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Original Article

Evaluation of the regenerative effect of chitosan scaffold and hyaluronic acid with and without mesenchymal stem cells on wound healing in rats

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Abstract

This study aimed to evaluate the restorative effect of chitosan hyaluronic acid scaffold (CHAS) with and without mesenchymal stem cells (MSCs) on the wound healing process in rats. The different wound treatment groups were as follows: no treatment or control (C), wound treatment with CHAS, wound covering with CHAS with MSCs. The wound healing effect was measured by measuring the wound area in each mouse on days 3, 5, 9, and 14. Then, for histopathological evaluation in the above days, each wound and 5 mm of normal skin tissue around each wound were separated and fixed. The results demonstrated that on the third and fifth days after the wound formation, the area of the remaining wound in the CHAS group was significantly smaller than the CHAS with MSCs but no significant difference was observed in the group C. Also, the area of the remaining wound on the ninth and fourteenth days in the studied groups did not show a significant difference. However, on day 14, the mean wound area in the CHAS group with MSCs was smaller than the other two groups. Histological examinations of the wound site were studied in terms of collagen arrangement, inflammation, vascular formation, granulation tissue, and epithelial regeneration. Studies in terms of collagen arrangement, granulation tissue formation, and vascular formation showed that on the third day. There was a significant difference between the groups, while no statistically significant difference was found between the groups in terms of inflammation and epithelial regeneration on the studied days. All these results demonstrate that there is no significant difference between the CHAS group and the CHAS group with MSCs as well as in group C.

Key words: Chitosan, Hyaluronic acid, Mesenchymal stem cell, Wound healing

Introduction

Wounds are one of the most common health problems and their complete healing represents a major challenge worldwide (Boateng and Catanzano, 2015). Wound healing is traditionally divided into four sequential phases: hemostasis, which lasts from a few minutes to a few hours after a skin injury, acute inflammation, which lasts 1- 3 days, Proliferation, generally lasting several days to a month, and eventual skin

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remodeling or scarring (Raziyeva et al, 2021). The inflammatory phase is characterized by the presence of pro-inflammatory neutrophils and macrophages at the lesion site. The proliferative phase of wound healing involves keratinocytes, fibroblasts, macrophages and endothelial cells whose active cooperation promotes re-epithelialization, angiogenesis and fibroplasia (Oliveira et al, 2022). Tissue remodeling mainly involves fibroblasts which are responsible for replacing the fibrin clot with scar tissue, slowing down angiogenesis and changing collagen composition (Yang et al, 2021). Several strategies have been proposed to improve wound healing. They can be broadly divided into biological agents, biomaterials, and cellular strategies (Son et al, 2019). Many studies have shown that cell therapies improve wound healing by improving angiogenesis and re-epithelialization to utilize cellular technologies, bone marrow-derived mesenchymal stem cells, adipose-derived cells, epidermal cells, and others (Shojaei et al, 2019).

Mesenchymal stem cells (MSCs) are involved to varying degrees in all three phases of wound healing. An important element of the mechanism of action of MSCs is that they directly reduce the inflammatory response and modulate immune system activity (Jiang and Xu, 2020). Studies have shown that the addition of MSCs to an active immune response reduces the secretion of the pro-inflammatory cytokines TNF-α and interferon while increasing the production of the anti-inflammatory cytokines, interleukin-10 and IL-4. This demonstrates the importance of the anti-inflammatory and immunomodulatory effects of MSCs in wound healing the detailed mechanisms of which have been described in several reviews (Harrell et al, 2019). In addition, MSCs have an antimicrobial effect which is very important in cleaning the wound from infections. The antimicrobial activity of MSCs occurs through two mechanisms: directly through the secretion of antimicrobials such as LL-37 and indirectly through the secretion of immune factors that regulate bacterial killing and phagocytosis by immune cells (Johnson et al, 2022). Current data suggest that the persistence and survival of MSCs at the lesion site are limited and need to be overcome by tissue engineering (TE) approaches (Shafiq et al, 2021).

One of the goals of skin tissue engineering is to use engineering techniques to facilitate the natural wound healing cascade by providing appropriate physicochemical and biochemical factors via natural or synthetic polymers (Nour et al, 2021). Chitosan (CS) is a de-acetylated derivative of chitin found primarily in the exoskeletons of arthropods including shrimps, crabs, and insects. In fact, CS is a polysaccharide composed of two di-acetyl units (D-glucosamine linked to β-(1-4)) and acetylated units (N-Acetyl-D-glucosamine) (Bakshi et al, 2020). The glucosamine moieties of CS have been reported to be a potent accelerator of wound healing. CS promotes wound healing through two main pathways (Eivazzadeh-Keihan et al, 2022). First, the N-Acetyl-D-glucosamine moiety of CS contributes to fibroblast proliferation and collagen production. Its positive charge interacts electrostatically with glycosaminoglycans leading to the uptake of growth factors. In the second phase, macrophages are activated by N-Acetyl-D-glucosamine to phagocytose and release mediators such as TGF-β1 and platelet-derived growth factor (Shariatinia, 2019). Furthermore, CS controls the production of IL-1 which controls fibroblast proliferation and collagen synthesis (Ribeiro, 2021). CS has also been reported to induce IL-8 secretion by fibroblasts leading to angiogenesis and neutrophil migration. CS can act as a hemostatic agent, promoting blood clotting by absorbing fibrinogen and plasma proteins. In addition, it can block nerve endings, thereby relieving pain (Guo et al, 2023). Therefore, it contributes to rapid wound healing and prevents scarring.

Numerous studies have shown that components and mimetics of the extracellular matrix play an important role in the repair of various tissues (Amorim et al, 2021). Scientists believe that hyaluronic acid (HA) can improve the mechanical properties and cell affinity of scaffolds through chemical combinations and surface modifications. HA is an important component of the synovial fluid and extracellular matrix with a disaccharide structure consisting of di-glucuronic acid and N-acetyl glucosamine (Chen et al, 2021). HA influences cell proliferation, differentiation and tissue repair. Long-chain HA activates fibroblast proliferation and migration and increases collagen deposition (Prajapati and Maheriya, 2019). During the wound healing process, HA can bind to fibrinogen during clot formation. At high concentrations, hyaluronic acid forms a porous network structure that allows the diffusion and migration of cells and proteins. During angiogenesis, HA binds to CD44 which promotes the formation of new blood vessels (Graça et al, 2020). It can also lead to wound healing through proliferation, migration, and increased collagen deposition in keratinocytes (Kawano et al, 2021). HA relieves pain by reducing nerve sensitivity (Li et al, 2020).

In fact, the speed and quality of repair of damaged tissue are very important (Kolimi et al, 2022). Therefore, this study was designed and implemented with the aim of evaluating the regenerative effect of chitosan and hyaluronic acid scaffold with and without MSCs on wound healing in rats.

Material and Methods

Preparation steps of 2% chitosan (CS) scaffold:

Medium molecular weight Sigma CS powder (Sigma, USA) was prepared, and the powder was dissolved in 0.5 molar acetic acid to produce and fabricate porous tissue. To produce 0.5 molar acetic acid according to the formula N1 V1 = N2 V2, 2.89 cm3 of this acid was poured into a 100 cm3 volumetric flask and the solution was then filled up with water. Then 2 grams of chitosan powder were added to this solution. To completely dissolve the CS-powder, this solution was stirred for 5 hours at 50 degrees Celsius on a Steer device using a magnet. After this time, a clear solution was obtained. The resulting solution was placed in the refrigerator at -4 °C for one day, then placed in the freezer at -18 °C for one day, and after leaving the freezer, it was placed in a freeze dryer for one day. The mechanism of this device is that when freezing at a temperature of about -50°C, porosity is created in the desired material using a vacuum pump. After completing these phases, a white, spongy and porous substance was finally obtained. In the final step, the obtained material was placed in a freezer at -18 °C for 24 hours and then placed in a non-sterile device for sterilization (Garg, Chanana & Joshi, 2012).

Scaffold impregnation with hyaluronic acid (HA)

To impregnate a series of scaffolds with HA, the first HA solution was prepared at a concentration of 3 mg/ml (0.3%). To avoid any contamination and to ensure the sterility of the solution, the preparation and impregnation phases of the solution were carried out under a laminar hood. In addition to using sterile PBS to prepare the HA solution, the resulting solution was also filtered using a filter on the syringe head. At this stage, the scaffolds are grown in containers. Six houses were set up, HA solution was added, and kept in an incubator at 37 °C for 24 hours (Figure 1).

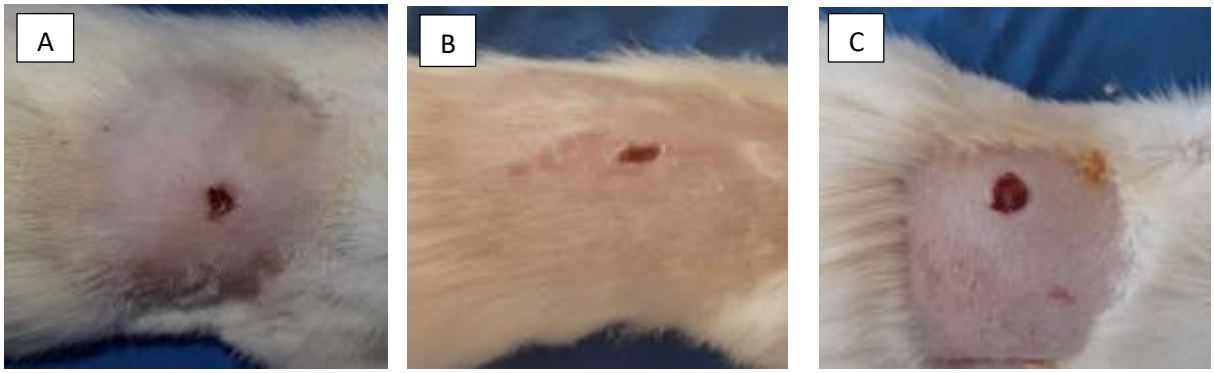

Figure 1: Wound site on the third day after creating a lesion: a) control group b) chitosan hyaluronic acid group c) chitosan hyaluronic acid + mesenchymal stem cells group

Mesenchymal Stem Cell (MSC) Culture

In this study, the cultured cells were human MSCs donated by Imam Khomeini Hospital in Tehran and transferred to the Animal Embryo Technology Research Institute of Shahrekord University in the frozen state during the first passage in order to produce there. Under the same conditions during the third passage, they were grown on research scaffolds. In the cell growth and proliferation study on the CH scaffold, the scaffolds were sterilized under UV- light for at least 2 hours and then placed in 24-well plates. In the third step, the cells were cultured on CH pieces in DMEM medium at 10% FBS for three days. Finally, they were transferred to the surgical department for use in the reconstruction process (Figures 2, 3).

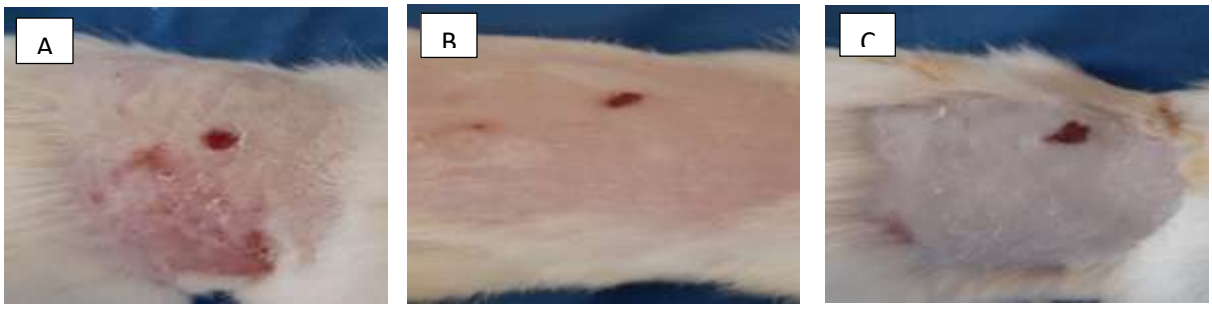

Figure 2: wound site on the fifth day a) control group b) chitosan hyaluronic acid group c) chitosan hyaluronic acid + mesenchymal stem cells group

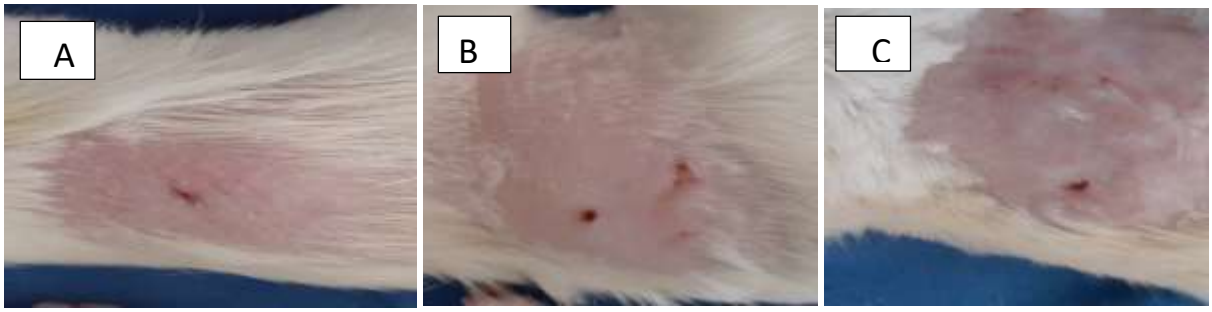

Figure 3: wound site on the ninth day a) control group b) chitosan hyaluronic acid group c) chitosan hyaluronic acid + mesenchymal stem cells group

Animal study

To carry out this research, the first 60 male rat pieces weighing about 200-250 gr were collected from the animal shelter of Shahrekord University. To adapt to the new environment, they were kept in cages for two weeks and given sufficient water and standard food. The animals were randomly divided into three groups of twenty animals each. In the negative control group (C), no compound was applied to the wound. In the second group, chitosan and hyaluronic acid (CH) scaffolds were placed on the wound, while in the third group, CH scaffolds with MSCs (CHM) were placed on the wound. It should be noted that the scaffold was sterilized under ultraviolet light for one hour before use.

To create a wound on the skin, the hair on both sides of the spine on the animal's back was first shaved. Subsequently, a combination of ketamine (at a dose of 40 to 87 mg/kg) and xylazine (at a dose of 5 to 13 mg/kg) was used to induce anesthesia. After setting the appropriate depth of anesthesia, the required wound area was disinfected with betadine scrub solution and the necessary measures were taken to establish sterile conditions. Before performing the wounds, the exact location of the wounds was marked. A 5 mm diameter skin punch device was used to create wounds of equal size (Figure 4). After dressing the wounds to prevent infection, the wound areas were covered with a sterile dressing and the rats were examined until complete healing. Each of them was then kept and cared for in a separate cage. After recovering and regaining consciousness, the animals were reintroduced into the herd and received enrofloxacin10%, by intramuscular injection at a dose of 10 mg/kg every three days. The day the wound formed was considered day zero.

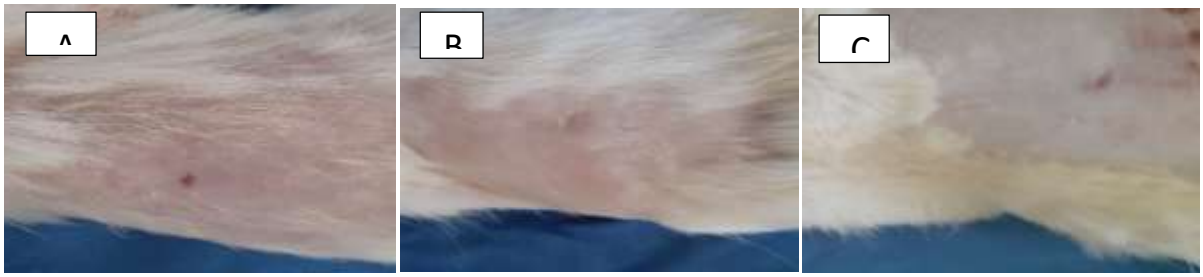

Figure 4: wound site on the fourteenth day a) control group b) chitosan hyaluronic acid group c) chitosan hyaluronic acid + mesenchymal stem cells group

Gross examination of wounds

On the 3rd, 5th, 9th and 14th days after the operation, wound location and wound appearance, such as inflammation, heat, redness, swelling and compatibility of materials with the wound were investigated. Photographs were then taken with a digital camera at a distance of 20 cm from the wound. The Image J software was used to evaluate and evaluate the prepared images.

Histopathological examination

Five samples were collected from each group on days 3,5,9 and 14 to prepare histopathological sections. Therefore, the rats were painlessly euthanized using the maximum anesthetic dose and appropriate tissue samples were collected from the wound. In this way, by cutting with a scalpel, a portion of the skin tissue at the edges of the wound, both healthy and damaged, was collected in its entire thickness and, after washing with

physiological serum, was fixed in container with buffered formalin to 10%. Five-micrometer sections were then prepared from these samples using a microtome and stained with hematoxylin-eosin as routine method. The wound healing process was evaluated and scored from the histopathological point of view of the prepared samples using evidence such as inflammation, fleshy tissue formation, collagen fiber alignment, angiogenesis and epithelial tissue regeneration (Gupta and Kumar, 2015).

Statistical analysis

The SPSS program was used to perform statistical tests. The macroscopic results obtained were analyzed using one-way anova and the microscopic results were analyzed using the nonparametric Kruskal-Wallis statistical test.

Results

The results of macroscopic examination of wound healing

On the third day after wound formation, there was no significant difference between the CH group and the control group, but there was a significant difference between this group and the CH group along with MSC (p=0.018). Also, there was a significant difference (p=0.018) between the control and CHM group. On day 5, there was no significant difference between the control group and CH group (p=0.065). The data show that there is a significant difference between the CH and the CHM group (P=0.045) and the wound area was smaller in the CH group. On the 9th day, no significant difference was observed between the groups, but the area of the wound in the CHM group was smaller than the other groups. On the 14th day, no significant difference was observed between the groups, but the wound area in the CH group was less that in the CHM group (Table 1, Figure 1).

Table 1: Area of wounds in mm2 scale in different groups

Groups	Days of study			
	3	5	9	14
Control	12.67±1.67[a]	8.14±1.89[a]	3.77±1.08[a]	1.40±0.44[a]
Chitosan/hyaluronic acid	12.63±3.36[a]	6.79±4.34[a]	3.70±1.01[a]	0.67±0.22[a]
Chitosan/hyaluronic acid /MSC	12.63±1.44[b]	8.42±1.49[b]	2.63±0.44[a]	0.95±0.65[a]
P value	P<0.05	P<0.05	p>0.05	p>0.05

The results of histopathology studies of wound healing

In the microscopic study of wound samples on days 3, 5, 9, and 14, the degree of wound healing was evaluated using evidence such as inflammation, formation of fleshy bud tissue, orientation of collagen fibers, vascularization, and epithelial tissue regeneration. A score of 0 to 4 was used to evaluate the healing of wounds based on the mentioned evidence.

The results of examining the arrangement of collagens

The results of examining the arrangement of collagens show that on the third day of the study, a significant difference can be seen among the studied groups (p<0.05). On the fifth, ninth, and fourteenth days of the study, the study of the collagen composition in the groups showed that there was no statistically significant difference between them (p<0.05) (Table 2).

Table 2: The results of the arrangement of collagen in the studied groups.

P≤0.05 indicates a significant difference. middle (minimum - maximum)

Groups	Days of study			
	3	5	9	14
Control	0(0-0)	1(1-2)	2(2-2)	3(3-3)
Chitosan/hyaluronic acid	1(1-1)	1(1-1)	2(2-3)	3(3-3)
Chitosan/hyaluronic acid /MSC	1(1-1)	2(2-2)	3(2-3)	3(3-4)
P value	0.030	0.061	0.067	0.368

The results of investigating the formation of granulation tissue

The results of examining the formation of granulation tissue show that there is a significant difference between the groups on the third day of the study ($p<0.05$). No significant difference was observed between the groups on days 5, 9 and 14 ($p<0.05$) (Table 3).

Table 3: The results of investigating the formation of granulation tissue in the studied groups. P≤0.05 indicates a significant difference. middle (minimum - maximum)

Groups	Days of study			
	3	5	9	14
Control	0(0-0)	3(3-2)	3(3-4)	3(3-4)
Chitosan/hyaluronic acid	1(1-1)	3(3-3)	4(3-4)	4(3-4)
Chitosan/hyaluronic acid /MSC	1(1-1)	3(3-4)	4(4-4)	4(4-4)
P value	0.018	0.102	0.264	0.565

Inflammation examination results

The results of the investigation of inflammation show that there is no significant difference between the investigated groups at all investigated times ($p<0.05$). On the ninth day, despite the absence of inflammation in the control and chitosan hyaluronic acid groups, no significant difference was observed between all groups ($p=0.67$) (Table 4)

Table 4: The results of investigating inflammation in the studied groups. P≤0.05 indicates a significant difference. middle (minimum - maximum)

Groups	Days of study			
	3	5	9	14
Control	2(2-2)	1(0-1)	0(0-0)	0(0-0)
Chitosan/hyaluronic acid	2(1-2)	1(2-1)	1(0-1)	0(0-0)
Chitosan/hyaluronic acid /MSC	1(1-1)	1(0-1)	0(0-0)	0(0-0)
P value	0.061	0.306	0.670	1

Results of vascularization

Examining the results of angiogenesis in the present study, it was observed that there was no significant difference between the investigated groups on the fifth, ninth and fourteenth day ($p<0.05$). But on the third day, angiogenesis in the hyaluronic acid chitosan group and the hyaluronic acid chitosan group with stem cells was better than the control group ($p<0.05$), and also the hyaluronic acid chitosan group was better than the hyaluronic acid chitosan group with stem cells ($p<0.05$) (Table 5).

Table 5: The results of investigating the formation of blood vessels in the studied groups.

P≤0.05 indicates a significant difference. middle (minimum - maximum)

Groups	Days of study			
	3	5	9	14
Control	0(0-0)	2(2-2)	2(2-1)	1(1-0)
Chitosan/hyaluronic acid	3(3-2)	2(2-3)	2(3-1)	2(1-2)
Chitosan/hyaluronic acid /MSC	2(3-2)	3(3-3)	2(3-2)	2(3-1)
P value	0.046	0.061	0.061	0.152

The results of examining the regeneration of the epithelium

The results of the examination of the regeneration of the epithelium are given in Table 6-4. As can be seen, there is no significant difference between the investigated groups in all investigated times ($p<0.05$) (Table 6).

Table 6: The results of investigating the regeneration of the epithelium in the studied groups. P≤0.05 indicates a significant difference. middle (minimum - maximum)

Groups	Days of study			
	3	5	9	14
Control	0(0-0)	1(2-1)	2(2-2)	3(4-3)
Chitosan/hyaluronic acid	0(0-0)	2(2-1)	3(3-3)	4(4-3)
Chitosan/hyaluronic acid /MSC	0(0-0)	2(2-2)	3(4-3)	4(4-4)
P value	1	0.102	0.110	0.061

Results of examining microscopic images of wounds in different groups

On the third day (Figure 5 A), there was a surface clot on the surface of the wound (star) and hyperemia was observed in the wound space in the control group (arrow). Also, the presence of a surface clot on the wound surface with a small accumulation of fibrin (star) and a small number of inflammatory cells in the wound space in the CH group (arrow) was observed (Figure 5 B). The presence of surface clot on the surface of the wound along with the accumulation of fibrin (star) and a small number of inflammatory cells and edema in the wound space in the CHM group (arrow) were recorded (Figure 5 C). On the fifth day, the presence of surface clot and the formation of edematous granulation tissue (star) and the formation of blood vessels in the wound space (arrow) were seen (Figure 6 A). The absence of surface clot (star) and the formation of granulation tissue with strings Irregular spots were recorded in the wound space in the CH group (arrow) (Figure 6 B). The Surface clot and filling of the wound space by irregular granulation tissue (star) and formation of blood vessels in the wound space were present in the CHM group (arrow) (Figure 6 C). On the 9th day, the filling of the wound space by immature granulation tissue (arrow) and the presence of newly formed vessels in the control group (star) were recorded (Figure 7 A). The formation of the epidermis and the filling of the wound space by fibrous granular tissue (arrow) were seen in CH group (Figure 7 B). Also, the filling of the wound space by relatively thick and regular granulation tissue was observed in the CHM group (arrow) (Figure 7 C). On the 14th day, in the control group, the formation of the epidermis was evident along with the filling of the wound space by relatively thick and irregular collagen fibers (arrow) (Figure 8 A). The formation of keratinized epidermis along with the filling of the wound space by relatively thick and regular collagen fibers (arrow) and the reduction of blood vessels were also seen in the CH group (star) (Figure 8 B). The formation of keratinized epidermis along with the filling of the wound space by thick and regular collagen fibers (arrow) and the reduction of blood vessels were also observed in the CHM group (star) (Figure 8 C).

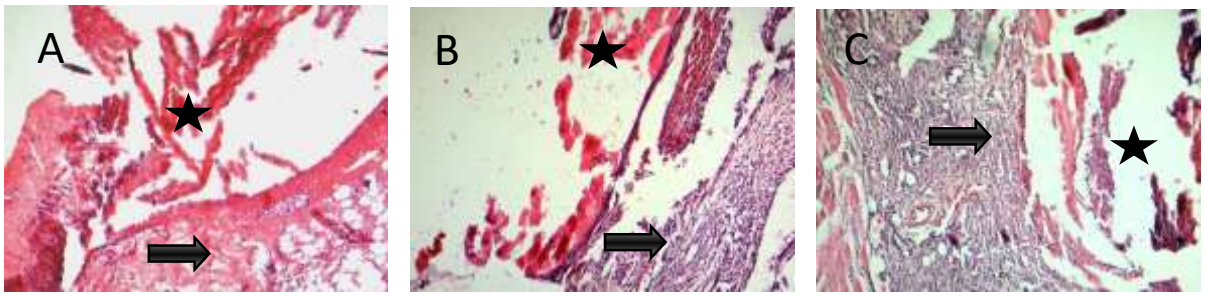

Figure 1: Microscopic sections of the wound site on day 3 after wound formation. (H&E, X10)

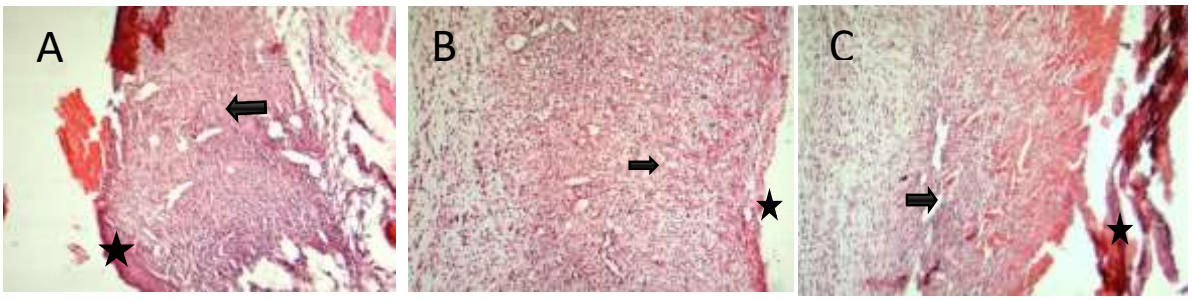

Figure 2: Microscopic sections of the wound site on the 5th day after wound formation. (H&E, X10)

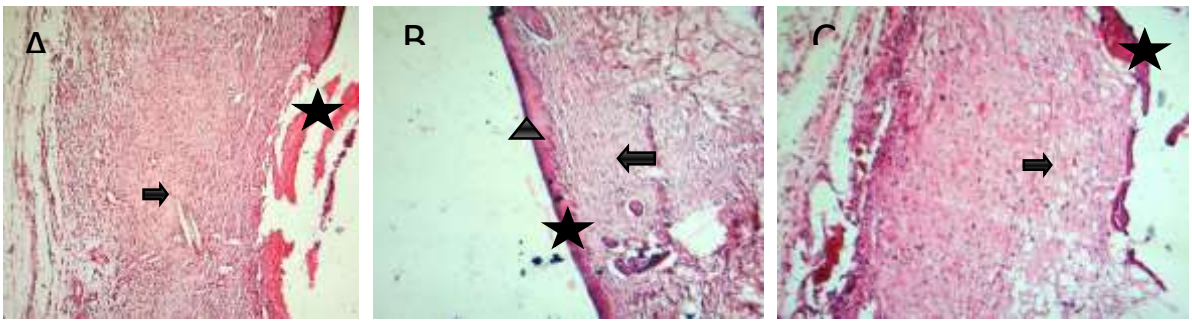

Figure 3: Microscopic sections of the wound site on the 9th day after wound formation. (H&E, X10)

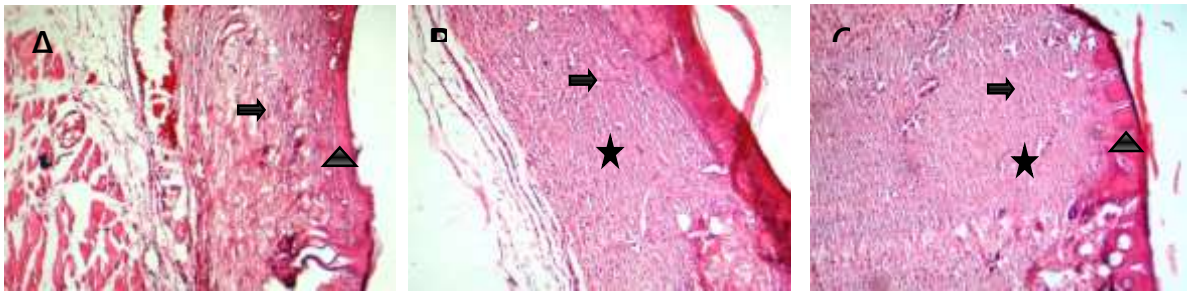

Figure 4: Microscopic sections of the wound site on the 14th day after wound formation

Discussion

Skin may be a delicate tissue that secures the entire body and inside tissues against warm, electrical adjust and physical harm (Kirwann and Pignataro, 2015). Skin dressings anticipate pathogens and organisms from entering the harmed zone and body water loss and lead to acceleration of wound closure and reduction of scar formation (Okur et al, 2020). The wound healing process may be a complex prepare, and in case of extreme tissue harm, the tissue reclamation may not lead to the return of the ordinary tissue structure, and the connective tissue may be damaged and a

scar or wound may be shaped (Tabassum et al, 2021). Nowadays, the utilization of dressings of biological origin plays a viable part in wound healing and scar decrease (Farahani and Shafiee, 2021). Moreover, broad considers on the utilization of topical treatments or modern dressings are continuously being conducted (Borda et al, 2016). Until presently, different dressings have been made for the treatment and healing of wounds, but most of them have issues such as incompatibility within the natural environment, need of antibacterial and biodegradable properties (Bianchera et al, 2020). In the new tissue designing strategies, engineered polymers such as chitin, chitosan, and collagen are utilized to create scaffolds due to their suitable biological properties such as adhesion, compatibility, and antimicrobial properties (Ahmed et al, 2018). MSCs have moreover attracted the consideration of numerous analysts due to their anti-bacterial properties and healing situations (Krasnodembskaya et al, 2010).

In this study, the effect of chitosan-hyaluronic acid scaffold with and without bone-derived mesenchymal stem cells (BMSC) on wound healing in rats was examined. On the third and fifth day, the region of the wound was significantly less compared to the CHM group. But there was no significant difference with the control group. On the 9th and 14th days of the study, there was no significant difference between the groups in terms of wound area. Sadik et al, (2015) examined chitosan gel and MSCs and found that chitosan improved wound healing compared to the control, but none of the wounds closed within 15 days. According to this study, the recovery and speed of wound healing was higher within the groups treated with MSCs, and it has also been shown that intradermal injection of MSCs has a faster healing rate than systemic injection (sadik et al, 2015). In addition to the positive effects of chitosan and hyaluronic acid on wound healing, bone-derived mesenchymal stem cells cause the growth and chemotaxis of fibroblasts, thereby increasing the speed of wound healing (Ha et al, 2020). In contrast to our results, in other studies, the effect of using hyaluronic acid, chitosan and MSCs in reducing the wound area is clear.

The results of examining the arrangement of collagen bundles in the present study showed that on the third day, CHM group and CH group had significantly better collagen arrangement than the control group. The local use of hyaluronic acid on the wound by increasing the movement of fibroblasts towards collagen sponges and the formation of collagenous tissue in the early stages accelerates the healing handle, and bone-derived stem cells increment type one and three collagens in the wound healing process (Thönes et al, 2019). In the study of Berce et al, (2018), the role of the coating containing chitosan and hyaluronic acid has been emphasized in reducing scar formation by improving the rearrangement of collagen bundles (Berce et al, 2018). Also, in the study of Sadik et al, (2018), it was shown that the systemic injection of MSCs in complete repair Collagen plays a significant role in wound healing and skin repair (sadik et al, 2015).

The results of the present study, in terms of examining the granulation tissue, showed that on the third day of the study, there was a significant difference between the CH group and the CHM group and the control group. In spite of the fact that the granulation tissue formation was better in the CH group on days five, nine and fourteen of the study than the other two groups, no significant difference was observed between the groups. HA increases vascularization, regeneration of epithelium and formation of granulation tissue, as well as migration of endothelial cells and improvement of regeneration of epithelium at the wound site (Hussain et al, 2017). BMSCs discharging different particular cytokines and chemokines, have higher amounts of VEGF-α, IGF-1, EGF, keratinocyte growth factor, angiopoietin-1,

derived factor 1 compared to skin fibroblasts. From the stroma, they secrete macrophage inflammatory protein-1 alpha and beta and erythropoietin. Also, the factors released by BM-MSC attract macrophages and endothelial cells into the wound and thus increase wound healing (Chakravorty and Shukla, 2023).

The results of the investigation of inflammation in the present study show that there is no significant difference between the studied groups. HA does not play a significant role in promoting inflammation and speeding up healing (Frenkel, 2014). Chitin, chitosan, its monomers and oligomers accelerated the wound healing process by increasing the activities of inflammatory cells such as polymorphonuclear leukocytes, macrophages and fibroblasts (Sharifi et al, 2022). It has been found that dressings impregnated with chitosan reduced inflammation and created significant antimicrobial effects compared to the control group. In fact, chitosan plays an effective role in faster wound healing by increasing the presence of multinucleated white blood cells, increasing the migration of fibroblasts to the wound surface and improving their growth and multiplication, and increasing the migration of macrophages (Yazarlu et al, 2021). The role of mesenchymal stem cells in reducing inflammation is related to the presence of cytokines and is very short-term (Van Buul et al, 2012).

In the present study, the results of angiogenesis show that there is a significant difference between the groups only on the third day of the study, and no critical difference was seen on the other days. It has been demonstrated that bone-derived stem cells increase epithelization and vascularization in the healing process of diabetic and non-diabetic wounds in mice (Pountos et al, 2014). It has also been found that chitosan induces inflammatory cells and increases blood vessels and collagen in the new tissue (Deng et al, 2010). In the study of Yang et al., 2021, the effect of nanoparticles containing hyaluronic acid on the inflammatory process and angiogenesis was evaluated and proved by measuring α-SMA and CD31 during the healing process of diabetic wounds (Yang et al, 2021).

The results of this study showed the appropriate performance of chitosan/hyaluronic acid scaffold impregnated with mesenchymal stem cells in wound healing, especially in the early days of wound healing. In fact, the nearness of progenitor cells, cytokines and growth factors in mesenchymal stem cells increases the performance of other components within the wound recuperating process. The manner and duration of use and the dose of the material used compared to other studies cause differences in the final results, and additional studies are needed in this field.

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Conflict of interest

The authors declare that there is no conflicts of interest regarding the authorship and publication of this research article.

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اثر بازسازی داربست کیتوزان و اسید هیالورونیک با و بدون سلول‌های بنیادی مزانشیمی بر بهبود زخم

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چکیده

این مطالعه با هدف بررسی اثر ترمیمی داربست اسید هیالورونیک/ کیتوزان (CHAS) با و بدون سلول‌های بنیادی مزانشیمی (MSCs) بر روند بهبود زخم در موش‌های صحرایی انجام شد. گروه‌های مختلف درمان زخم به شرح زیر بودند: بدون درمان یا کنترل (C)، درمان زخم با CHAS، پوشش زخم با CHAS همراه با سلول‌های بنیادی مزانشیمی. اثر ترمیم زخم با اندازه‌گیری مساحت ناحیه زخم در هر موش در روزهای ۳،۵،۹ و ۱۴ اندازه‌گیری شد. سپس برای ارزیابی هیستوپاتولوژیک در روزهای فوق، هر زخم و ۵ میلی‌متر از بافت نرمال پوست اطراف هر زخم جدا و فیکس شد. نتایج نشان داد که در روزهای سوم و پنجم پس از تشکیل زخم، سطح زخم باقی‌مانده در گروه CHAS به طور قابل توجهی کوچک‌تر از CHAS با سلول‌های بنیادی مزانشیمی بود، اما تفاوت معنی‌داری با گروه C مشاهده نشد. همچنین سطح زخم باقی مانده در روزهای نهم و چهاردهم در گروه‌های مورد مطالعه تفاوت معنی‌داری نشان نداد. با این حال، در روز ۱۴، میانگین ناحیه زخم در گروه CHAS با سلول‌های بنیادی مزانشیمی کوچک‌تر از دو گروه دیگر بود. بررسی‌های بافت‌شناسی محل زخم از نظر آرایش کلاژن، التهاب، تشکیل عروق، بافت گرانولاسیون و بازسازی اپیتلیال مورد مطالعه قرار گرفت. مطالعات از نظر آرایش کلاژن، تشکیل بافت گرانوله و تشکیل عروق نشان داد که در روز سوم، بین گروه‌ها تفاوت معنی‌داری وجود داشت، در حالی که تفاوت آماری معنی‌داری بین گروه‌ها از نظر التهاب و بازسازی اپیتلیال در روزهای مورد مطالعه مشاهده نشد. همه این نتایج نشان می‌دهد که تفاوت معنی‌داری بین گروه CHAS و گروه CHAS با سلول‌های بنیادی مزانشیمی و همچنین با گروه C وجود ندارد.

کلمات کلیدی: کیتوزان، هیالورونیک اسید، سلول‌های بنیادی مزانشیمی، ترمیم زخم

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