FULL LENGTH | Iranian Biomedical Journal 27 (5): 257-268 September 2023

Antimicrobial Effects of Mouse Adipose-Derived Mesenchymal Stem Cells Encapsulated in Collagen-Fibrin Hydrogel Scaffolds on *Bacteroides fragilis* Wound Infection in vivo

Mansoor Khaledi[1], Mohammad Hossein Ahmadi[1], Parviz Owlia[2], Horieh Saderi[1*]

[1]Department of Microbiology, Faculty of Medicine, Shahed University, Tehran, Iran; [2]Molecular Microbiology Research Center, Faculty of Medicine, Shahed University, Tehran, Iran

[Downloaded from ibj.pasteur.ac.ir on 2025-04-29]

[DOR: 20.1001.1.1028852.2023.27.5.3.9]

[DOI: 10.61186/ibj.27.5.257]

OPEN ACCESS

Received: 11 October 2022
Accepted: 25 June 2023
Published online: 27 June 2023

Citation:
Khaledi M, Ahmadi MH, Owlia P, Saderi H. Antimicrobial Effects of Mouse Adipose-Derived Mesenchymal Stem Cells Encapsulated in Collagen-Fibrin Hydrogel Scaffolds on *Bacteroides fragilis* Wound Infection in vivo. *Iranian biomedical journal* 2023; 27(5): 257-268.

ABSTRACT

Background: Anaerobes are the causative agents of many wound infections. *B. fragilis* is the most prevalent endogenous anaerobic bacterium causes a wide range of diseases, including wound infections. This study aimed to assess the antibacterial effect of mouse AD-MSCs encapsulated in CF hydrogel scaffolds on *B. fragilis* wound infection in an animal model.
Methods: Stem cells were extracted from mouse adipose tissue and confirmed by surface markers using flow cytometry analysis. The possibility of differentiation of stem cells into osteoblast and adipocyte cells was also assessed. The extracted stem cells were encapsulated in the CF scaffold. *B. fragilis* wound infection was induced in rats, and then following 24 h, collagen and fibrin-encapsulated MSCs were applied to dress the wound. One week later, a standard colony count test monitored the bacterial load in the infected rats.
Results: MSCs were characterized as positive for CD44, CD90, and CD105 markers and negative for CD34, which were able to differentiate into osteoblast and adipocyte cells. AD-MSCs encapsulated with collagen and fibrin scaffolds showed ameliorating effects on *B. fragilis* wound infection. Additionally, AD-MSCs with a collagen scaffold (54 CFU/g) indicated a greater effect on wound infection than AD-MSCs with a fibrin scaffold (97 CFU/g). The combined CF scaffold demonstrated the highest reduction in colony count (the bacteria load down to 29 CFU/g) in the wound infection.
Conclusion: Our findings reveal that the use of collagen and fibrin scaffold in combination with mouse AD-MSCs is a promising alternative treatment for *B. fragilis*. ***DOI: 10.61186/ibj.27.5.257***

Keywords: Bacteroides fragilis, Mesenchymal stem cells, Wound infection

Corresponding Author: Horieh Saderi
Department of Microbiology, Faculty of Medicine, Shahed University, Tehran, P.O. Box: 1476856681, Tehran, Iran; Mobile: (+98-912) 3278569; Fax: (+98-215) 1212644; E-mail: horiehsaderi@gmail.com, saderi@shahed.ac.ir

List of Abbreviations:
AD-MSC: adipocyte derived-mesenchymal stem cell; **AMP:** antimicrobial peptides; **ASC:** adipose stem cells; **ATCC:** American type culture collection; ***Bacteroides fragilis*:** *B. fragilis*; **BMSC:** bone marrow-derived stem cells; **$CaCl_2$:** calcium chloride; **CD:** cluster of differentiation; **CF:** collagen-fibrin; **DMSO:** dimethyl sulfoxide; ***E. coli*:** *Escherichia* coli; ***K. pneumoniae*:** *Klebsiella pneumoniae*; **FBS:** fetal bovine serum; **ISCT:** International Society for Cell Therapy; **MAPK:** Mitogen-Activated Protein Kinase; **MDR:** multidrug resistance; **MSC:** mesenchymal stem cell; **MTT:** 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; ***P. aeruginosa*:** *Pseudomonas aeruginosa*; **PBS:** phosphate-buffered saline; ***S. aureus*:** *Staphylococcus aureus*

[Downloaded from ibj.pasteur.ac.ir on 2025-04-29]

[DOR: 20.1001.1.1028852.2023.27.5.3.9]

[DOI: 10.61186/ibj.27.5.257]

INTRODUCTION

A wound is defined as a breach in the skin and the loss of skin integrity, which provides a wet, warm, and nutritive environment that is ideal for proliferation and colonization of pathogenic microorganisms[1]. The level of contamination in a wound is a determining factor in the probability of infection, with an estimated 50% of bacterial contaminations leading to an infection. Wound infections are classified as contaminated and colonized, as well as local, diffuse, and systemic (sepsis) infections skin and soft tissue infections, due to trauma, burns, and surgery, may cause the formation of exudates, which comprises of dead white blood cells, cell debris, and necrotic tissue[2]. Patients with wound complications often experience shock, prolonged hospitalization, and antibiotic-resistant infections. The emergence of antibiotic resistance can impose a high burden on the healthcare system and increase morbidity rates[3]. Chronic wounds affect approximately 20 million individuals worldwide annually, which treatment and management of those cost over $31 billion[4].

Anaerobes are a major group of endogenous microorganisms that are responsible for wound infections. *B. fragilis* is the most common anaerobic bacterium in endogenous clinical infections[5]. Since most wound infections are polymicrobial, neglecting the anaerobic endogenous microorganisms results in a high treatment failure rate[6]. Other factors that contribute to the failure of treatment include the improper and illogical use of antibiotics, as well as the inherent and acquired resistance mechanisms of bacteria.[7]. The significant increase in antibiotic resistance and treatment failure rates for anaerobic wound infections has led researchers to focus on nonantibiotic treatments[8], including hyperbaric oxygen therapy, ultrasound treatment, laser resurfacing, and skin grafting[9]. The utilization of MSCs is one of the most recent nonantibiotic techniques, which considered an advanced technology in treating wounds and traumas[9]. MSCs can differentiate into cell lineages, such as keratinocytes, adipocytes, chondrocytes, myocytes, and osteoblasts[10].Differentiation of MSCs possibly restores the functional components of the skin, as well as cytokines. MSCs can be isolated from various sources (such as bone marrow, adipose tissue, and cord blood) and have the ability to promote the development of neighboring cells and regulate the immune system reaction to effectively manage inflammation[10]. BMSCspromote tissue repair by producing growth factors, cytokines, and extracellular matrix, as well as by promoting the migration of other cells[11]. ASCs, which are multipotent cells present in adipose tissue and share similarities with BMSCs, offer the advantage of being easy to obtain and causing minimal donor morbidity, unlike BMSCs that require an invasive procedure and have a low[12]. Since the first report by Zuk et al.[13], many studies have confirmed the similarity of the therapeutic effects of ASCs to BMSCs on wound healing, immunoregulatory and antiapoptotic activity[14]. MSCs can be placed on different scaffolds to be used locally. Scaffolds harbor the extracellular matrix properties, including adhesion, migration, proliferation, differentiation, and supporting tissue creation. MSCs and scaffolds have previously been used to restore the damaged tissues for organ transplantation[15].

Fibrin, an essential protein for blood clotting, supports the growth of new blood vessels (angiogenesis) and facilitates the restoration of blood supply. This protein is able to enhance cell adhesion, while exhibiting high levels of biocompatibility and biodegradability. Upon contact with fibrin, cells gradually replace the fibrin scaffold with an adult tissue-specific extracellular matrix[16]. Collagen is another primary protein found in the extracellular matrix and is responsible for providing structural support. Collagen is a critical component of many tissues and benefits from multipleproperties, including low immunogenicity, high porosity and permeability, excellent biocompatibility and bio-degradability, as well as ability to regulate cell behavior such as morphology, adhesion, migration, and differentiation[17]. All these superb features make these two natural polymers promising biomaterials for scaffolding in tissue engineering.

MSCs encapsulated in scaffolds have recently been used to heal bacterial infections such as *P. aeruginosa* and *S. aureus*[18]. Previous studies have indicated that MSCs hold promise for wound healing and possess antimicrobial properties, which could be advantageous in addressing the complex treatment of *B. fragilis* wound infections mentioned earlier. Our goal was to investigate the antibacterial efficacy of adipose tissue-derived MSCs and hydrogel scaffolds made of fibrin and collagen against anaerobic wound infections caused by *B. fragilis*.

MATERIALS AND METHODS

Isolation of MSCs from adipose tissue

For isolation of MSCs from mouse adipose tissue, 24 male mice (6-8 weeks old; 150-200 g) were provided by the Pasture Institute of Iran (Tehran). After the mice were anesthetized with chloroform, the abdomen and inguinal region were excised, and 5 g of subcutaneous adipose tissue was dissected and cut into small pieces. Then the tissues were washed twice with PBS and

[Downloaded from ibj.pasteur.ac.ir on 2025-04-29]

[DOR: 20.1001.1.1028852.2023.27.5.3.9]

[DOI: 10.61186/ibj.27.5.257]

centrifuged at 2500 ×g for 5 minutes. The supernatant was resuspended in PBS in a sterile tube and centrifuged again. Next, the supernatant was collected and cultured in DMEM (INOCLON, Tehran, Iran) low-glucose complete medium supplemented with 15% heat-inactivated FBS (Gibco [Life Technologies, USA]) and 1% penicillin-streptomycin, and 1% L-glutamine (Gibco [Life Technologies]) solution. The tube was further centrifuged at 2500 ×g for 5 minutes to remove the collagenase. The pellet was then washed with PBS and resuspended in DMEM again. The tubes were incubated in 5% CO_2 and 95% humidity at 37 °C. After 24 h, the flasks were washed with PBS to remove the nonadherent cells, and 10 ml of fresh DMEM-supplemented medium was added. When the adherent cells reached 70-90% confluency, the cells were harvested by 0.25% trypsin-0.02% ethylene diamine tetra acetic acid (Gibco [Life Technologies]). The MSCs were passaged three times, and the cells of the third passage were used for further experiments. Characterization of MSC according to ISCT guidelines was performed by the observation of morphology, immunophenotyping, and differentiation potential[19].

Investigation of MSC differentiation ability to adipocytes

DMEM was supplemented with 15% FBS, dexamethasone (100 nM), indomethacin, and insulin (50 g/ml) to differentiate MSCs from adipocytes. DMEM containing 15% FBS was added to the wells as a negative control. The medium was discarded, and a fresh medium was added to each well every three days. The MSCs were incubated in 5% CO_2 at 37 °C for 14 days. The cells were fixed with 4% formalin at room temperature for 1 hour and then stained with oil red solution 0.5% in isopropanol 99% for 10-15 minutes. Afterwards, the cells were washed three times with ethanol 70% and visualized under a light microscope at a magnification of 40×[20].

Identification of the isolated MSCs by flow cytometry

After three passages of mouse AD-MSCs, cell surface markers were evaluated to confirm the population of AD-MSCs. To this end, AD-MSCs were suspended in PBS. Cell suspensions and monoclonal antibodies against CD105, CD45, and CD90 (eBioScience, Inc., San Diego, CA, USA) were used to determine the presence of surface markers using a FACS Caliber flow cytometer (FACScan; Becton Dickinson, San Jose, CA, USA)[21].

Synthesis of collagen and fibrin scaffold

To prepare the collagen scaffold, commercial type I collagen powder, L-glutamine, sodium bicarbonate, DMEM, and 1 ml of saline were mixed to a final 1 mg/ml concentration. To polymerize the collagen, 1 ml of the prepared solution was added to each well of a six-well microplate and then incubated at 37 °C for 30 min[22]. Fibrin hydrogel scaffolds were constructed using specific concentrations of $CaCl_2$ and thrombin (Sigma, USA). $CaCl_2$ and thrombin were filtered through a 0.22 µm filter before using. A 10% w/v fibrinogen solution (1.5 mg of fibrinogen + 0.5 ml of M199 solution; Sigma, USA) was obtained by dissolving fibrinogen powder in a sterile PBS solution and filtering using a 0.22 µm filter. A 1 U/ml of thrombin solution was prepared by dissolving thrombin powder in 0.1 ml of PBS to polymerize fibrinogen to fibrin. Finally, 10-well plates were covered with PBS and incubated at room temperature for 24 h[22,23].

Synthesis of CF scaffold

The collagen and fibrinogen solutions were mixed at a 2:1 ratio, and the mixture was stirred until homogenous. Then the pH of the CF solution was adjusted to 7.4. CF hydrogels were formed by polymerizing the single components in the mixture. All gel solutions were cast into Teflon annular ring molds (15.5 mm O.D., 11.5 mm I.D.) and incubated at 37 °C overnight[23].

Encapsulation of AD-MSCs in constructed scaffolds

A total of 2×10^6 cell/ml MSCs at the third passage were loaded on each scaffold. The scaffolds were incubated for 30 minutes. Then 1 ml of DMEM containing 15% FBS was added and incubated at 37 °C for 72 hours[24]. The growth and proliferation of AD-MSCs on collagen and fibrin scaffolds were examined using the MTT assay on days 1, 3, and 7 after seeding. To do this, 20 µl of MTT reagent (5 mg/ml) and 200 µl of DMEM was added to each well and incubated at 37 °C for 4 h. Thereafter, the MTT and medium were discarded, and 600 µl of DMSO was added to each well. Finally, the absorbance of the cells was evaluated using a plate reader at 570 nm. The seeded cells without scaffolds were used as negative controls[25].

Analysis of MSC proliferation on scaffolds by MTT assay

The growth and proliferation of MSCs on collagen, fibrin, and CF scaffolds were assessed using the MTT test. After the transplantation of MSCs into the scaffold, on days 1, 3, and 7 after seeding, the propagation power of scaffold was compared to the control. For this test, 20 µl/well of 5 mg/ml of MTT was poured onto the scaffold, and the plates were completely covered with foil and placed in the incubator at 37 °C for 4 h. After the formation of purple crystals, the solution was dissolved in 600 µl of DMSO solution, and the

absorbance of the solution was calculated by ELISA at a wavelength of 570 nm. As a control, the cells were cultivated directly in the wells without scaffolds[25].

In vivo assay

Preparation of bacterial suspension

The proposed bacterium *B. fragilis* ATCC 23745 was obtained from the Pasteur Institute of Iran. The standard strain was cultured on Columbia agar and incubated under anaerobic conditions at 37 °C for 48 h. A 0.5 McFarland (1.5×10^8 CFU/ml) concentration of bacterial suspension (OD = 0.08-0.13 absorbance at 625 nm) was prepared to induce wound infection.

Development of puncture wound infection in the animal model

A total of 24 8-10-week-old male rats (250-350 g) were selected to induce infection in puncture wound. Animals were housed for five days before undergoing any experiment. Cages were maintained in a room at a temperature of 24 ± 1 °C, with 12 h of lightness and 12 h of darkness, with constant ventilation and free access to water and food supply. Twelve rats were randomly divided into four experimental groups. Each experimental group consisted of three rats; the triplicate method was used. The groups included rats with infected wounds receiving AD-MSCs (group 1), AD-MSCs on the collagen scaffold (group 2), AD-MSCs on the fibrin scaffold (group 3), and AD-MSCs on the CF scaffold (group 4). The remaining 12 rats were randomly divided into four control groups, including rats with infected wounds receiving no treatment (group 1), collagen scaffold (group 2), fibrin scaffold (group 3), and CF scaffold (group 4). Rats were first anesthetized with xylazine (20 mg/kg) and ketamine (100 mg/kg) and their dorsal hair was removed, and then the body surface was cleaned with povidone-iodin. A 2 × 2 cm wound was created using a punch measuring, and after 30 minutes, 50 µl of the standard dilution (0.5 McFarland) of *B. fragilis* was applied to the puncture wound site. Twenty-four hours later, the wound infection was developed. After one day, the considered treatment for each group was applied to the wound, and the rats were observed for one week.

Colony count of microbial infection after treatment of infected rats

To assess the antibacterial effects of the AD-MSCs and the scaffolds, all the rats were euthanized with CO_2 after one week. Subsequently, 1 g of the wound tissue was dissected and homogenized. Following the preparation of a serial dilution, ranging from 10^{-1} to 10^{-14} of the wound tissue in PBS, 50 µl of each dilution was cultured on Columbia agar and incubated under anaerobic conditions at 37 °C for 24 hours. The next day, the number of colonies of each dilution was counted, and the bacterial load was calculated in CFU/g[26].

Statistical analysis

All results were expressed as mean ± SEM. Data obtained from different groups were compared using a one-way analysis of variance (ANOVA), followed by either the Turkey's post hoc multiple comparison tests. All statistical analyses were performed using SPSS version 26.0. Differences with a 95% or higher confidence level were considered statistically significant at $p < 0.05$.

RESULTS

Morphological and differential characterization of AD-MSC

MSCs were characterized by morphological monitoring, immunophenotyping, and differentiation potential. MSCs attached to the culture substrate demonstrated a fibroblast-like appearance and spindle-shaped structure and were frequently propagated and maintained when examined using an inverted microscope (Fig. 1A). Additionally, using oil Red staining, lipid droplet adipocytes were dyed in order to detect mineralized matrix in cell cultures (Fig. 1B). Cells were analyzed for the presence of the CD marker using flow cytometry, which confirmed the positivity of cells for CD45, CD90, and CD105 as standard MSC surface antigens (95% purity; Fig. 2), but they were negative for CD34 as a standard hematopoietic surface marker (Fig. 3). Overall, our results confirmed that AD-MSC met the criteria of ISCT.

Cell proliferation assessment using MTT assay

Cell proliferation was evaluated by MTT assay, and analysis of variance was assessed at a significant level of $p < 0.05$. In all three scaffolds, a gradual rise was observed in cell proliferation and stimulation over time (Fig. 4). Based on the results, our scaffolds were biocompatible and completely nontoxic for MSCs, since no significant cytotoxicity was observed in the hydrogel culture conditions.

Process of wound healing in infected rats

The wound (2 × 2 cm) developed in rats was infected with *B. fragilis*, and then the AD-MSCs and the scaffolds were applied. The rats that were administered MSCs and CF scaffold exhibited the most significant outcomes, with the 2 cm incision healing and the infection disappearing by day 20 (Fig. 5).

[Downloaded from ibj.pasteur.ac.ir on 2025-04-29]
[DOR: 20.1001.1.1028852.2023.27.5.3.9]
[DOI: 10.61186/ibj.27.5.257]

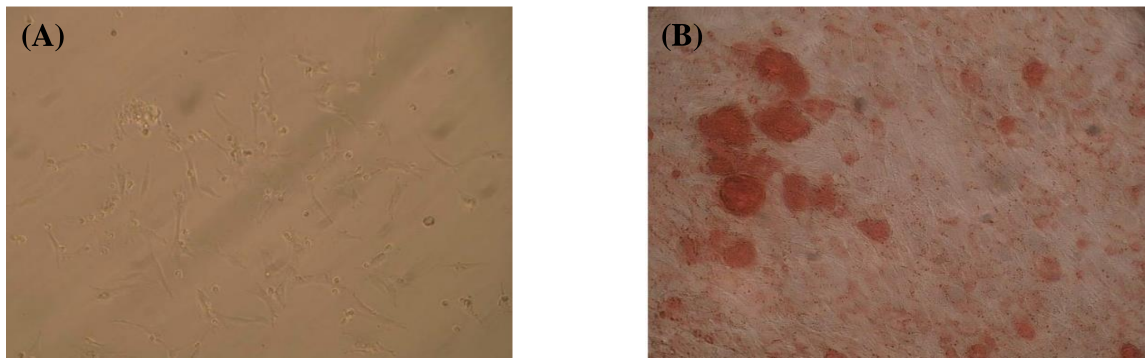

Fig. 1. (A) Morphology of AD-MSCs (×40); (B) AD-MSC differentiation into adipocytes using oil Red staining (×40).

Comparison of colony count among the experimental groups

The bacterial load in the infected wound of rats in each group was assessed by counting the bacterial colonies, and the results were then compared. The colony count on the dilutions 10^{-12}, 10^{-13}, and 10^{-14} is presented in Table 1. In all three dilutions, the highest number of colonies was observed in the control group 1, which received no treatment, while the lowest count was detected in the experimental group 4, which received AD-MSCs encapsulated in a CF scaffold. The results showed that the mean difference among the studied groups was statistically significant ($p < 0.001$) at the 95% confidence level (Table 2). Therefore, we used

Fig. 2. Flow cytometric analysis of CD45, CD90, CD105 markers of AD-MSCs. The green graph represents the expression level of the surface marker in the cell, and the black graph represents the control isotype.

[Downloaded from ibj.pasteur.ac.ir on 2025-04-29]
[DOR: 20.1001.1.1028852.2023.27.5.3.9]
[DOI: 10.61186/ibj.27.5.257]

[Downloaded from ibj.pasteur.ac.ir on 2025-04-29]
[DOR: 20.1001.1.1028852.2023.27.5.3.9]
[DOI: 10.61186/ibj.27.5.257]

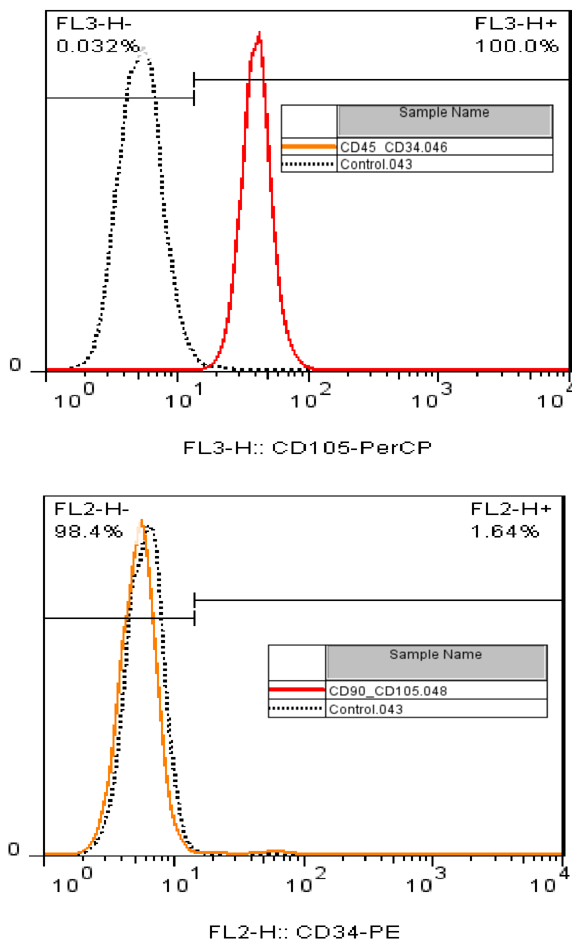

Fig. 3. Flow cytometric analysis of CD45 and CD90 markers of AD-MSCs. The red graph shows the expression level of the surface marker in the cell, and the black graph represents the control isotype.

the LSD post-hoc test to confirm the pairwise comparisons (Table 3). Three experimental groups that received AD-MSCs on different scaffolds were compared. It was observed that among these three groups, rats receiving AD-MSC encapsulated in a CF scaffold had the lowest colony count of *B. fragilis* on their wound. One-way ANOVA test results showed that the mean difference between the three studied groups was statistically significant at the 95% confidence level ($p < 0.001$; Table 4). Therefore, we used the LSD post hoc test to confirm the pairwise comparisons. The results are demonstrated in Table 5.

Comparison of colony count among control groups

Four control groups consisting of group 1 (no treatment), group 2 (collagen scaffold), group 3 (fibrin scaffold), and group 4 (CF scaffold) were compared in terms of colony count of *B. fragilis*. Control group 1 had the highest number of colonies. In contrast, control 4 had the lowest number of colonies, confirming the highest healing effects of the CF scaffold among other scaffolds. One-way ANOVA test results showed that the mean difference between the four studied groups is statistically significant at the 95% confidence level ($p < 0.001$; Table 6). Similarly, the LSD post hoc test confirmed this result (Table 7).

DISCUSSION

Anaerobic bacteria such as *B. fragilis* are one of the most important factors in wound infection and have largely been neglected due to limitations in identifying these bacteria[27]. The increased rate of antibiotic resistance among these bacteria has also restricted the available choices for their treatment. Thus, scientists have recently been looking for nonantibiotic treatments for anaerobic bacteria[28]. Cell therapy using hydrogel scaffolds is one of the novel therapeutic approaches for treating antibiotic-resistant bacteria. This approach encapsulates the MSCs in hydrogel scaffolds[29].

Evidence showed that MSCs can directly influence the immunological properties of macrophages and neutrophils by secreting cytokines, including PGE2, IL-6, IL-8, or IFN-β. Exposure to macrophages with these cytokines increases phagocytosis[30]. On the other hand, neutrophils exposed to the MSC-treated medium are resistant to apoptosis and have increased migration. Studies on infection animal models have shown that human MSCs can increase monocyte proliferation and reduce excessive neutrophil influx and neutrophil elastase production, particularly in murine models of cystic fibrosis and pulmonary *P. aeruginosa* infection. MSCs also produce AMPs and short peptides, often found on neutrophils or epithelial cells[30]. AMPs kill bacteria directly by disrupting the integrity of the microbial membrane, as well as by inducing the release

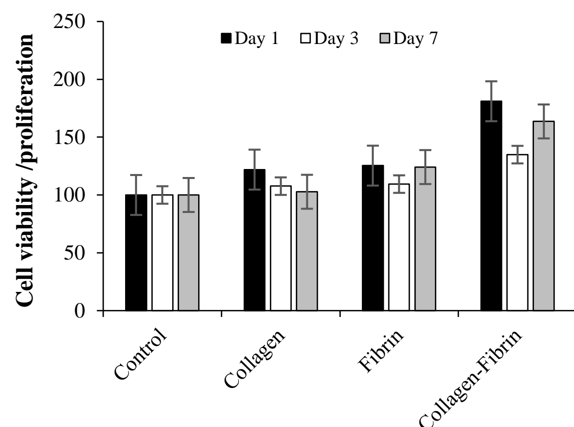

Fig. 4. Cell proliferation evaluation using the MTT assay for MSCs in different rat groups on days 1, 3, and 7. A gradual rise was observed in the cell proliferation seven days after seeding.

[Downloaded from ibj.pasteur.ac.ir on 2025-04-29]

[DOR: 20.1001.1.1028852.2023.27.5.3.9]

[DOI: 10.61186/ibj.27.5.257]

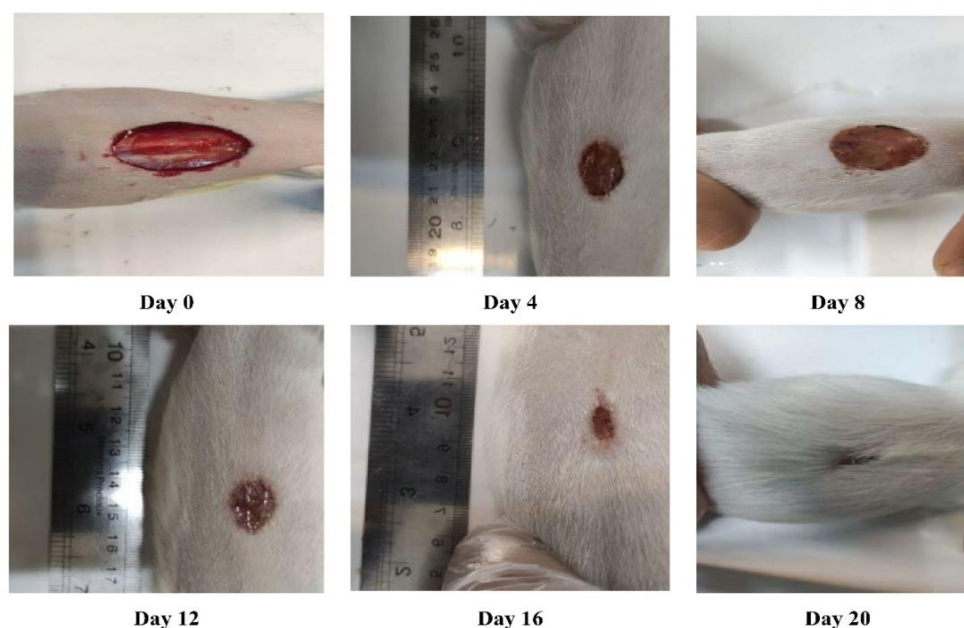

Fig. 5. Process of wound healing in the infected rats receiving the AD-MSCs encapsulated in collagen-fibrin scaffold.

of proinflammatory cytokines and, in turn, the proliferation of immune cells. Human MSCs have been shown to produce several AMPs, including the cathelicidin peptide LL-37[31], hepcidin[32], β-defensin, and lipocalin[33]. Various in vivo mouse models have investigated the effect of MSCs on acute bacterial infections. For instance, human MSCs reduced bacterial counts in mice with *E. coli* pneumonia[31] and mice with *K. pneumoniae* pneumosepsis[34]. Human MSCs also reduced *P. aeruginosa*-related mortality in a murine model of peritonitis and sepsis[35]. MSCs have revealed the ability to increase the effects of antibiotic therapy through the secretion of LL-37 in cystic fibrosis mice[36]. Moreover, MSC instillation into the airways of the explanted lungs decreased *E. coli* burden and attenuated acute lung injury and inflammation[37]. MSCs have been extensively analyzed experimentally and clinically for their ability to promote wound healing and suppress inflammation. However, the use of MSCs in anti-infective therapy has not well been studied[38].

Results of the present study indicated that AD-MSCs encapsulated in collagen and fibrin scaffolds had an amazing therapeutic effect on the rats infected with *B. fragilis* and could reduce the bacterial load in wound infection. The use of AD-MSCs encapsulated in collagen hydrogel scaffold has also been discovered to offer a therapeutic effect that outweighs those of AD-MSCs encapsulated in fibrin hydrogel scaffold. In a number of studies, carriers other than fibrin and collagen have been combined with MSCs. For instance, chitosan nanoparticles have been studied by Saberpour et al.[39] as a carrier of MSCs for their antimicrobial and antibiofilm effects on multidrug resistant *Vibrio cholera.* They concluded that their established model

Table 1. Results of colony count in three final dilutions in four experimental groups and four control groups

Groups	Number of colonies in each dilution (mean)		
	10^{-12}	10^{-13}	10^{-14}
Experiment			
AD-MSCs + collagen	124	89	54
AD-MSCs + fibrin	166	128	97
AD-MSCs + collagen-fibrin	84	36	29
AD-MSCs	198	161	138
Control			
No treatment	Full	412	315
Collagen	274	261	224
Fibrin	287	272	241
Collagen-fibrin	227	185	154

Table 2. One-way ANOVA analysis to compare the colony count of control and experimental groups

Groups	N	Mean	Standard deviation	Minimum	Maximum	p value
AD-MSCs + collagen	3	54.00	3.000	51.00	57.00	
AD-MSCs + fibrin	3	97.00	15.394	84.00	114.00	
AD-MSCs + collagen-fibrin	3	29.00	5.291	25.00	35.00	
AD-MSCs	3	138.00	10.816	129.00	150.00	<0.001
Control 1	3	315.00	5.567	309.00	320.00	
Control 2	3	224.00	6.557	218.00	231.00	
Control 3	3	241.00	4.358	236.00	244.00	
Control 4	3	154.00	9.643	147.00	165.00	

(chitosan nanoparticles) could be a promising option for treating multidrug *Vibrio cholera* in clinical settings. Similarly, Saeedi et al.[40] have reported that pretreatment with lipopolysaccharides offers an innovative development strategy. Lipopolysaccharides improve the survival rate of the murine sepsis model after MSC transplantation and protects cells from apoptosis and organ damage. A different investigation by Shahabadi et al.[41] confirmed the notable antibacterial properties of collagen scaffolds containing curcumin against *Streptococcus mutans.* Another study by Kim et al.[42] revealed that administration of MSCs either alone or in combination with an antibiotic improved survival and organ dysfunction associated with reduction in proinflammatory cytokines (e.g. TNF and IL-6) and chemokines (e.g. CXCL2, CCL5 and KC/IL-8) in peripheral blood and/or bronchoalveolar lavage fluid. Hackstein et al.[43] also reported for the first time the feasibility and in vivo immunomodulatory capacity of prospectively defined MSC in pneumonia. They showed that PαS MSC therapy after respiratory *K. pneumoniae* infection significantly improved the overall survival rate compared to mock-treated (NaCl only) animals and showed that MSCs have the ability to fight bacterial pneumonia due to their antimicrobial properties. Hackstein's study is consistent with the current study in this field. Guerra et al.[44] discovered that preconditioning MSCs with a specific antibiotic can boost their differentiation capabilities and decrease necrosis caused by *S. aureus*. In a review paper published in 2019, Regmi et al.[45] showed that the proinflammatory effect of MSCs is beneficial in the early stages of inflammation and microbial infections; however, their anti-inflammatory effects are helpful in later stages because overactivation of the immune system causes tissue damage or injury. In another review article conducted in 2023 on diabetic foot ulcer, it has been demonstrated that MSC therapy reinforces the healing process in nonhealing diabetic foot ulcers by activating cellular and molecular pathways. Moreover, MSCs secrete molecules with potential antimicrobial effects[46]. The results of that study are completely consistent with our findings. Furthermore, in a recent investigation, it has been reported that combined intravenous administration of AD-MSCs and antibiotics induced a stronger antibacterial effect than antibiotic monotherapy in the methicillin-sensitive *S. aureus*-infected periprosthetic joint infection rat model. It seems that this strong antibacterial effect is related to the increased cathelicidin and decreased inflammatory cytokine expressions at the site of infection[47]. Therefore, it is speculated that the combination of AD-MSCs and antibiotics may have a stronger effect on wound infection compared to the effect of each alone, which requires further analysis in future studies. Antimicrobial and antioxidant properties of collagen against *S. aureus* and *E. coli* as well as its antifungal activity against *Candida albicans* have been recently investigated and confirmed[48]. It has also been suggested that fibrin hydrogel scaffold is able to provide a relatively stable sterile environment for cell adhesion, proliferation, and migration and prolongs cell survival at the wound site, ultimately leading to wound healing and limitation of infection [49]. Similarly, in our study, collagen and fibrin hydrogels showed a high antibacterial effect on *B. fragilis* wound infection compared to the control group (rats with infected wounds receiving no treatment) that received no treatment.

The use of nonantibiotic therapies is critical since bacteria are becoming more and more resistant to antibiotics. One innovative way to treat bacteria is the use of MSCs with biocompatible hydrogel scaffolds. Anaerobic bacteria's role in hospital-acquired infections is underestimated, making research in this area increasingly important and specialized. Our findings demonstrated that one of these innovative and successful antibacterial treatment techniques is the therapeutic use of MSCs. Herein, the AD-MSCs encapsulated in collagen and fibrin scaffolds indicated a considerable therapeutic effect on rats infected with *B. fragilis*. This effective treatment can be used to reduce microbial infection, heal wounds caused by infection and regenerate the skin at the site of an infection.

[Downloaded from ibj.pasteur.ac.ir on 2025-04-29]
[DOR: 20.1001.1.1028852.2023.27.5.3.9]
[DOI: 10.61186/ibj.27.5.257]

[Downloaded from ibj.pasteur.ac.ir on 2025-04-29]

[DOR: 20.1001.1.1028852.2023.27.5.3.9]

[DOI: 10.61186/ibj.27.5.257]

Table 3. LSD post hoc test to confirm the significant difference in colony count among four control and four experimental groups

Group (I)	Group (J)	Mean difference (I-J)	p value
AD-MSCs + collagen	AD-MSCs + fibrin	-43.00	0.000
	AD-MSCs + collagen-fibrin	25.00	0.002
	AD-MSCs	-84.00	0.000
	Control 1	-261.00	0.000
	Control 2	-170.00	0.000
	Control 3	-187.00	0.000
	Control 4	-100.00	0.000
AD-MSCs + fibrin	AD-MSCs + collagen-fibrin	68.00	0.000
	AD-MSCs	-41.00	0.000
	Control 1	-218.00	0.000
	Control 2	-127.00	0.000
	Control 3	-144.00	0.000
	Control 4	-57.00	0.000
AD-MSCs + collagen-fibrin	AD-MSCs	-109.00	0.000
	Control 1	-286.00	0.000
	Control 2	-195.00	0.000
	Control 3	-212.00	0.000
	Control 4	-125.00	0.000
AD-MSCs	Control 1	-177.00	0.000
	Control 2	-86.00	0.000
	Control 3	-103.00	0.000
	Control 4	-16.00	0.035
Control 1	Control 2	91.00	0.000
	Control 3	74.00	0.000
	Control 4	161.00	0.000
Control 2	Control 3	-17.00	0.026
	Control 4	70.00	0.000
Control3	Control 4	87.00	0.000

I-J indicate group I is subtracted from group J. The mean difference for all the groups was significant at the 0.05 level.

Table 4. One-way ANOVA analysis to compare the colony count of three experimental groups

Experimental groups	N	Mean	Std.	Minimum	Maximum	p value
AD-MSC + collagen	3	54.00	3.00	51.00	57.00	
AD-MSC + fibrin	3	97.00	15.39	84.00	114.00	<0.001
AD-MSC + collagen-fibrin	3	29.00	5.29	25.00	35.00	

Table 5. LSD post hoc test to confirm the significant difference in colony count among three experimental groups

Group (I)	Group (J)	Mean difference (I-J)	p value
Adipose-collagen	Adipose-fibrin	-43.00*	0.001
	Adipose-collagen-fibrin	25.00	0.019
Adipose -fibrin	Adipose-collagen-fibrin	68.00*	<0.001

I-J indicate group I is subtracted from group J.

Table 6. One-way ANOVA analyses to compare the colony count of four control groups

Control groups	N	Mean	Standard deviation	Minimum	Maximum	p value
Control 1	3	315.00	5.567	309.00	320.00	
Control 2	3	224.00	6.557	218.00	231.00	
Control 3	3	241.00	4.358	236.00	244.00	<0.001
Control 4	3	154.00	9.643	147.00	165.00	

[Downloaded from ibj.pasteur.ac.ir on 2025-04-29]
[DOR: 20.1001.1.1028852.2023.27.5.3.9]
[DOI: 10.61186/ibj.27.5.257]

Table 7. LSD post hoc test to confirm the significant difference in colony count among four control groups

Group (I)	Group (J)	Mean difference (I-J)	p value
Control 1	Control 2	91.00	0.000
	Control 3	74.00	0.000
	Control 4	161.00	0.000
Control 2	Control 3	-17.00	0.016
	Control 4	70.00	0.000
Control 3	Control 4	87.00	0.000

I-J indicate group I is subtracted from group J. The mean difference for all the groups was significant at the 0.05 level.

DECLARATIONS

Acknowledgements

Authors would like to express their deep thanks to all laboratory staff of Shahed University, Tehran, Iran.

Ethical statement

This study was conducted in compliance with all the ethical principles of working with animals in research. All protocols used for animal experiments were approved by the Ethics Committee of Shahed University, Tehran, Iran (ethical code: IR.SHAHED. REC.1400.118).

Data availability

The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

Author contributions

MK: design of study, acquisition of data, evaluation of data, preparation of the manuscript, and assessment of data; MHA: acquisition of data, evaluation of data, preparation of the manuscript, and assessment of data; PO: design of study, acquisition of data, evaluation of data, preparation of the manuscript, and assessment of data; HS: acquisition of data, evaluation of data, preparation of the manuscript, and assessment of data. All authors have read and approved the final version of the manuscript.

Conflict of interest

None declared.

Funding/support

This research was supported by the budget of research projects of the Shahed University (Tehran, Iran). Funding was used to purchase equipment and tools.

REFERENCES

1. Iregbu K, Uwaezuoke N, Nwajiobi-Princewill I, Eze S, Medugu N, Shettima S, Modibbo Z. A profile of wound infections in National Hospital Abuja. *African journal of clinical and experimental microbiology* 2013; **14**(3): 160-163.
2. Omole IA, Stephen E. Antibiogram profile of bacteria isolated from wound infection of patients in three hospitals in Anyigba, Kogi State, Nigeria. *FUTA journal of research in sciences* 2014; **2**: 258-266.
3. Pal N, Sharma N, Sharma R, Hooja S, Maheshwari RK. Prevalence of multidrug (MDR) and extensively drug resistant (XDR) Proteus species in a tertiary care hospital, India. *International journal of current microbiology and applied sciences* 2014; **3**(10): 243-252.
4. Nussbaum SR, Carter MJ, Fife CE, DaVanzo J, Haught R, Nusgart M, Cartwright D. An economic evaluation of the impact, cost, and medicare policy implications of chronic nonhealing wounds. *Value in health* 2018; **21**(1): 27-32.
5. Singh C, Sood A, Bala K, Tandup C, Ray P, Angrup A. Anaerobic infections in patients admitted in various surgical units of a tertiary care hospital of north India: neglected but important. *Iranian journal of microbiology* 2021; **13**(3): 274-281.
6. Shenoy PA, Vishwanath S, Gawda A, Shetty S, Anegundi R, Varma M, Mukhopadhyay C, Chawla K. Anaerobic bacteria in clinical specimens–frequent, but a neglected lot: a five year experience at a tertiary care hospital. *Journal of clinical and diagnostic research* 2017; **11**(7): DC44-DC48.
7. Aftab S, Tarik MM, Siddique MA, Yusuf MA. Clinical and microbiological aspect of wound infection: a review update. *Bangladesh journal of infectious diseases* 2014; **1**(2): 32-36.
8. Smulski S, Turlewicz-Podbielska H, Wylandowska A, Włodarek J. Non-antibiotic possibilities in prevention and treatment of calf diarrhoea. *Journal of veterinary research* 2020; **64**(1): 119-126.
9. Goodarzi P, Alavi-Moghadam S, Sarvari M, Tayanloo Beik A, Falahzadeh K, Aghayan H, Payab M, Larijani B, Gilany K, Rahim F, Adibi H, Arjmand B. Adipose tissue-derived stromal cells for wound healing. *Advances in experimental medicine and biology* 2018; **1119**: 133-149.

10. Pittenger M, Discher D, Pé ault BM, Phinney DG, Hare JM, Caplan AI. Mesenchymal stem cell perspective: cell biology to clinical progress. *Npj regenerative medicine* 2019; **4**: 22.
11. Kim H, Hyun MR, Kim SW. The effect of adipose-derived stem cells on wound healing: comparison of methods of application. *Stem cells international* 2019; **2019**: 2745640.
12. Abdulrazzak H, de Coppi P, V Guillot P. Therapeutic potential of amniotic fluid stem cells. *Current stem cell research and therapy* 2013; **8**(2): 117-124.
13. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, Benhaim P, Lorenz HP, Hedrick MH. Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue engineering* 2001; **7**(2): 211-228.
14. Kim CG, Lee JJ, Jung DY, Jeon J, Heo HS, Kang HC, Shin JH, Cho YS, Cha KJ, Kim CG, Do BR, Kim KS, Kim HS. Profiling of differentially expressed genes in human stem cells by cDNA microarray. *Molecules and Cells* 2006; **21**(3): 343-355.
15. Benayahu D. Mesenchymal stem cell differentiation and usage for biotechnology applications: tissue engineering and food manufacturing. *Biomaterials translational* 2022; **3**(1): 17-21.
16. Sheykhhasan M, Qomi RT, Ghiasi M. Fibrin scaffolds designing in order to human adipose-derived mesenchymal stem cells differentiation to chondrocytes in the presence of TGF-β3. *International journal of stem cells* 2015; **8**(2): 219-227.
17. Sananta P, Rahaditya I, Imadudin MI, Putera MA, Andarini S, Kalsum U, Mustamsir E, Dradjat RS. Collagen scaffold for mesencyhmal stem cell from stromal vascular fraction (biocompatibility and attachment study): Experimental paper. *Annals of medicine and surgery* 2020; **59**: 31-34.
18. Mirshekar M, Afkhami H, Razavi S, Masjedian Jazi F, Darban-Sarokhalil D, Ohadi E, Mottaghi Nezhad M, Karimi R. Potential antibacterial activity and healing effect of topical administration of bone marrow and adipose mesenchymal stem cells encapsulated in collagen-fibrin hydrogel scaffold on full-thickness burn wound infection caused by Pseudomonas aeruginosa. *Burns* 2023. Available at: https://doi.org/10.1016/j.burns.2023.01.005.
19. Boregowda SV, Krishnappa V, Phinney DG. Isolation of mouse bone marrow mesenchymal stem cells. *Methods in molecular biology* 2016; **1416**: 205-223.
20. Hu Y, Lou B, Wu X, Wu R, Wang H, Gao L, Pi J, Xu Y. Comparative study on *in vitro* culture of mouse bone marrow mesenchymal stem cells. *Stem cells international* 2018; **2018**: 6704583.
21. Ramos TL, Sánchez-Abarca LI, Muntión S, Preciado S, Puig N, López-Ruano G, Hernández-Hernández A, Redondo A, Ortega R, Rodríguez C, Sánchez-Guijo F, del Cañizo C. MSC surface markers (CD44, CD73, and CD90) can identify human MSC-derived extracellular vesicles by conventional flow cytometry. *Cell communication and signaling* 2016; **14**: 2.
22. Hasanzadeh E, Ebrahimi-Barough S, Mirzaei E, Azami M, Tavangar SM, Mahmoodi N, Basiri A, Ai J. Preparation of fibrin gel scaffolds containing MWCNT/PU nanofibers for neural tissue engineering. *Journal of biomedical materials research part A* 2019; **107**(4): 802-814.
23. Montalbano G, Toumpaniari S, Popov A, Duan P, Chen J, Dalgarno K, Scott 3rd WE, Ferreira AM. Synthesis of bioinspired collagen/alginate/fibrin based hydrogels for soft tissue engineering. *Materials science and engineering: C* 2018; **91**: 236-246.
24. Chan BP, Hui TY, Yeung CW, Li J, Mo I, Chan GCF. Self-assembled collagen–human mesenchymal stem cell microspheres for regenerative medicine. *Biomaterials* 2007; **28**(31): 4652-4666.
25. Dao TT, Nguyen CT, Vu NB, Le HT, Nguyen PD, Van Pham P. Evaluation of proliferation and osteogenic differentiation of human umbilical cord-derived mesenchymal stem cells in porous scaffolds. *Advances in experimental medicine and biology* 2019; **1084**: 207-220.
26. Minnich KE, Stolarick R, Wilkins RG, Chilson G, Pritt SL, Unverdorben M. The effect of a wound care solution containing polyhexanide and betaine on bacterial counts: results of an in vitro study . *Ostomy wound manage* 2012; **58**(10): 32-36.
27. Yekani M, Rezaee MA, Beheshtirouy S, Baghi HB, Bazmani A, Farzinazar A, Memar MY, Sóki J. Carbapenem resistance in Bacteroides fragilis: A review of molecular mechanisms. *Anaerobe* 2022; **76**: 102606.
28. Falagas ME, Siakavellas E. Bacteroides, Prevotella, and Porphyromonas species: a review of antibiotic resistance and therapeutic options. *International journal of antimicrobial agents* 2000; **15**(1): 1-9.
29. Guo J, Yao H, Li X, Chang L, Wang Z, Zhu W, Su Y, Qin L, Xu J. Advanced hydrogel systems for mandibular reconstruction. *Bioactive materials* 2023; **21**: 175-193.
30. Sutton MT, Fletcher D, Episalla N, Auster L, Kaur S, Gwin MC, Folz M, Velasquez D, Roy V, van Heeckeren R, Lennon DP, Caplan AI, Bonfield TL. Mesenchymal stem cell soluble mediators and cystic fibrosis. *Journal of stem cell research and therapy* 2017; **7**(9): 400.
31. Krasnodembskaya A, Song Y, Fang X, Gupta N, Serikov V, Lee JW, Matthay MA. Antibacterial effect of human mesenchymal stem cells is mediated in part from secretion of the antimicrobial peptide LL-37. *Stem cells* 2010; **28**(12): 2229-2238.
32. Alcayaga-Miranda F, Cuenca J, Martin A, Contreras L, Figueroa FE, Khoury M. Combination therapy of menstrual derived mesenchymal stem cells and antibiotics ameliorates survival in sepsis. *Stem cell research and therapy* 2015; **6**: 199.
33. Cong Y, Yang S, Rao X. Vancomycin resistant Staphylococcus aureus infections: A review of case updating and clinical features. *Journal of advanced research* 2020; **21**: 169-176.
34. Perlee D, deVos AF, Scicluna BP, Mancheño P, de la Rosa O, Dalemans W, Nürnberg P, Lombardo E, van der Poll T. Human adipose-derived mesenchymal stem cells modify lung immunity and improve antibacterial defense in pneumosepsis caused by Klebsiella pneumoniae. *Stem cells translational medicine* 2019; **8**(8): 785-796.
35. Krasnodembskaya A, Samarani G, Song Y, Zhuo H, Su

[Downloaded from ibj.pasteur.ac.ir on 2025-04-29]
[DOR: 20.1001.1.1028852.2023.27.5.3.9]
[DOI: 10.61186/ibj.27.5.257]

X, Lee JW, Gupta N, Petrini M, Matthay MA. Human mesenchymal stem cells reduce mortality and bacteremia in gram-negative sepsis in mice in part by enhancing the phagocytic activity of blood monocytes. *American journal of physiology-lung cellular and molecular physiology* 2012; **302**(10): L1003-L10013.

36. Sutton MT, Fletcher D, Ghosh SK, Weinberg A, van Heeckeren R, Kaur S, Sadeghi Z, Hijaz A , Reese J, Lazarus HM, Lennon DP, Caplan AI, Bonfield TL. Antimicrobial properties of mesenchymal stem cells: therapeutic potential for cystic fibrosis infection, and treatment. *Stem cells international* 2016; **2016**: 5303048.
37. Lee JW, Krasnodembskaya A, McKenna DH, Song Y, Abbott J, Matthay MA. Therapeutic effects of human mesenchymal stem cells in ex vivo human lungs injured with live bacteria. *American journal of respiratory and critical care medicine* 2013; **187**(7): 751-760.
38. Chow L, Johnson V, Impastato R, Coy J, Strumpf A, Dow S. Antibacterial activity of human mesenchymal stem cells mediated directly by constitutively secreted factors and indirectly by activation of innate immune effector cells. *Stem cells translational medicine* 2020; **9**(2): 235-249.
39. Saberpour M, Bakhshi B, Najar-Peerayeh S. Evaluation of the antimicrobial and antibiofilm effect of chitosan nanoparticles as carrier for supernatant of mesenchymal stem cells on multidrug-resistant Vibrio cholerae. *Infection and drug resistance* 2020; **13**: 2251-2260.
40. Saeedi P, Halabian R, Fooladi AAI. Antimicrobial effects of mesenchymal stem cells primed by modified LPS on bacterial clearance in sepsis. *Journal of cellular physiology* 2019; **234**(4): 4970-4986.
41. Shahabadi AB, Memar MY, Dizaj SM, Sharifi S. Antimicrobial effects of curcumin containing collagen scaffolds against Streptococcus mutans. *Journal of advanced chemical and pharmaceutical materials* 2019; **2**(4): 197-198.
42. Kim H, Darwish I, Monroy MF, Prockop DJ, Liles WC, Kain KC. Mesenchymal stromal (stem) cells suppress pro-inflammatory cytokine production but fail to improve survival in experimental staphylococcal toxic shock syndrome. *BMC immunology* 2014; **15**(1): 1.
43. Hackstein H, Lippitsch A, Krug P, Schevtschenko I, Kranz S, Hecker M, Dietert K, Gruber AD, Bein G, Brendel C, Baal N. Prospectively defined murine mesenchymal stem cells inhibit Klebsiella pneumoniae-induced acute lung injury and improve pneumonia survival. *Respiratory research* 2015; **16**(1): 123.
44. Guerra AD, Rose WE, Hematti P, Kao WJ. Minocycline modulates NFκB phosphorylation and enhances antimicrobial activity against Staphylococcus aureus in mesenchymal stromal/stem cells. *Stem cell research and therapy* 2017; **8**(1): 171.
45. Regmi S, Pathak S, Kim JO, Yong CS, Jeong JH. Mesenchymal stem cell therapy for the treatment of inflammatory diseases: challenges, opportunities, and future perspectives. *European journal of cell biology* 2019; **98**(5-8): 151041.
46. Mahmoudvand G, Rouzbahani AK, Razavi ZS, Mahjoor M, Afkhami H. Mesenchymal stem cell therapy for non-healing diabetic foot ulcer infection: New insight. *Frontiers in bioengineering and biotechnology* 2023; **11**: 1158484.
47. Yamamuro Y, Kabata T, Nojima T, Hayashi K, Tokoro M, Kajino Y, Inoue D, Ohmori T, Yoshitani J, Ueno T, Ueoka K, Taninaka A, Kataoka T, Saiki Y, Yanagi Y, Tsuchiya H. Combined adipose-derived mesenchymal stem cell and antibiotic therapy can effectively treat periprosthetic joint infection in rats. *Scientific reports* 2023; **13**(1): 3949.
48. Berechet MD, Gaidau C, Nešić A, Constantinescu RR, Simion D, Niculescu O, Stelescu MD, Sandulache I , Râpă M. Antioxidant and antimicrobial properties of hydrolysed collagen nanofibers loaded with ginger essential oil. *Materials* 2023; **16**(4): 1438.
49. Hu L, Zhou J, He Z, Zhang L, Du F, Nie M, Zhou Y, Hao H, Zhang L, Yu S, Zhang J, Chen Y. In situ-formed fibrin hydrogel scaffold loaded with human umbilical cord mesenchymal stem cells promotes skin wound healing. *Cell transplantation* 2023; **32**: 09636897231156215.

[Downloaded from ibj.pasteur.ac.ir on 2025-04-29]
[DOR: 20.1001.1.1028852.2023.27.5.3.9]
[DOI: 10.61186/ibj.27.5.257]