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Research Article

The effect of adipose-tissue derived mesenchymal stem cells with Tragacanth gum hydrogel and human amniotic membrane on skin wound in rat

Short Running Title: MSCs on scaffold in wound healing

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Amniotic membrane, MSCs, Rats, Scaffold, Wound healing

Abstract

Wound healing and finding a solution for its fast healing is one of the major issues of today's world. This study aims to scrutinize the effect of using Tragacanth gum hydrogel as a three-dimensional scaffold of Mesenchymal stem cells (MSCs) along with a wound dressing of human amniotic membrane in the healing of full-thickness skin wounds in rats. In this study, 54 Albino female rats (150 g) were divided into control, hydrogel, and hydrogel+stem cell groups. Under general anesthesia, two bilateral full-thickness wounds were created on the dorsal area by a 9.8-millimeter biopsy punch. Rats were euthanized on days 3, 10, and 21 for histopathology and cell tracking with PCR evaluation of tissue samples. The histopathological results showed that no significant difference was seen on days 3 and 21, and there were significant differences only on day 10. In terms of epithelialization between treatment groups with the control group and in terms of granulation tissue formation between the hydrogel+MSCs group with the control group, they were statistically significant. Cell tracking results with PCR on days 3, 10, and 21 in the hydrogel+MSCs group showed that MSCs were found only on day 3. The results of the present study show that, in general, the use of stem cells together with the Tragacanth gum hydrogel as a scaffold and the use of human amniotic membrane as a dressing can cause early healing of full-thickness wounds.

Introduction

The skin acts as the first defense barrier of the body against external injuries, pathogens, and water loss, and it is also a thermoregulator [1,2]. In the management of wounds, the best method should be selected according to the size of the wound, the amount of tissue damage, the

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presence of infection, and the length of time that the wound is caused [3]. Naturally, the body's speed in response to injury and wound healing should be such as to prevent infection and severe inflammation or organ failure [4]. In addition, open wounds can be treated by two methods: primary (suturing) and secondary healing. Sometimes, due to the large size of the wound, the amount of tissue damage, the presence of infection, and economic conditions, it is treated with secondary healing methods (contraction and reepithelization), which will be a more appropriate and practical method [5]. Although a variety of local treatments with different substances are used for secondary wound healing, today, cell therapy is used as a new treatment with minimal side effects in the treatment of various diseases, including wound healing. Among the cells with different origins, mesenchymal stem cells (MSCs) have potential ability. MSCs are found in most tissues with different sources and have been used to manage the wound healing and regeneration process [6]. Studies showed that adipose-derived mesenchymal stem cells (AD-MSC) have effective properties such as easier separation, affordability, and lack of ethical issues, and they have high differentiation ability into different types of cells, including skin cells and myocytes [7].

The second solution that can be used in wound healing is the use of biological scaffolds as a carrier for the transfer of cells, drugs, and various compounds. One of these biological scaffolds is hydrogels, which can be made from various natural polysaccharides. Natural polysaccharides as abundant polymers are hydrophilic and environmentally friendly. Due to their structure and high hydrophilicity, they are more similar to the extracellular matrix and rarely cause inflammatory responses and cytotoxicity [8,9]. Tragacanth gum (TG), as an anionic polysaccharide, is a natural polymer that is extracted from renewable sources of agrochemicals. Its advantages include wound healing and drug delivery to control and improve the microstructure, texture, stability, and viscosity of cosmetics, biological products, food, and pharmaceutical formulations. Also, they are used as emulsifiers and thickeners [10–12].

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Tragacanth gum hydrogels are hydrophilic polymers with three-dimensional structures that have a high capacity to absorb water and are used as a controlled drug release and wound dressing [13–15]. Also, they have biocompatibility features in the presence of different fluids and tissues of the body. They are similar to the native extracellular matrix (ECM) in terms of lack of toxicity, subtlety, non-allergenic power, and rubbery nature [16,17].

One of the protective dressings that are widely used in wound healing today, and its effectiveness has been confirmed in various studies, is the use of the human amniotic membrane (HAM). The HAM causes the migration and proliferation of epithelial cells of the epidermis [18]. It has anti-inflammatory, antibacterial, and non-immunological properties and causes the wound to contract faster. Also, it is impregnated with a number of essential growth factors [19].

Considering that all these valuable substances alone are effective in wound healing, in this study, the simultaneous effect of mesenchymal stem cells and Tragacanth gum hydrogel on wound healing and the use of human amniotic membrane as dressing have been investigated.

Results

MSCs Characterization and Differentiation

In passage 0, the cell colonies of MSCs were heterogeneously visible. As the passage numbers were increased, the cells became more homogeneous and morphologically elongated (Figure 2A, B).

In this study, the cells were placed in the third passage in the differentiation media, inducing fat and bone cells. After 21 days of culture for differentiation into fat and 14 to 17 days for differentiation into bone, their successful differentiation towards fat and bone cells was proved by Oile Red O (bright red fat vacuoles) and Alizarin Red (Calcium ions deposited in the external matrix of cells) staining methods, respectively. Also, examining the activity of alkaline phosphatase was indicative of the differentiation of these cells towards bone cells (Figure 2C-H).

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DAPI staining of HAM

The results of this staining showed that the cell removal was completely done, and there was no blue dot on the membrane, which indicated the presence and accumulation of epithelial cells of the amniotic membrane, and the membrane was entirely uniformly black under the fluorescent microscope (Figure 3A-C).

Histological wound healing assessment

Epidermal cells regeneration

The process of wound healing three days after surgery in rats of the control and hydrogel groups was observed only in the form of thickening of the epidermis tissue at the cut edges. In some rats of the hydrogel+MSCs group, the epidermis was completely regenerated, and the entire wound was covered with keratinocyte cells. Also, the amniotic membrane was observed as a serous layer that was covered with squamous cells on the wound scab in this group. In general, between treatment groups with the control group were significant ($p< 0.05$) on the 10 day, and at this time, complete regeneration of the epidermis was done only in the hydrogel+MSCs group (Figure 4A, 5A-D).

Inflammation response

According to the results, the amount of granulomatous inflammation was high in all groups at 3 and 10 days. On the 10th, in the hydrogel and control groups, the presence of more granulomatous inflammation and giant cells was confirmed. On the 21st day, hydrogel+MSCs had the least amount of granulomatous inflammation and inflammatory cells, but these differences were not significant ($p> 0.05$) (Figure 4B, 5E-G).

Granulation tissue formation

According to the total evaluation scores of granulation tissue formation on days 3 and 10, the samples of hydrogel+MSCs showed the highest rate of granulation tissue formation than the other groups, but only on day 10 was significant with the control group ($p< 0.05$). On the

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21st day, it was highest in the hydrogel and control groups than hydrogel+MSCs but was not significant (Figure 4C, 5H-J).

PCR analysis of male DNA in female tissue

Sex mismatch between donor and recipient animals was designed to determine the persistence of MSCs in the wound tissue. Female rats were injected with a hydrogel containing AD-MSCs of male rats at the wound site. In examining the results obtained from PCR, it was observed that MSCs were present until the third day only in the wound of the group receiving hydrogel+MSCs. However, no bands were observed on the 10th and 21st days and also in two other groups (Figure 6).

Discussion

MSCs can differentiate into other cells and secrete or suppress the growth hormones or essential cytokines in the wound environment. AD-MSCs in large quantities are easily isolated and cultured and have great potential in therapeutic applications [20]. In the present study, the regeneration of the epithelium was completed on day 3 in the hydrogel+MSCs group, and in the other groups, it was completed on day 10. In different studies, the start of epithelialization was different, and most of them had significant differences with the control group. It has been shown in one study that AD-MSCs increase blood supply and rate of granulation tissue formation in wounds, survive in the wound for up to 14 days, and have lasting effects on the wound [21].

Contrary to the above studies, one study showed that between the control and treatment groups, there was no significant difference in terms of the amount of collagen, epithelialization, angiogenesis, and number of fibroblasts and macrophages. They showed that AD-MSCs have a significant effect in reducing the size of the wound, but their effect on the severity of skin lesions and pathological factors was not confirmed. Compared to BMSCs, they have less ability

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to differentiate into endothelial cells [22]. Also, Karimi et al. reported that AD-MSCs had no significant improvement in acute burn wound healing [23].

The present study showed that the hydrogel+MSCs group had the highest amount of granulation tissue and angiogenesis on days 3 and 10, and this amount decreased on day 21. Lotfi et al, (2019) stated that the most granulation tissue thickness in the keratinocyte/MSCs/scaffold group was increased in the first week, and it decreased significantly in the second week compared to other groups [24]. In the present study and Lotfi's study, polymers made from natural materials such as hydrogels provide a suitable environment and direct cell contact. In the present study, the hydrogel group had the highest amount of granulation tissue on day 21 compared to the hydrogel+MSCs group, which could indicate the critical role of stem cells that have paracrine signaling properties, which decrease inflammation, increase angiogenesis and cell proliferation [24].

In this study, we used allogenic AD-MSCs. It has been shown in the studies that autologous MSCs have more accelerated cicatrization than allogeneic MSCs. However, in burn injuries, allogeneic MSCs can be the only option available [25]. The results of one study showed that the intradermal injection of allogenic AD-MSCs in burn wounds caused a significant difference on the 14th with the control group [16].

In the detection of the SYR gene by PCR, the band of this gene was seen only on day 3 in the hydrogel+MSCs group. Hanson et al. (2016) injected allogenic male AD-MSCs intradermally in partial-thickness of female minipigs. In female tissues, male DNA content was evaluated via PCR amplification of a 377 bp segment from chromosome Y. They observed Y chromosome bands with a decreasing trend on days 0, 7, and 10 [26]. Based on this study, it would have been better to include the cell tracking investigations of day 7 in the present study to understand this decreasing process better. Other reasons for the difference between the present study and the above study may be due to the type of animal modeling, the method, and

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the amount of stem cell injection. The reason for the decrease in the presence of MSCs in the wound site is unknown. However, it can be because of MSCs migration from the wound site, MSCs phagocytosis by macrophages, or mechanisms involved in cell processing and tissue regeneration [26].

Tragacanth gum hydrogel can be a suitable scaffold for AD-MSCs. It accelerates the proliferation and differentiation of cells and provides a suitable space for the support and adhesion of cells. It is also capable of expressing genes for up to 21 days and maintains the original morphology of cells. In the present study, in terms of epithelialization, between the hydrogel group and the control group was significant on day 10. Although no significant difference was observed in terms of inflammation and granulation tissue formation between the hydrogel and other groups, on the 10 and 21 days, the rate of granulation tissue formation in the hydrogel group was higher. One of the reasons is the high concentration of hydrogel, and the cause of reduction of inflammation in the stem cell group is due to the presence of MSCs. In one study similar to the present study, it was shown that wound closure occurs faster in the PCL-GT-stem cells group than in the PCL-GT scaffolds group. Actually, granulation tissue, collagen synthesis, and angiogenesis were improved in the PCL-GT-stem cells. They stated that GT accelerates the transition from the inflammatory and germinal phases as well as the maturation of scar tissue [27–29]. One study showed that creams made from Tragacanth gum with a concentration of 6% had the most effect on rabbit wound healing compared to the control group [27]. In another study, with the daily application of the Tragacanth gel, a significant difference was observed in terms of epithelialization, inflammation, and granulation tissue on the 10th day compared to the control group. Similar to the present study, they showed that Tragacanth gum caused most of the wounds to close on the 10th day by accelerating wound contraction [28].

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A proper dressing should increase epithelial regeneration, control the amount of exudate, prevent material leakage, reduce inflammation and infection, and finally be comfortable for the patient. In this study, amniotic membranes as economically reasonable alternative biomaterial were used to benefit from the above characteristics and also to prevent hydrogel leakage. In some studies, the use of amniotic membranes alone in wound healing was ineffective [30], but in others, it had no significant difference with the control group or other treatment groups [31]. Studies have shown that the use of the amniotic membrane alone is effective for wounds with a shallow extent, and in wide and deep wounds such as full-thickness wounds and third-degree burns, a more effective solution is required [32].

Studies showed that the application of MSCs with cellular/acellular amniotic membrane multiplies the rate of wound healing compared to the use of amniotic membrane alone [33]. In this study, on the three days in the stem cell group, acellular human amniotic membrane was observed as a serous layer covered with squamous cells on the wound scab, which is a sign of the effective role of MSCs.

In summary, in this study, the synergism effect of MSCs, Tragacanth gum hydrogel, and using of human amniotic membrane as a dressing were investigated, and histopathology results showed that the combination of stem cells and Tragacanth gum hydrogel have an influential role in the immediate wound closure, and the human amniotic membrane has a supporting role for them.

Materials and Methods

Ethical statement

All procedures and experiments performed on animals according to the guidelines of the Animal Care Committee and approved by the Research Ethics Committees of Ferdowsi University of Mashhad (IR.UM.REC.1400.333).

Isolation and Cell Expansion of ADSCs

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Adipose tissue source was prepared from a Wistar male rat (8 weeks old). After general anesthesia with ketamine 10% (50 mg kg^{-1}, Bremer Pharma GmbH, Warburg, Germany) and xylazine 2% (5 mg kg^{-1}, Alfasan, Woerden, Netherlands) and then under aseptic condition, laparotomy was made. 2-3 ml of accumulated fat from the retroperitoneal, perirenal, and inguinal regions were dissected bilaterally and collected with at least manipulation. These samples were transferred to the laboratory in sterile conditions and in the shortest time for subsequent steps. Adipose tissue was washed with phosphate-buffered saline (PBS) (Gibco, Eggenstein, Germany) containing 1% of the penicillin–streptomycin (Gibco, Eggenstein, Germany) until to separate the vessels and connective tissues attached to the fat pieces as much as possible and it is repeated if necessary. For enzymatic digestion, 1 mg/ml of collagenase type IA (Sigma-Aldrich (St. Louis, MO)) and 10 mg of bovine serum albumin (BSA) (Invitrogen (Carlsbad, CA)) were dissolved in PBS. After adding 2 mM CaCl2 to this solution, it was sterilized using 0.2 micron filters (Jet Biofil/ Orange, Canada). The collagenase solution was added to the 3 ml of adipose tissue fragment that was obtained from the washing of the previous step. The mixture of fat and collagenase was incubated at 37°C for 1 h in a shaking water bath (Memmert. Germany). After the enzyme digestion step, the adipose tissue was diluted 1:3 with PBS and centrifuged (800g, 10 min). After centrifuging, 4 phases are visible. Then, the suspended fats, adipose tissue and PBS in the three upper phases were removed from the test tube with a pipette without disturbing the cells. In the DMEM / low glucose medium (DENAzist Asia's DMEM-LG (Low Glucose)), the cell pellet was resuspended, and centrifugation (at 400 g for 6 min) of the solution was done. The Sediment (the pellet, which contained MSCs) was resuspended with DMEM/LG and cultured in DMEM-LG with 10% fetal bovine serum and 1% penicillin/streptomycin. Then, it was transferred to a 75T flask and maintained at a temperature of 37˚C with 95% humidity and 5% CO_2. After 48 h, the culture medium was replaced and changed each day until the confluence of cells in the flask reached 80-90%. Then, the previous

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culture medium was removed, and cells were washed with PBS. Cells were then detached by the application of trypsin–EDTA for subculture [24,34–37]. Briefly, after adding trypsin to the flask, it was incubated for 5 minutes, a new culture medium containing 10% FBS was added to the flask to neutralize the effect of trypsin, and the contents of the flask centrifuged at 400 g for 6 minutes. The supernatant medium was removed, a new medium was added to it, and pipetting with a sampler several times to the cells were suspended. After cell counting using trypan blue, 75×10^4 cells were transferred into a new 75T flask and after adding culture medium, the flask was transferred to the incubator.

Characterization of MSCs

The capability of the cells to differentiate into osteoblasts and adipocytes was analyzed. Flow cytometry was performed in a previous study to assay the immunophenotype [24].

Adipogenic and Osteogenic Differentiation of MSCs

ADSCs at 80-90% confluence, after 3 passages, were cultured in the adipogenic differentiation medium (DMEM supplemented with 10% FBS, 100 μmol/L indomethacin, 10 mM β-Glycerol phosphate, and 1 μM dexamethasone) (Sigma, Germany)) for 21 days. After changing this medium, at an interval of 2-3 days, the cells were stained with Oil red O at the end of this differentiation period. Also, for osteogenic differentiation, the cells were cultured in the osteogenic differentiation medium (DMEM supplemented with 10% FBS, 10 mM ascorbic acid, 10 mM β-Glycerol phosphate, and 0.1 μM dexamethasone) (Sigma, Germany)) for 14-17 days and then this induction medium was refreshed at an interval of every 2-3 days and cells were stained with Alizarin red solution. All these steps were performed on MSCs without induction by differentiation medium as a control. After staining, the cells were observed under the iX70 inverted microscope (Olympus, Japan)[37].

Alkaline phosphatase assay

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The level of alkaline phosphatase activity increases with the differentiation of MSCs into osteoblast cells. Therefore, alkaline phosphatase activity can be used as an indicator to investigate the differentiation of these cells that can be easily detected by the substrate of this enzyme, i.e. BCIP/NBT. Briefly, after differentiation, the cells were washed with PBS buffer and fixed in 4% paraformaldehyde. The paraformaldehyde solution was removed and washed, then a BCIP/NBT tablet ((5-Bromo-4-chloro-3-indolyl phosphate/Nitro blue tetrazolium) (Roche, USA) was dissolved in 10 ml of deionized water and 1 ml was poured on the differentiated cells and the control group and kept at room temperature for 5 to 10 minutes. For better penetration of the substrate into the cells, 0.05% Tween-20 was added to the PBS buffer. Then, they were washed with PBS buffer and examined with an inverted light microscope [37].

Preparation of Tragacanth gum hydrogel

To prepare Tragacanth gum hydrogel 6 % (6g/100 mL), after passing sterilization steps under the hood and UV lamp, 1/5 g of Tragacanth powder (Missouri, United States) is mixed with 25 ml of culture medium of DMEM-LG containing FBS and then it was shaken for at least 2hrs.

Preparation of Human Amniotic Membrane (HAM)

The Ethics Committee of the Ferdowsi University of Mashhad approved the use of HAM for this research. After obtaining written informed consent, placentas were obtained during elective cesarean section. Viral diseases were negative for all donors in the serological results. Dulbecco's modified Eagle's medium (DMEM; Gibco, Scotland) contained 100 U/mL penicillin, 100 µg/mL streptomycin, and 2.5 µg/mL amphotericin B solution (Biosera UK) was used to place the placental tissue in it. The placenta was washed several times in phosphate-buffered saline (PBS) with an antibiotic/antimycotic solution. Blunt dissection was performed to detach the HAM from the chorion. HAM was divided into approximately 2.5×2.5 cm pieces. To perform de-epithelialization, 0.5 M NaOH (Sigma-Aldrich) was used with a cotton-tipped

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applicator to rub the cells, followed by washing with sterile PBS in a shaker for 10–15 min. To confirm the cell removal of the amniotic membrane, DAPA staining (2µg/ml PBS) was done [38,39].

In vivo wound healing experiments in an animal model

In total, 54 female rats at 1.5 months of age and ~150 g were randomly divided into control, Tragacanth gum hydrogel (hydrogel) and Tragacanth gum hydrogel+MSCs (hydrogel+MSCs) groups. The rats were anesthetized by an intraperitoneal injection of 50 mg kg^{-1} ketamine and 5 mg kg^{-1} xylazine. To evaluate the effects of these compounds on wound healing, two full-thickness circular wounds of equal sizes (8.7×8.7 mm^2) using biopsy punches were aseptically generated on either side of the dorsal area of each rat. The rats in the control group were treated with a hydrofilm dressing; in the hydrogel group, the amniotic membrane was sutured on the wound, 0.1 ml of hydrogel was injected, and then hydrofilm dressing was placed on them. In the hydrogel+MSCs group, after the amniotic membrane was sutured, 0.1 ml of gel-containing cells (3×10^5) was placed on the wound, and finally, the hydrofilm dressing was glued on the set (Figure 1).

During the entire period of this research, the rats were kept in the animal house. They were placed individually in cages under the conditions of maintaining a temperature of 24 ˚C and a cycle of 12 hours of light and 12 hours of darkness with free access to water and food.

Histopathology

On the 3, 10, and 21 days after surgery, six rats were selected, and after euthanasia with chloroform, sampling was done under sterile conditions. Randomly, one of the two wounds was selected, and the skin wound and surrounding skin were fixed in 10% formaldehyde, embedded in paraffin, and cut into 5 µm sections. Standard HE staining was performed, and the samples were evaluated quantitatively and qualitatively in terms of the amount of regeneration of the epithelium, the number of inflammatory cells in the area, and the amount of granulation tissue

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formation. The scoring of the evaluated factors was done as follows: regeneration of the epithelial tissue (beginning of regeneration of the epithelial tissue or thickening of the cut edges = 1, coverage of less than half of the wound = 2, coverage of more than half of the wound = 3, coverage of the entire wound with low thickness = 4 , covering the entire wound with keratinization and normal thickness = 5); degree of inflammation (absence of inflammation = 0, mild inflammation = 1, moderate inflammation = 2, severe inflammation = 3); degree of formation and maturation of granulation tissue (absence of granulation tissue = 0, low granulation tissue formation = 1, moderate granulation tissue formation = 2, abundant granulation tissue formation = 3, abundant granulation tissue formation and with tissue maturity = 4)[40].

Polymerase chain reaction (PCR)

To evaluate the persistence of MSCs injected in the local tissue wound bed, tissue samples were taken in sterile condition and frozen (-80˚C). As a pre-extraction step, these sections were crushed in liquid nitrogen using sterile pestles and mortars, and the obtained powder was used for DNA extraction. Genomic DNA was isolated using the Animal DNA Isolation Kit (DENAzist Asia Co., Mashhad, Iran) according to the manufacturer's instructions. To trace the mesenchymal stem cells of male rat adipose used in the wound site, the Y chromosome was selected by the SYR gene [41]. Because living rats are female, the mentioned gene could be traced. Primers that were used to amplify the SRY gene (PCR product size: 273 bp; Gene ID: 103694554) were 5′ - GTAGGTTGTTGTCCCATTGC-3′ and 5′ - GAGAGAGGCACAAGTTGGC-3′. The PCR protocol consisted of an initial denaturation step at 94°C for 15 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 57°C for 30 s, and extension at 72°C for 20 s. 5m final extension at 72°C was applied at the end of the PCR reaction. The quantification of the extracted DNA was measured by a spectrophotometer at wavelengths of 260 nm and 280 nm. After DNA extraction, a 1.5%

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agarose gel was used, which consisted of 0.75 g of agarose powder, a solution containing 1 ml of TAE buffer, 49 ml of distilled water, and 1μL of ethidium bromide. For the preparation of the Master mix solution, 20 ng per PCR sample was taken from the extracted DNA, and the final volume was adjusted to 10.5 μL with distilled water. The samples were then spun, and 14.5 μL of master mix and primer solution was added to each sample. After another round of spinning, the samples were ready for use.

Statistical analysis

Statistical analyses were performed using SPSS (Ver. 26). Appropriate tests (Kruskal-Wallis Test and Mann–Whitney U-test) were used to analyze the data. $p< 0.05$ was considered significant.

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Figure legends

Figure 1. Schematic representation of the study. Created with BioRender.com.

Figure 2. Characteristics of rat adipose tissue-derived mesenchymal stem cells (adMSCs). Phase contrast photomicrographs show the morphology of colonies of rat adMSCs at passage 0 (A) and expanded rat adMSCs at passage 1 (B). The cells represent spindle-like morphology. Rat adMSCs at passages 1-3 were characterized by adipogenic differentiation via Oil Red O staining that represents the lipid vesicles within differentiated cells (C) versus the control cell without adipogenic induction media (D). The osteogenic differentiation potential of rat adMSCs was also examined by Alkaline phosphatase activity assay (E) and Alizarin red staining (G) against their respective controls (F and H). All the images except A were taken by ×100 magnification.

Figure 3. Confirmation of amniotic membrane decellularization by DAPI fluorescence dye. A: Amniotic membrane before decellularization, B: amniotic membrane during decellularization, C: amniotic membrane after decellularization. In this staining, the blue dots represent the nucleus of the amniotic membrane cells, and the black parts indicate the decellularized amniotic membrane.

Figure 4. A: Average evaluation of regeneration of epidermal cells, B: intensity of inflammation and C: granulation tissue formation in different days and groups.

Figure 5. A: Re-epithelialization (arrow) and moderate granulation tissue formation (star) at the wound edge of the hydrogel+MSCs group on day 3 (H&E staining, 10× magnification). B: Amniotic membrane covered with squamous cells (arrow) on the wound scab in the hydrogel+MSCs group on day 3 (star) (H&E staining, 10× magnification). C: Complete regeneration of the epidermis (arrow) in the wound site of the hydrogel+MSCs group on day 10 (H&E staining, 4× magnification). D: Lack of complete regeneration of the epidermis (star) at the wound site in the control group on day 10 (H&E staining, 10× magnification). E: Diffuse granulomatous inflammation in the dermis area at the wound repair site in the hydrogel+MSCs on day 10 (H&E staining, 10× magnification). F: Diffuse granulomatous reaction at the base of the wound in the hydrogel group on day 21. (H&E staining, 10× magnification). G: Granulomatous reaction around the unrecognizable structure in the control group on day 21. (H&E staining, 10× magnification). H: Hyperemia and the beginning of the angiogenesis process at the base of the wound (arrow) and the absence of granulation tissue formation (star) in the control group on day 3 (H&E staining, 10× magnification). I: Two foci of granulation tissue formation in the center of the wound (star) and the amnion membrane on the surface of the wound in the hydrogel+MSCs group on day 3 (arrow) (H&E staining, 4× magnification). J: Lack of complete regeneration of the epidermis and the presence of a scab on the surface of the wound (black arrow), the formation of abundant granulation tissue and angiogenesis (star), and the presence of granulomatous reaction in the base of the wound (white arrow) (H&E staining, magnification ×10).

Figure 6. Representation of the gel electrophoresis to trace the SRY gene in order to check the presence of MSCs in the wound healing site on day 3. From left to right: 1) MSCs isolated from the fat of male rats. 2) Samples were isolated from the hydrogel+MSCs group on day 3. 3) ladder. 4) Replication of three samples from the same group with higher DNA concentration.

Online supplemental material

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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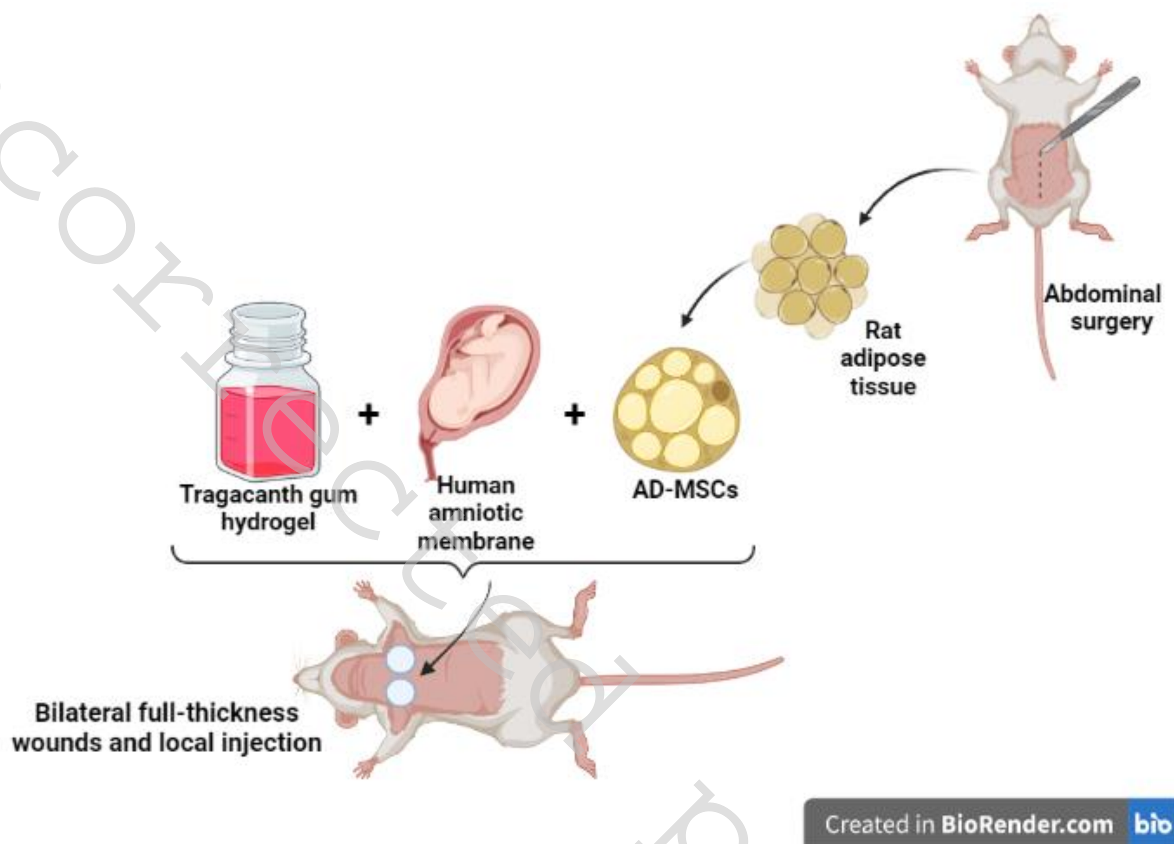

Figure. 1

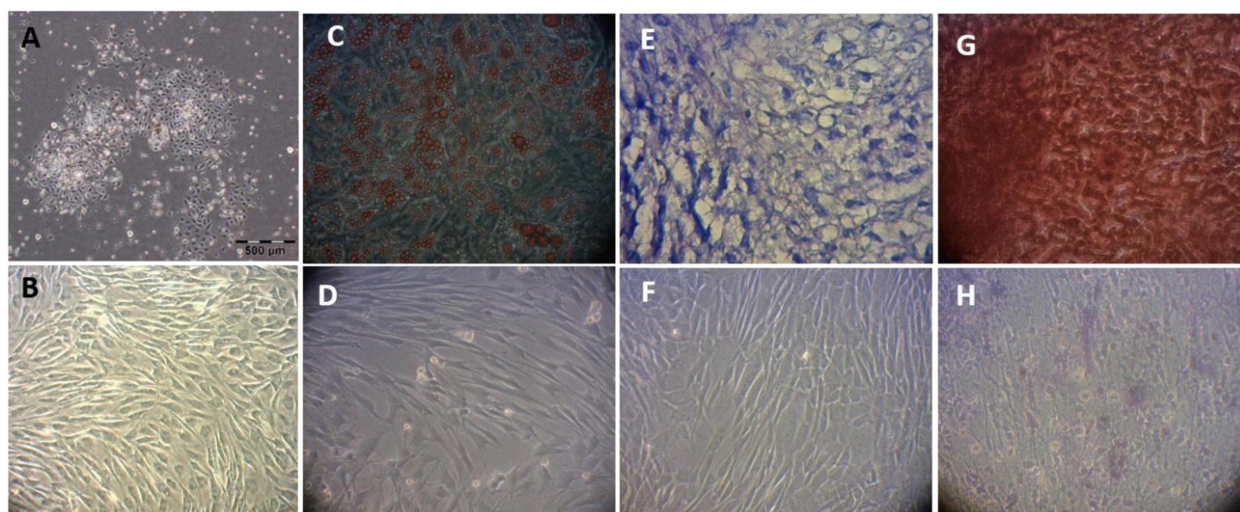

Figure. 2

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Figure. 3

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Figure. 4A

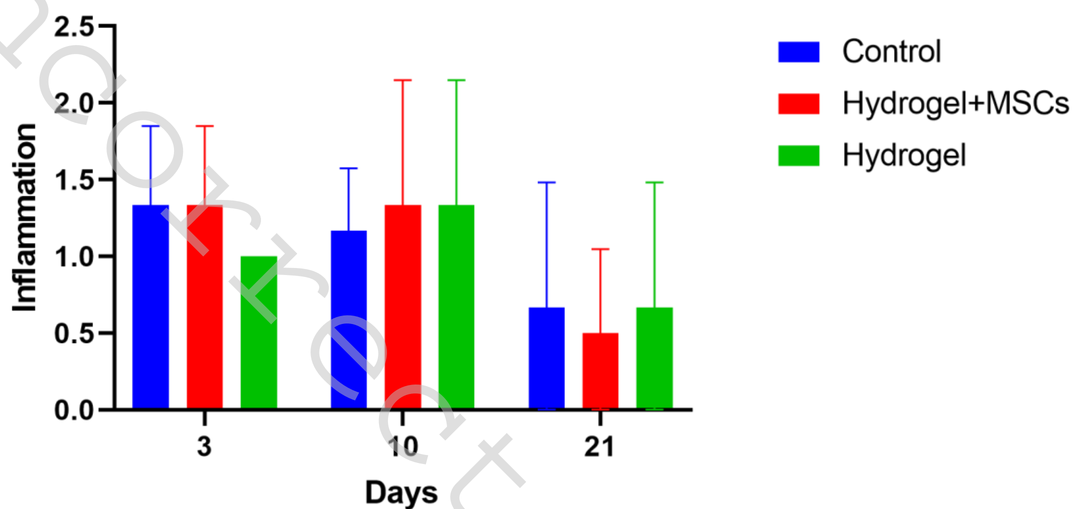

Figure. 4B

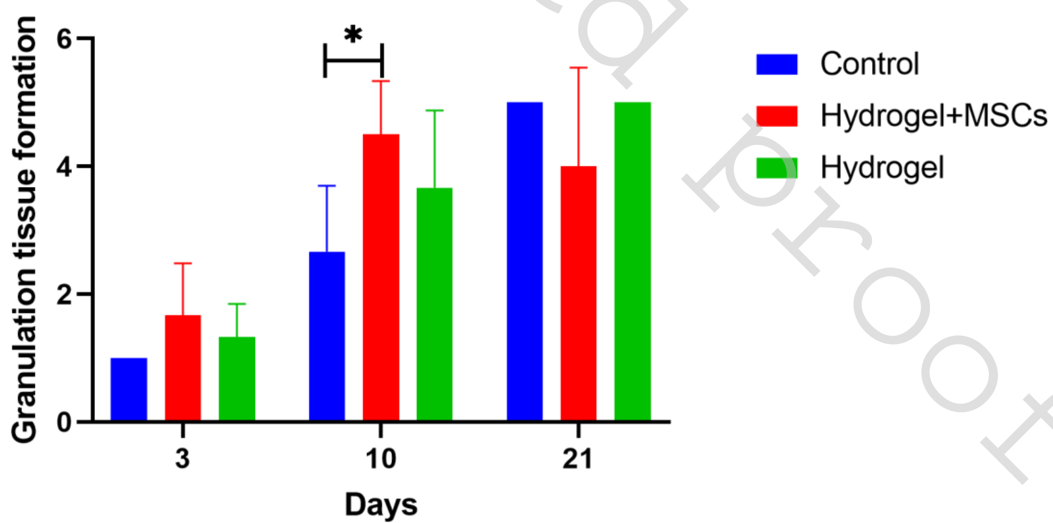

Figure. 4C

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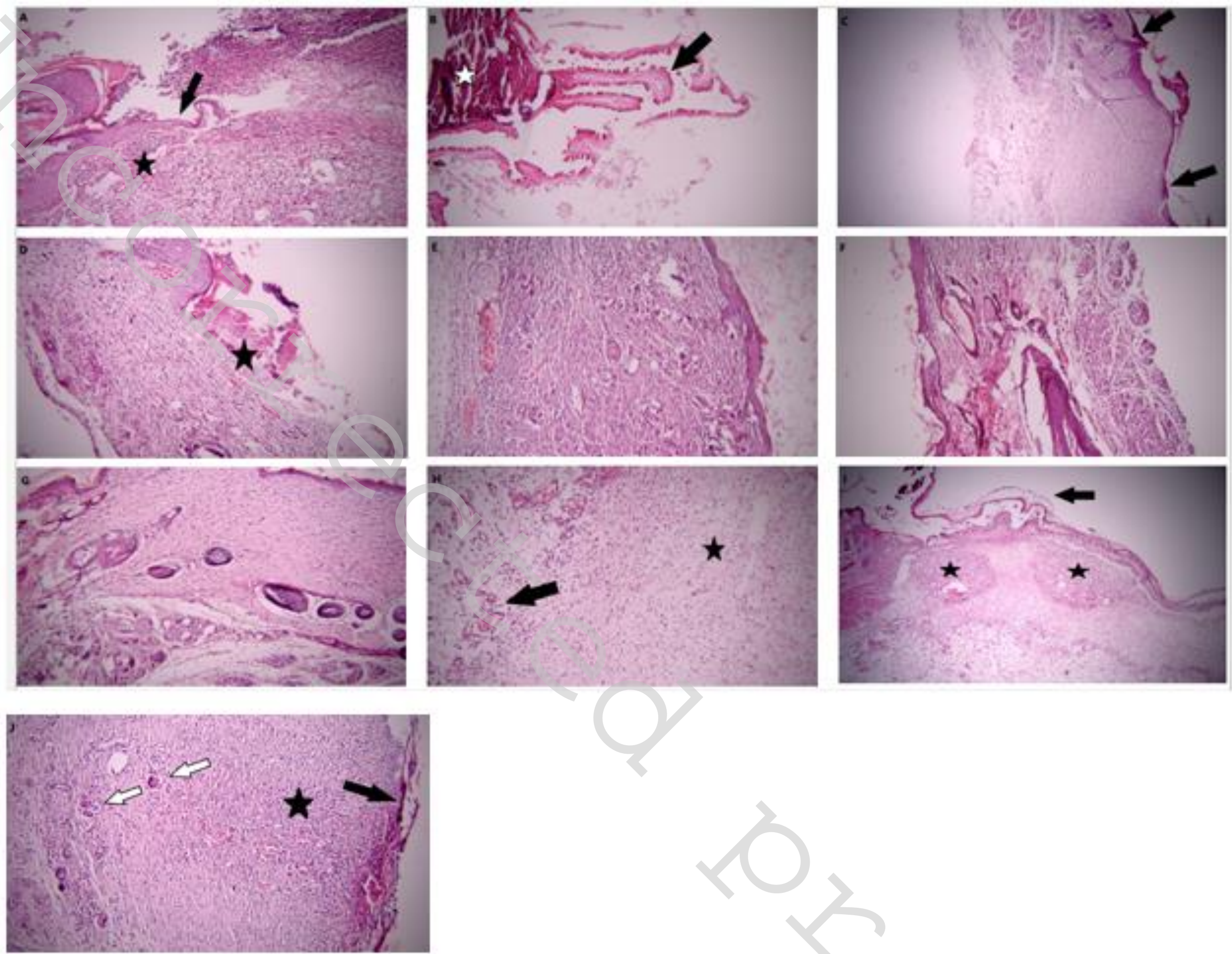

Figure. 5

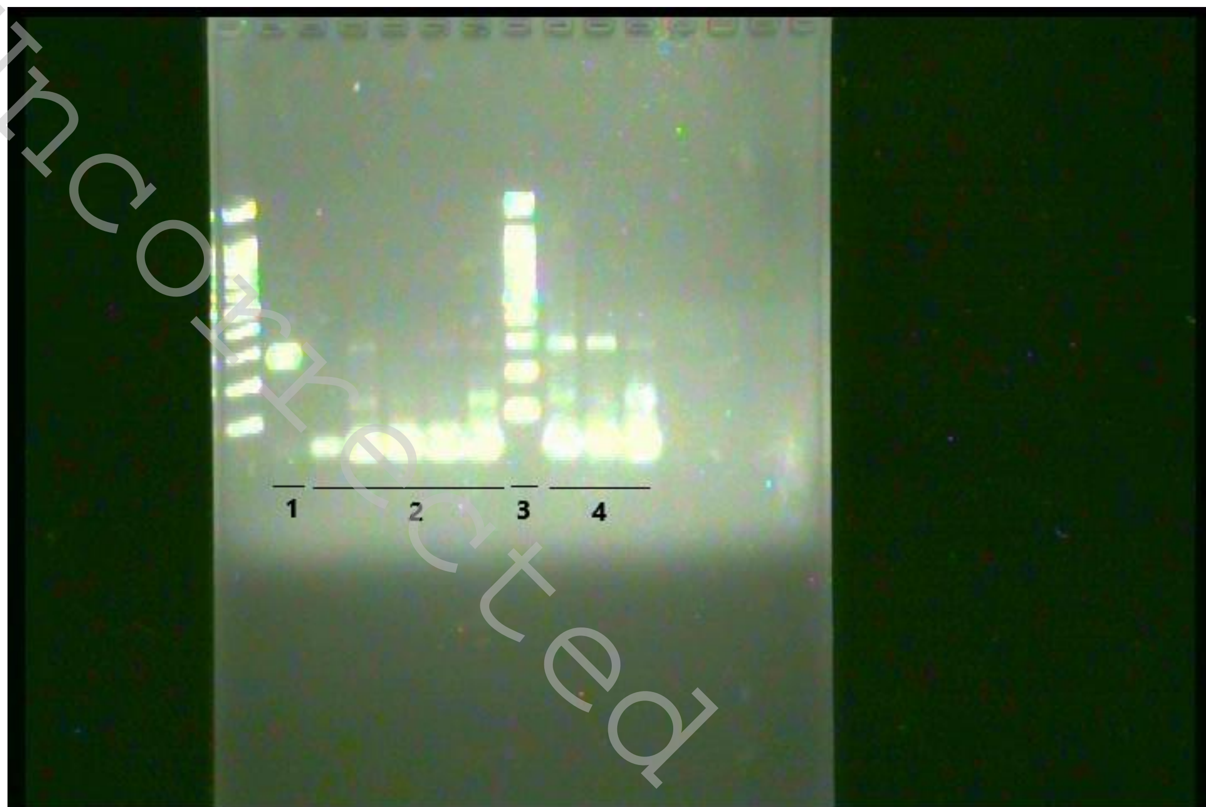

Figure. 6

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عنوان

ارزیابی آسیب‌شناسی التیام زخم پس از استفاده از سلول‌های بنیادی مزانشیمی گرفته شده از بافت چربی با هیدروژل صمغ کتیرا، و غشای آمنیوتیک انسانی به عنوان پانسمان

کلید واژه ها

غشای آمنیوتیک، سلول‌های بنیادی مزانشیمی، رت‌ها، داربست، ترمیم زخم

چکیده

بهبود زخم و یافتن راه حلی برای بهبود سریع آن یکی از مسائل مهم دنیای امروز است. این مطالعه با هدف بررسی دقیق تأثیر استفاده از هیدروژل صمغ کتیرا به عنوان داربست سه بعدی سلول‌های بنیادی مزانشیمی همراه با پانسمان زخم غشای آمنیوتیک انسانی در ترمیم زخم‌های پوستی با ضخامت کامل در رت انجام شد. در این مطالعه 54 رت ماده نژاد آلبینو (150 گرم) به گروه‌های کنترل، هیدروژل و هیدروژل+سلول‌های بنیادی تقسیم شدند. تحت بیهوشی عمومی، دو زخم دو طرفه با ضخامت کامل در ناحیه پشتی توسط پانچ بیوپسی 9/8 میلی‌متری ایجاد شد. رت‌ها در روزهای 3، 10 و 21 برای ارزیابی بافت‌شناسی و ردیابی سلول با ارزیابی PCR در نمونه‌های بافتی تحت آرام‌کشی قرار گرفتند. نتایج آسیب‌شناسی نشان داد که در روزهای 3 و 21 تفاوت معنی‌داری مشاهده نشد و تنها در روز 10 تفاوت معنی‌داری وجود داشت. از نظر تشکیل بافت پوششی، بین گروه‌های درمان با گروه کنترل و از نظر تشکیل بافت جوانه‌ای، بین گروه هیدروژل+سلول‌های بنیادی مزانشیمی با گروه کنترل از نظر آماری معنی‌دار بود. نتایج ردیابی سلولی با PCR در روزهای 3، 10 و 21 در گروه هیدروژل+سلول‌های بنیادی مزانشیمی نشان داد که تنها در روز 3، سلول‌های بنیادی مزانشیمی یافت شدند. نتایج مطالعه حاضر نشان می‌دهد که به طور کلی استفاده از سلول‌های بنیادی همراه با هیدروژل صمغ کتیرا به عنوان داربست، و استفاده از غشای آمنیوتیک انسانی به عنوان پانسمان، می‌تواند باعث ترمیم زودهنگام زخم‌های تمام ضخامت شود.