials, aseptic filling was done using a fully automated system with constant shaking at a constant speed. Immediately after the filling and before the lyophilization, 8 vials were randomly selected from all vials and cells were counted on the Thoma slide using Trypan blue. It was confirmed that each vial contained 1.5 million cells. Consistency between vials after lyophilization was ensured by measuring the total protein amount using the Lowry method in the 8 randomly selected vials. Lyophilization is the process of freeze-drying of cells. Passaged cells were removed with trypsin in the final passage and centrifuged at 400 rpm for 10 min at +4 °C. The supernatant was discarded and saline solution was added. After the Pellet was suspended, the supernatant was discarded and saline solution was added by centrifuging again at 400 RPM at 10 min +4 °C. The supernatant of the cell culture harvest, which was centrifuged at 400 rpm for 10 min at +4 °C for the last time, was discarded and suspended with saline solution. The lyophilization additive was then added. The cell suspension supplemented with lyophilization additive was divided into 15X107 cells per vial. Vials were lyophilized with a half plug. A vial of 1.5 million MSC-lyophilisate contains; the freeze-drying medium was composed of 30% Polyvinylpyrrolidone (Sigma Aldrich, CAS Number 9003-39-8), 100 mmol/L trehalose (Merck, CAS Number: 6138-23-4) and 100 mmol/L sucrose (Sigma Aldrich, CAS Number: 57-50-1) and serum-free DMEM/F-12 (Gibco 11320074, Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12) as solvent.

2.4. Histopathological evaluation

Before sacrificed animals at the end of the 7th-day tissue samples taken from were fixed in 10% formol solution for 1 week. The 7 μm (μm) thick sections were obtained by using the Thermo Shandon Finesse ME microtome device (Thermo Fisher Scientific, Cheshire, UK) from paraffin blocks prepared by the routine histological follow-up (consisting of alcohol, xylene and paraffin series) with the automatic tissue tracking device (Leica TP1020, Nussloch, Germany) were stained with Masson trichrome and toluidine blue staining and examined under the light microscope. The tissue layers were examined and scored in terms of endothelial cell loss, inflammation, thrombus formation, necrosis and fiber degeneration in the muscular layer. Carl Zeiss Axiocam ERc5 model (Carl Zeiss Microscopy GmbH 07745 Jena, Germany) digital camera clip-on microscope device was used in the evaluations.

2.4.1. Histopathological scoring

This study was evaluated semi-quantitatively using Abramov's histologic scoring system [20]. In the evaluation, it was graded as 0 (none), 1 (scant), 2 (moderate) or 3 (abundant). In the study, findings such as endothelial cell loss, inflammation, necrosis and smooth muscle fiber degeneration were examined. Each parameter was independently evaluated by an expert histologist in the field.

2.4.2. Histomorphometric analysis

For histomorphometric analysis, 7 μm thick serial sections were taken from the tissue sample with 1/10 sampling for the layer thickness and lumen diameter analysis of the artery wall. The thickness and lumen diameter of the layers were measured in 6 serial sections taken for each vascular tissue. ImageJ Software program was used for analysis.

2.5. Biochemical analysis

2.5.1. Malondialdehyde

The amount of lipid peroxidation was measured according to the concentration of thiobarbituric acid reagent kinds [21]. One volume of sample (plasma) and two volumes of stock solution (0.375% thiobarbituric acid and 15% trichloroacetic acid in 0.25 N HCl) were mixed in the centrifuge tube. The solution was heated in boiling water for 15 min and then cooled. Separation was achieved by centrifuging the precipitate at 4500 rpm for 10 min. Then, test samples were read at 532 nm. The amount of lipid peroxidation is stated as nmol/g. Shimadzu, UV-1280 and UV/VIS Spectrometer devices were used in the experimental study. The amount of MDA is indicated as an indicator of lipid peroxidation, and it increases in the presence of oxidative stress [22].

2.5.2. Reduced glutathione

The amount of GSH was measured at 412 nm using Sedlak and Lindsay method (Sedlak 1968). 0.5 ml of sample was taken from the sample (plasma) and 0.4 ml TCA was added to it. It was then centrifuged at 1000 rpm for 5 min. Next, 2 ml of Tris-EDTA buffer (0.2 M, pH = 8.9) and 0.1 ml of 0.01 M 5.5′-dithiobis-2-nitrobenzoic acid were added onto 0.5 ml of supernatant. This mixture was left at room temperature for 5 min and its absorbance was measured at 412 nm in a spectrophotometer. Shimadzu, UV-1280 and UV/VIS Spectrometer devices were used in the experimental study. GSH-related metabolisms are an important mechanism to protect cells against agents that cause oxidative stress [22].

2.6. Ethics

The study was approved by the Adıyaman University Animal Experiments Local Ethics Committee (Protocol No: 2020/061, Decision No: 4). MSCs lyophilization process steps were transmitted by cold chain method at VETAL Incorporated Company with the permission of Acıbadem University Ethics Committee (Protocol No: 53005807–12.11.2017). The study has been reported in line with the STROCSS criteria [23]. The study was registered at www.researchregistry.com (researchregistry7146), https://www.researchregistry.com/register now#home/registrationdetails/613d0453a1292a001e3dea71/)

2.7. Data analysis

The data analysis was performed by using IBM SPSS Statistics 25. Results were evaluated at a 95% confidence interval, and the p-value is less than 0.05 significance level. The variables in the study were described with descriptive statistics such as frequency, percentage, arithmetic mean, standard deviation, confidence intervals and graphics. Whether the data showed normal distribution was determined by Kolmogorov-Smirnov and Shapiro-Wilk normality tests. The data that did not show normal distribution were performed with Kruskal-Wallis, a nonparametric test, and comparisons between groups were performed using Tamhane's test. The upper limit of significance level was taken as $p = 0.05$. The data were analyzed with IBM SPSS Statistics 25.

3. Results

3.1. Histopathological evaluation results

3.1.1. Control group

In the control group, light microscopy of the femoral artery revealed that the vessels had a thin and smooth endothelium, a thin curved internal elastic lamina and concentrically located smooth muscle cells. Adventitia was made of connective tissue, and collagen fiber structures were apparent (Fig. 1A, B and C). No pathology was found in the intima, media and adventitia layers. Mast cell density was normal (Fig. 1D).

3.1.2. Sham (physiological saline) group

In the light microscopic examination of the femoral artery in the sham group, it was observed that the normal vascular wall structure was disrupted, unlike the control group. Also distortion of the folded structure of the inner elastic lamina in some areas, deformity of the lumen and marked thickening of the vessel wall thickness, deterioration in the endothelial integrity, fiber degeneration in the muscle layer and tissue loss areas were found, and these were apparent (Fig. 2A, B and C). Inflammation areas were detected in the adventitia layer. There was no

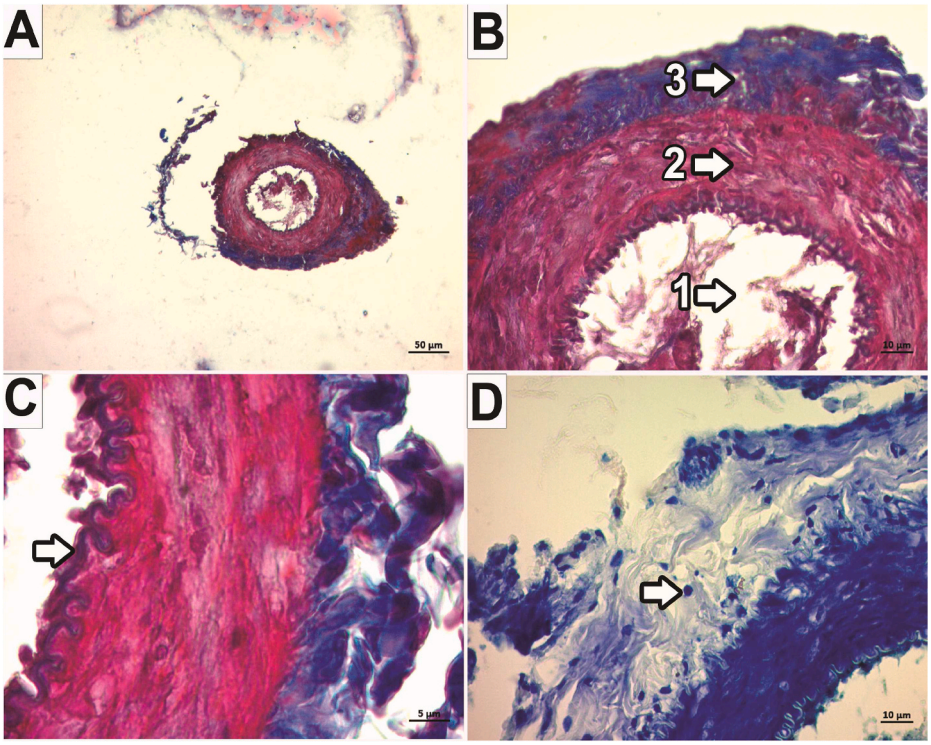

Fig. 1. Histological appearances in the control group. A: Masson's trichrome staining and appearance at x10 objective magnification. B: Masson's trichrome staining and appearance at x40 objective magnification. First arrow; lumen, second arrow; muscular layer, third arrow; adventitia layer. C: Masson's trichrome staining and appearance at x100 objective magnification. Arrow; inner elastic lamina. D: Toluidine blue staining and appearance at x40 objective magnification. Arrow; mast cell. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

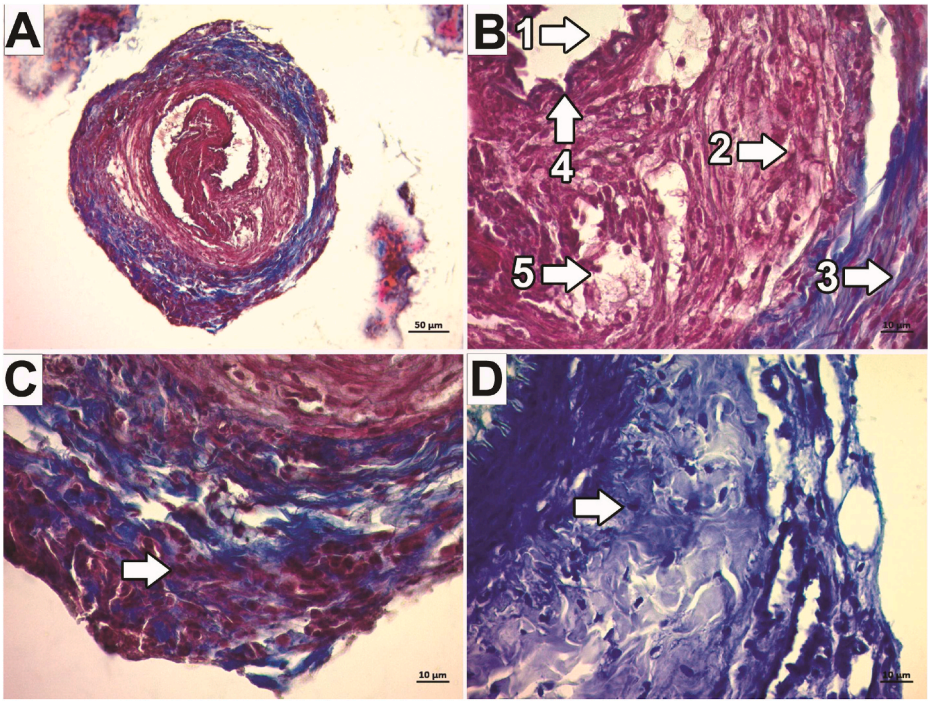

Fig. 2. Histological appearances in the sham group. A: Masson's trichrome staining and appearance at x10 objective magnification. B: Masson's trichrome staining and appearance at x40 objective magnification. First arrow; lumen, second arrow; muscular layer, third arrow; adventitia layer; fourth arrow; inner elastic lamina; fifth arrow; muscular degeneration. C: Masson's trichrome staining and appearance at x100 objective magnification. Arrow; inflammation in the adventitia layer. D: Toluidine blue staining and appearance at x40 objective magnification. Arrow; mast cell. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

increase in mast cell density (Fig. 2D).

3.1.3. Lyophilized stem cell group

In the experiment group, in which lyophilized stem cells were used, it was observed that findings similar to the sham group were dominant. Normal vessel wall structure was found to be impaired in this group as well. It was also observed that there were flattening in the inner elastic lamina in places, changes in the morphological structure and vacuolization of the muscle fibers in the media layer, and increased inflammation in the adventitia layer compared to the other groups. In addition, a significant thickening was detected in the thickness of the vessel wall and a dense thrombus was observed (Fig. 3A, B and C). No increase in mast cell density was observed (Fig. 3D).

3.1.4. Histopathologic scoring result

In the study, no pathological finding was found because a normal tissue structure was dominant in the control group (0-none). Significant pathological findings such as endothelial damage, flattened areas where the folded structure in the inner elastic lamina disappeared, muscle fiber degeneration and inflammation in the adventitia were observed in tissue samples belonging to the sham and stem cell groups (2–3, moderate-abundant). In addition, there were thrombus formation in tissue

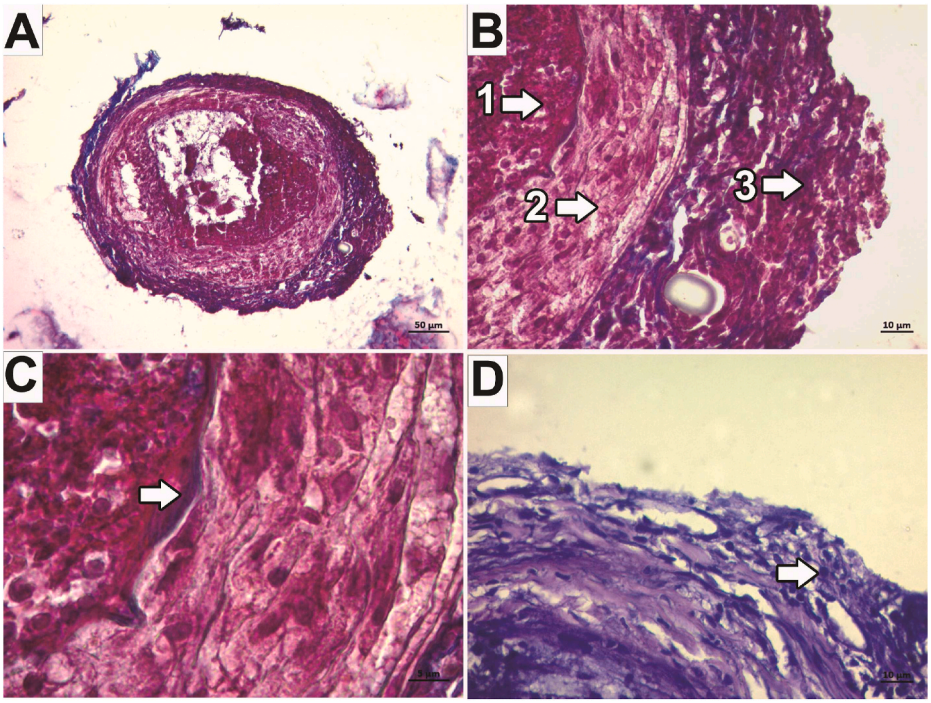

Fig. 3. Histological appearances in the Lyophilized Stem Cell group. A: Masson's trichrome staining and appearance at x10 objective magnification. B: Masson's trichrome staining and appearance at x40 objective magnification. First arrow; thrombus in the lumen, second arrow; edema in the muscular layer, third arrow; inflammation in the adventitia layer; C: Masson's trichrome staining and appearance at x100 objective magnification. Arrow; flattening in the inner elastic lamina. D: Toluidine blue staining and appearance at x40 objective magnification. Arrow; mast cell. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

samples belonging to the stem cell group (3 abundant). The scoring data obtained from the evaluation result were shown in Table 1.

3.2. Histomorphometric analysis results

7 μm thick serial sections were taken from the tissue sample with 1/10 sampling for the layer thickness and lumen diameter analysis of the artery wall. The thickness and lumen diameter of the layers were measured in 6 serial sections taken for each vascular tissue. ImageJ Software program was used for the analysis, and it was presented in Fig. 4.

There was a significant decrease in the thickness of the intima layer in the lyophilized stem cell group compared to the other groups ($p < 0.05$). It was found that the thickness of the media layer increased significantly in the lyophilized stem cell group compared to the other groups ($p < 0.05$). Likewise, an increase in the thickness of the adventitia layer was determined in the lyophilized stem cell group according to the analysis result ($p < 0.05$). As a result of lumen diameter measurements, although a decrease was detected in the sham and lyophilized stem cell group compared to the control group, this difference did not indicate a statistically significant result ($p > 0.05$). Analysis results were shown in Table 2.

3.3. Biochemical analysis results

3.3.1. Malondialdehyde

When Malondialdehyde (MDA) levels in plasma samples were examined, an increase was observed in the lyophilized stem cell group compared to the control group ($p < 0.05$). There was no significant difference in MDA levels between sham and control groups ($p > 0.05$). The increase in MDA level in the lyophilized stem cell group supports the presence of oxidative stress (Table 3).

3.3.2. Reduced glutathione

When the reduced glutathione (GSH) levels in plasma samples were examined, an increase was detected in the lyophilized stem cell group, but it was found that these data did not create a statistically significant difference between the groups ($p > 0.05$) (Table 3).

4. Discussion

It has been stated that MSCs have the potential to transform into endothelial-like cells in both in vivo and in vitro conditions and have immunoregulatory properties [17,24–29]. In the light microscopy examination of the femoral artery of 9 rats in each group, the vascular endothelium, its location and smooth muscle cells were observed. No pathology was found in the control group. However, in the sham group, deterioration of the inner elastic lamina, deformity of the lumen and marked thickening of the vascular wall thickness, deterioration in endothelial integrity, fiber degeneration in the muscle layer and tissue loss were detected. Similar results were found in the experimental group as in the sham group.

From a pathophysiological point of view, sources of information are inadequate due to the lack of evidence on signaling molecules, receptors, stimulants, inhibitors, or endothelial differentiation. MSCs endothelial differentiation feature is also related to the nanomechanical aspect [30,31]. Although the vital importance of neoangiogenesis and the therapeutic MSCs' restoration of aged, worn or damaged tissues seems promising for Endothelial Cell (EC) production [24,32], it has been reported that the culture conditions related to the differentiation of

Table 1
Results of histopathological scoring performed on tissue samples of the groups.

Groups	Control	Sham	MSC-lyophilisate
Endothelial dysfunction	0.33 ± 0.516	1.5 ± 0.548[b]	2.17 ± 0.753[a]
Smooth muscle fiber degeneration	0.5 ± 0.548	2.17 ± 0.408[c]	2.33 ± 0.816[a]
Necrosis	0.17 ± 0.408	2.00 ± 0.632[c]	2.17 ± 0.753[a]
Adventitial inflammation	0.17 ± 0.408	2.00 ± 0.632[c]	2.33 ± 0.816[a]
Thrombus formation	0.50 ± 0.837	0.67 ± 0.816	2.55 ± 0.548[a,d]

[a] It expresses the highly significant difference between the lyophilized stem cell group and the control group. ($p < 0.05$).
[b] It expresses the significant difference between the sham group and the control group. ($p < 0.05$).
[c] It expresses the highly significant difference between the sham group and the control group. ($p < 0.05$).
[d] It expresses the highly significant difference between the sham group and the lyophilized stem cell group. ($p < 0.05$).

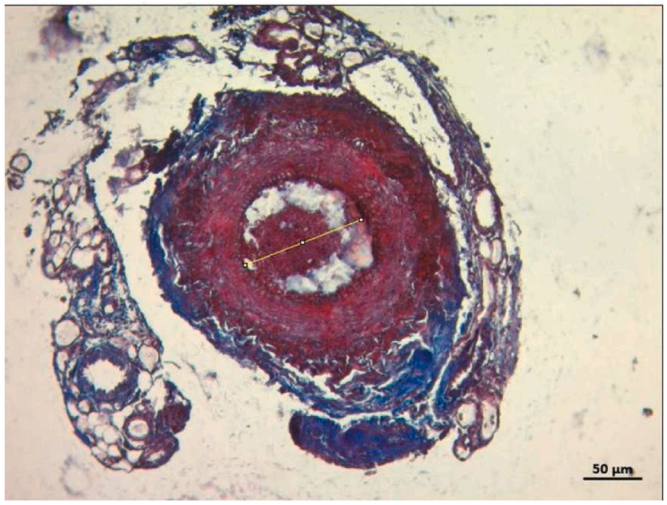

Fig. 4. Image j program.

Table 2
Histomorphometric evaluation results of vascular wall structures in tissue samples of the groups.

Groups	Control	Sham	MSC-lyophilisate
Tunika intima thickness (micrometers)	4.17 ± 0.703	3.47 ± 0.987	1.50 ± 0.240[a,b]
Tunika media thickness (micrometers)	27.91 ± 7.42	25.07 ± 4.68	39.36 ± 8.69[a,b]
Tunica adventitia thickness (micrometers)	28.56 ± 3.23	25.98 ± 6.91	53.54 ± 6.75[b,c]
Lumen diameter (micrometer)	93.23 ± 12.02	68.25 ± 8.16	74.80 ± 29.23

[a] It expresses the highly significant difference between the lyophilized stem cell group and the control group ($p < 0.05$).
[b] It expresses the highly significant difference between the lyophilized stem cell group and the sham group ($p < 0.05$).
[c] It expresses the highly significant difference between the lyophilized stem cell group and the control group ($p < 0.01$).

Table 3
Biochemical parameter results of tissues belonging to the groups.

Groups	Control	Sham	MSC-lyophilisate
MDA (nmol/ml)	5.15 ± 1.63	6.74 ± 0.93	7.20 ± 1.12[a]
GSH (μmol/ml)	128.51 ± 4.31	125.41 ± 10.85	124.88 ± 6.72

[a] It expresses the significant difference between the lyophilized stem cell group and the control group ($p < 0.05$).

MSCs to ECs and the properties of the target tissue have effects on potential effectors determining the survival time of MSCs [30].

In the experiment, it was found that the femoral artery wall structure was disrupted after the administration of MSC-lyophilisate. Flattening of the inner elastic lamina, morphological changes and vacuolization in muscle fibers, inflammation and significant vascular wall thickening in the adventitia were observed. In addition, according to the histopathological scoring results obtained in our study, it was found that there was a normal tissue structure and no pathological finding (0-none). Significant pathological findings such as endothelial damage, flattened areas where the folded structure in the inner elastic lamina disappeared, muscle fiber degeneration and inflammation in the adventitia were observed in tissue samples belonging to the sham and stem cell groups (2–3, moderate-abundant).

In studies on the differentiation of MSCs into endothelial-like cells, it has been determined that the EC-specific marker panel (Kinase Insert Domain Receptor-KDR, Fms-related Tyrosine Kinase-FLT-1 and vWF) has expression and integrin modulation at both gene and protein levels [32,33]. On the other hand, in the endothelial differentiation of MSCs obtained from human amnion, although cells with some angiogenic features were observed, it was determined that the formation/differentiation of mature and healthy EC could not be achieved. The down-regulation of pro-angiogenic factors (tenascin C, Tie-2, VEGF, and FGF2 and up-regulation of anti-angiogenic factors, sprinkle F1, sprouty1, angioarrestin, and endostatin) might be the reason for this [32]. In our study, it was determined that MSC-lyophilisate applied layers of the experimental group could not preserve tissue integrity and thrombus developed in tissue-specific differentiation (histopathological scoring result: 3-abundant). It has been found that trophoblast debris (residue) is transported from the placenta to the target organ in vivo and causes problems such as microembolism or hypertension while triggering endothelial cell activation in the transported organ [34]. Ultimately, placental stromal cells significantly increase the coagulation cascade [35]. In our study, thrombus formation may have been caused by the physiology of placental stromal cells.

In the literature, there are details that when certain conditions (hypoxic or osteogenesis-inducing conditions) occur, MSCs secrete the pro-angiogenic factor VEGF and the endothelial marker VEGFR1 (FLT-1) is expressed [36]. In addition, current studies indicate that EC-related markers are expressed after the injection of MSCs into damaged tissues [37,38]. In a study, the effect of MSC-lyophilisates experimental mechanisms on Premature Ovarian Insufficiency was examined. In the same study, an in vitro study was conducted using Human Ovarian Microvascular Endothelial Cells (HOVECs) to take advantage of the angiogenic properties and regenerative effects of MSC-lyophilisates. As a result of the aforementioned study, MSC-lyophilisates were found effective in the treatment of human primary HOVECs, and the expression of angiogenesis markers was monitored (Endoglin, Tie-2, and VEGF) [39].

Although it has been emphasized that VEGF stimulates the differentiation of human and rat MSCs into endothelial-like cells via the Rho/myocardin-related transcription factor-A (MRTF-A) family, it has been stated that MRTF-A selectively ablates the differentiation of MSCs to EC [39]. In other studies, it was emphasized that the up-regulation of the myocardin transcription factor failed to differentiate human-derived MSCs in smooth muscle cells into ECs only in non-human species [41,42].

In our study, no increase in mast cell density was observed in all three groups. Therefore, it is possible to say that the intervention applied in the experimental group did not affect the immune regulation properties. In a similar study, human-sourced MSCs and ECs were cultured together, then it was determined that the femoral artery network was successfully formed and MSCs preserved the differentiation ability. However, in the same study, it was determined that osteogenic and adipogenic induction damaged previously formed microvascular structures and jeopardized the ability of MSCs to secrete angiogenic factors [43]. In the literature, it has been stated that maternally derived MSC-lyophilisate is beneficial in improving kidney damage [44,45] and regeneration of atrophied muscles [46]. In this regard, it has been determined that exosomes produced from the human umbilical cord improve oxidative stress and cell apoptosis and increase cell proliferation in rats with Acute Kidney Injury [45].

To the best of our knowledge this is the first study that have examined the effects of MSC-lyophilisates obtained from maternal sources on femoral artery anastomosis. In this study, where we examined its effectiveness in rats, while MSC-lyophilisates were expected to increase vascular regeneration, they resulted in thrombus formation, morphological changes and inflammation. It is clear that the results obtained from this research will guide future studies and will be an important source of information to conduct new research designs. The limitations of the study can be listed as being tested in rats, the lack of an optimal number of similar studies, and the number of small samples.

5. Conclusion

This experimental study, conducted to determine the efficacy of the use of maternal origin MSC-lyophilisate in the femoral artery anastomosis, which is preferred because it is an easily accessible, storable and inexpensive method in addition to its strong effects on tissue regeneration, has the feature of being an important source of information. Since the number of experimental studies on the use of MSC-lyophilisate is insufficient in terms of quality and number, rats are generally preferred as the sample group. One of the most important results we obtained in our study is that thrombus developed in the femoral arteries of the rats in the experiment group after MSC-lyophilisate application. Therefore, intravenous heparin may be recommended in addition to the administration of MSCs for future studies. In this way, it will be possible to control the tendency of placental stromal cells to coagulation without ignoring the endothelial cell activation feature.

It can be concluded that the deterioration of the femoral artery wall structure, morphological changes in muscle fibers, and significant vascular wall thickening caused thrombus formation, and this was due to possible partial obstruction and circulatory failure in the experiment group. Apart from the current estimates, it would be useful to investigate the causes of vacuolization, inflammation in the adventitia and significant vessel wall thickening in more detail. In this regard, the suitability of the application method of MSC-lyophilisate to physiology remains unclear. Awareness of the benefits of MSC-lyophilisate is high. However, it is important to examine the method of administration, confounding variables, possible complications and adverse events in depth. We evaluate that it will be meaningful to determine the dose amounts of MSC-lyophilisates for hemostasis without creating thrombus after anastomosis in future studies. The findings of our study; emphasize that we have found a safe way to achieve hemostasis. However, when the appropriate dose study of MSC-lyophilisate is done, it can be a hemostatic product that can be used safely in the future without the need for additional suturing to the vessel in femoral artery anastomosis.

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Ethical approval

Since the study did not include human subject ethical approval was not taken.

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Author contribution

FNK: Designing the study, analysis and interpretation of data, collecting data and final approval of the version.

EA: Review the written material and edited. Revising critically.

Both of the authors have read and approved the final manuscript.

Consent

None.

Registration of research studies

1. Name of the registry:
2. Unique Identifying number or registration ID:
3. Hyperlink to your specific registration (must be publicly accessible and will be checked):

Guarantor

MD. Fatma Nilay TUTAK.

Declaration of competing interest

The authors certify that he has affiliations with or involvement in any organization or entity with any financial interest.

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