The Effect of encapsulated autologous adipose-derived stem cells in chitosan/PRPCryogel on healing of grade-II burn injuries

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Background: Wound healing in grade II burns is one of the treatment priorities. Advances in new sciences, such as stem cell therapy, biological scaffolds, and growth factors, have renewed hopes in this area. The present study aimed to assess the impact of ADSCs encapsulated in PRP/Chitosan-based gel and PRP/Chitosan cell-free gel on the healing of grade-II burn wounds in rats as compared to the control group.

Methods: Thirty rats were randomly assigned to one of three groups. Following adipose stem cell extraction, a deep grade II burn was induced in the back of the rats using a standardized catheter. The first group served as a control and received simply routine treatments. The second group received a gel based on PRP/Chitosan and ADSCs, and the third group received a gel free of PRP/Chitosan cells. The results were based on response to treatment, observable granulation tissue formation, or epithelialization at 7, 14, 21, and 28 days after treatment.

Results: The findings revealed that the use of a cell-based PRP/Chitosan scaffold or cell-free PRP/Chitosan scaffold reduced the rate of necrosis and inflammation and increased the rate of epithelialization, granulation, and neovascularization compared to the control group ($P < 0.05$). Moreover, the use of stem cells in scaffolds resulted in greater wound healing than the cell-free scaffolds group ($P < 0.05$).

Conclusion: Due to their porosity and the improved efficacy of stem cells placed in them, PRP/Chitosan scaffolds could have a positive impact on healing and speed up the wound healing process.

Keywords: stem cell, therapy, biological, scaffolds, burns

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INTRODUCTION

Burn wounds are devastating injuries that have a high cost for both individuals and the healthcare system. Deep grade II burn wounds cause dermal injury, which might exacerbate if infected [1]. Standard burn therapies include debridement, dressing, and post-treatment skin grafting. However, clinical trials have shown that they are ineffective in terms of

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delayed wound healing, scar formation, and tissue necrosis [2]. Scientists are becoming more interested in stem cell-based regenerative medicine.

MSCs are self-renewing cells that can differentiate into a variety of mesoderm lineages, including osteocytes, adipose, and chondrocytes [3,4]. Mesenchymal stem cells (MSCs) were demonstrated to have significant potential in tissue regeneration, with the ability to restore multiple injured tissues such as the heart, blood vessels, cartilage, bone, and skin [5]. Various MSC populations, including adipose-derived stem cells (ADSCs) [6], bone marrow mesenchymal stem Cells (BMSCs) [7,8], and umbilical cord-derived stem cells (UC-MSCs) [9,10], have been used in therapeutic practice. ADSCs provide several distinct advantages, including more sources, the avoidance of ethical issues, and faster amplification [11]. Due to these characteristics, ADSCs become an attractive stem cell source for wound healing.

The combination of mesenchymal stem cells (MSCs) and platelet-rich plasma (PRP) could be a promising therapy option for burn wounds [12] and chronic wounds [13]. The differentiation of MSCs into fibroblasts, keratinocytes, and epithelial cells contributes to wound healing. Furthermore, their paracrine functions boost angiogenesis, re-epithelialization, neovascularization, and collagen formation [14,15]. Platelet-rich plasma (PRP) is a platelet and leucocyte-rich solution. PRP contains a number of growth factors and cytokines, including platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and transforming growth factor (TGF), making it an attractive candidate for cell transplantation [16]. These growth factors stimulate angiogenesis by assisting in promoting proliferation, migration, and extracellular matrix (ECM) remodeling. Platelets have also been linked to antimicrobial and pain-relieving properties [17].

Cells, on the other hand, must be delivered to the wound site on a scaffold that allows them to attach, develop, and differentiate. This type of scaffold promotes better organization, incorporation, and the development of new tissue [18]. Cell encapsulation is a popular method of combining cells with scaffolds. The capsule environment stimulates cell proliferation, differentiation, and new tissue growth while also providing structural support for cells [19]. An organic biopolymer is called chitin, which makes up the majority of the exoskeleton of crustaceans. Chitin is N-deacetylated, resulting in chitosan, a cationic polymer. Chitosan is a well-known biopolymer due to its distinctive features, which include being nontoxic, non-antigenic, physiologically adhesive, biodegradable, biocompatible, and hemostatic [20]. In this study, a well-formed platelet gel, also known as PRPgel or PRPCryogel, and chitosan gel as two distinct scaffolds with the ability to enclose cells as wound healing accelerators were chosen. In a rat model of grade II superficial burn lesions, we assessed the regeneration effects of three treatment groups: (a) control (received only regular treatments), (b) PRPCryogel-autologous ADSCs, and (c) PRPCryogel containing chitosan without cell. The effect of this method on wound healing in comparison to the control group was assessed by measuring the wound surface with a caliper on days 7, 14, 21, and 28 after treatment, examining the presence or absence of scars, and comparing the scar with the control group.

METHODS

Rat ADSCs isolation and culture

In brief, animals (7–8 weeks old Wistar rats) were anesthetized, and their inguinal adipose tissues (2-3 g) were removed and washed with phosphate-buffered saline (PBS), before being transferred to a culture room, and placed in a small container. Fat was divided into small pieces and 0.2% Type I collagenase (Invirogen, USA) was utilized to digest the tissue pieces for 50 minutes at 37 °C. Excess collagenase was then removed, and fragments were placed near the well of the 6-wall plate. In such a way that one side adhered to the floor and one to the wall. The goal was for the adipose-derived MSCs to come out of the adipose and stick to the bottom of the well. Therefore, only 0.5 mL of FBS was poured into each well and spread slowly. Finally, the plate was transferred to the incubator. The first two days were always checked for cell adhesion, and as soon as the adherent cell was seen, tissue and FBS were removed, and a culture medium was added to promote cell growth. Cell passages were performed when the cells achieved 90% confluency using Trypsine-EDTA solution (Gibco, USA).

Preparation of Chitosan/Platelet gel and cell encapsulation

Before collecting the blood from the donors, written

informed consent was obtained from all the individuals. Platelet-rich plasma was extracted from the blood of the donors. Following that, 5 mL of platelet-rich plasma and calcium gluconate (2 M, 25 µL) were mixed. The prepared samples were incubated at 37 °C for 20 minutes to make PRPCryogel. A 5% chitosan (Sigma-Aldrich, USA) solution in 43% acetic acid was prepared and stirred for 48 hours with a magnet on a stirrer to achieve a homogenous gel, which was then added to PRPCryogel. To encapsulate ADSCs in PRP/ chitosan Cryogel, the cells were added to the gel just before it was completely formed and then re-incubated for 3 minutes.

Animal modeling

40 male Wistar rats, aged 7-8 weeks and weighing 200-250 g were purchased from Shahrekord University of Medical Sciences' animal nests to induce grade II burn wounds. The rats utilized in this investigation were kept housed in a 12-hour light-dark cycle with free access to food and drink. amount of G6PD enzyme. This study was approved by the Ethics Committee of Shahrekord University of Medical Sciences (ethical code: IR.SKUMS.REC.1398.090). The animal care and experimental procedures were performed according to the national guidelines, in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. To begin, rats were anesthetized with intraperitoneal (IP) injections of ketamine 10% (100 mg/Kg) and xylazine 2% (10 mg/Kg). Next, the dorsal side of each rat was shaved and disinfected with ethanol 75%. The shaved skin was then scorched with a 2 × 2 cm hot plate heated to 190° using electricity. The heated plate was placed on the rats' skin for 20 seconds without applying pressure, resulting in a superficial grade II burn (Figure 1).

Experiment groups and treatment

After creating the burn models, the animals were randomly divided into three groups of ten rats each: (a) control (received only routine treatments, including Vaseline gauze), (b) PRPCryogel-autologous ADSCs (received 40 g PRPCryogel+2 106 ADSCs), and (c) routine treatment with PRPCryogel containing chitosan without cell. Following transplantation;

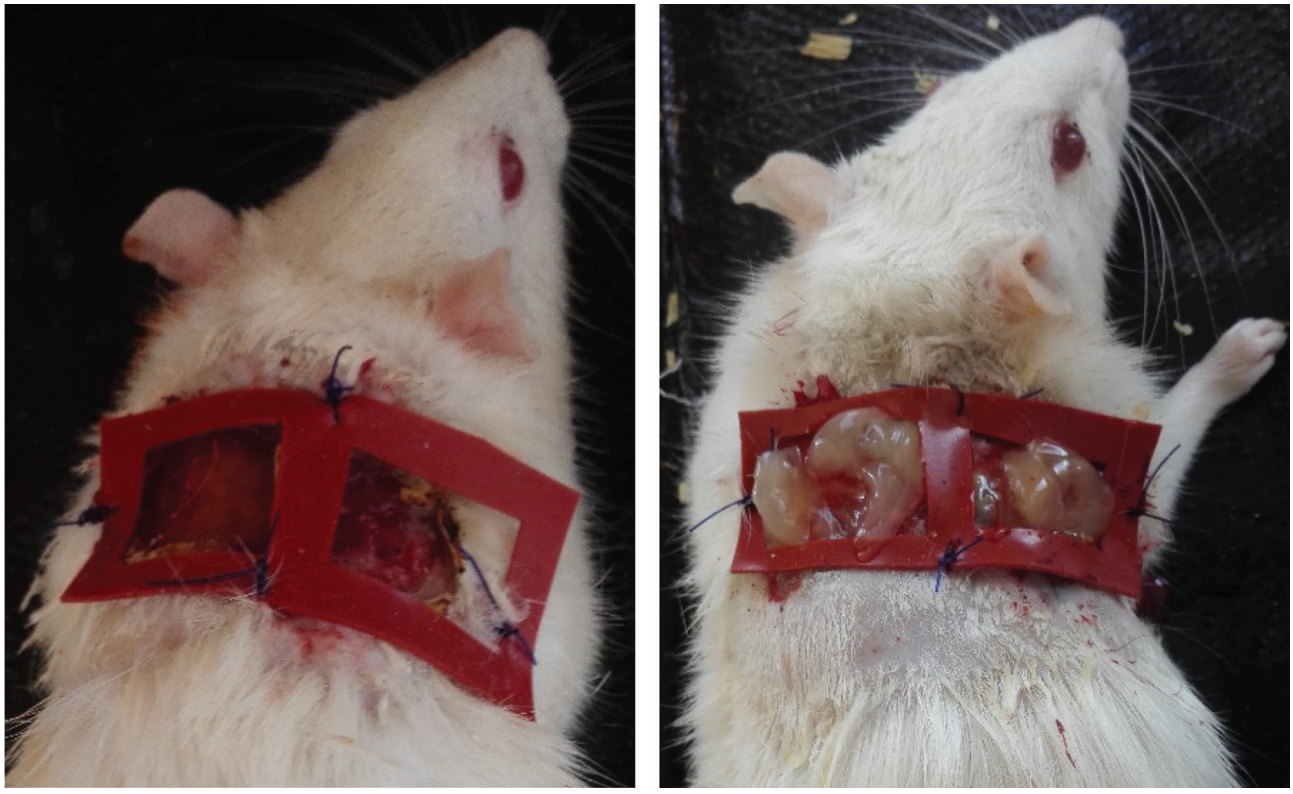

Figure 1. Animal models of grade-II burn. (a) Superficial grade-II burn, wrap around the wound site with a silicone washer. (b) The wound site is covered with PRPCryogel.

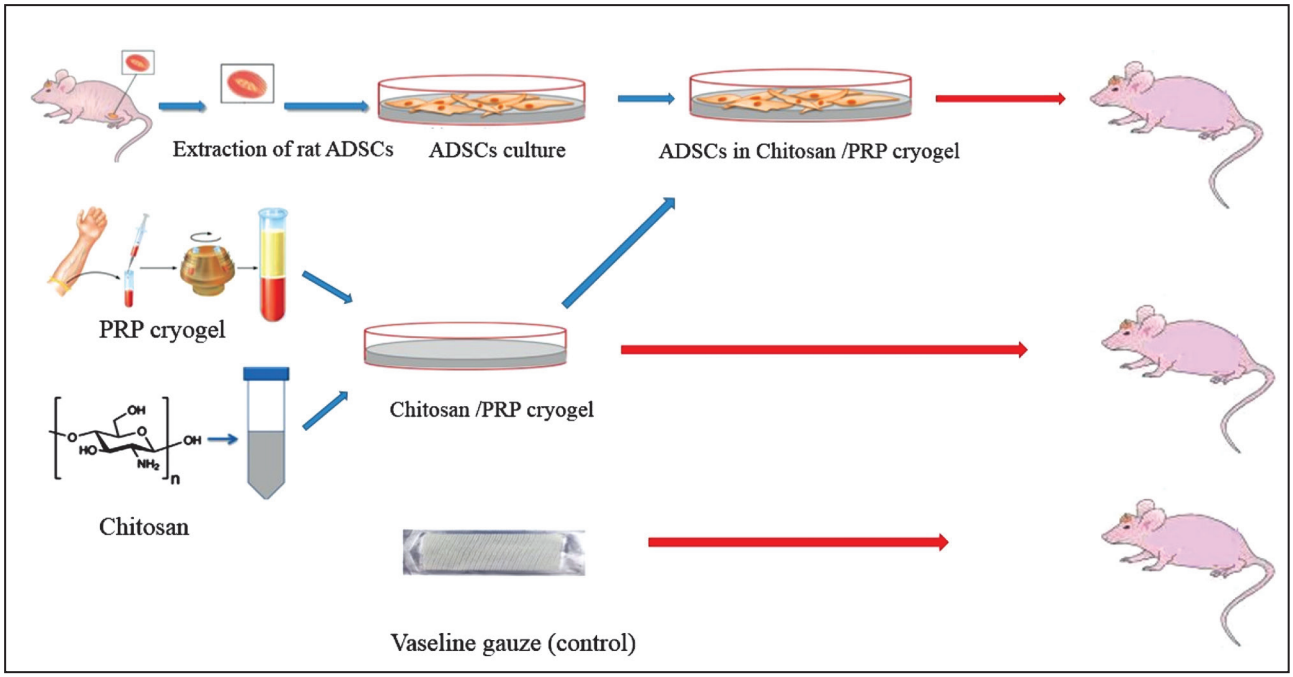

Figure 2. The diagram showed experimental groups for the treatment of grade II burn injuries in rats. All groups received their treatment plus Vaseline gauze, except the control group, which received simply vaseline gauze. ADSCs: Adipose-Derived Stem Cells.

the wounds were dressed with sterile bandages. The following investigations were conducted on 7, 14, 21, and 28 days after treatment. Images of wound sites were recorded at regular intervals, and rats were sacrificed (Figure 2).

Statistical analysis

All data were reported as means ± standard deviations (SD). SPSS software version 22 (SPSS, Chicago, IL) was used for all statistical analysis, using two-way ANOVA for multiple group comparisons. Graph pad Prism Software, version 8 (Graphpad Software, California, USA) was used to make the graphs. evaluated < 0.05 was considered statistically significant.

RESULTS

Wound closure rate

According to the images of the wound sites, the wound closure rate was accelerated in both treatment groups as compared to the control group that received only Vaseline gauze. However, the cell-based PRP/chitosan scaffold groups exhibited a greater wound-healing effect on days 21 and 28 post-treatment. On day 28 post-treatment, cell-based PRP/chitosan scaffolds demonstrated complete wound healing with minimal scar formation (Figure 3).

Microscopic results

As shown in Figures 4 and 5, different parameters, including granulation, epithelialization, neovascularization, inflammation, and necrosis were investigated. Cell-free and cell-based scaffolds resulted in significantly higher granulation on day 28 than the control group ($P < 0.05$). Besides, the use of cell-based PRP/chitosan scaffolds increased the granulation rate compared to the group that received cell-less scaffolds. However, this difference was not statistically significant (Figure 4A). As indicated in Figure 4B, the results showed that the use of scaffolds with and without cells significantly increased epithelialization compared to the group receiving routine treatment ($P < 0.05$, $P < 0.01$). The rate of neovascularization increased with the use of cell-free and cell-based scaffolds when compared to the control group, with a statistically significant difference on days 14 and 28 ($P < 0.05$, $P < 0.01$). The findings also demonstrated that, on day 14, neovascularization was faster in the group receiving cell-based scaffolds than in the group receiving cell-free scaffolds (Figure 4C).

As shown in Figure 4D, the use of cell-free and cell-based PRP/chitosan scaffolds reduced inflammation compared to the control group, which was statistically

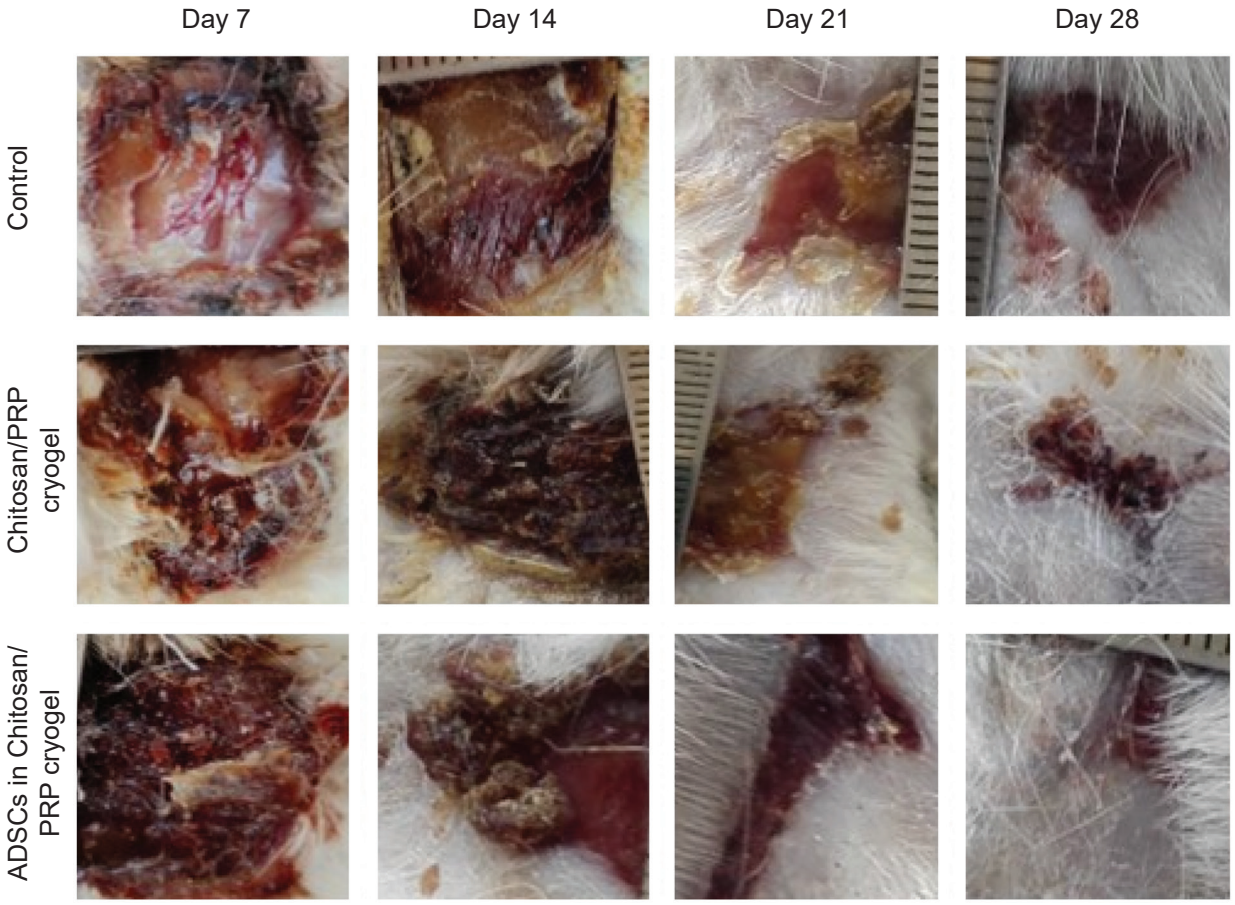

Figure 3. Macroscopic images of wound sites, the rate of wound closure in burn wounds treated with cell-based PRP/chitosan scaffolds, cell-free scaffolds, and control groups on days 7, 14, 21, and 28 post-treatment.

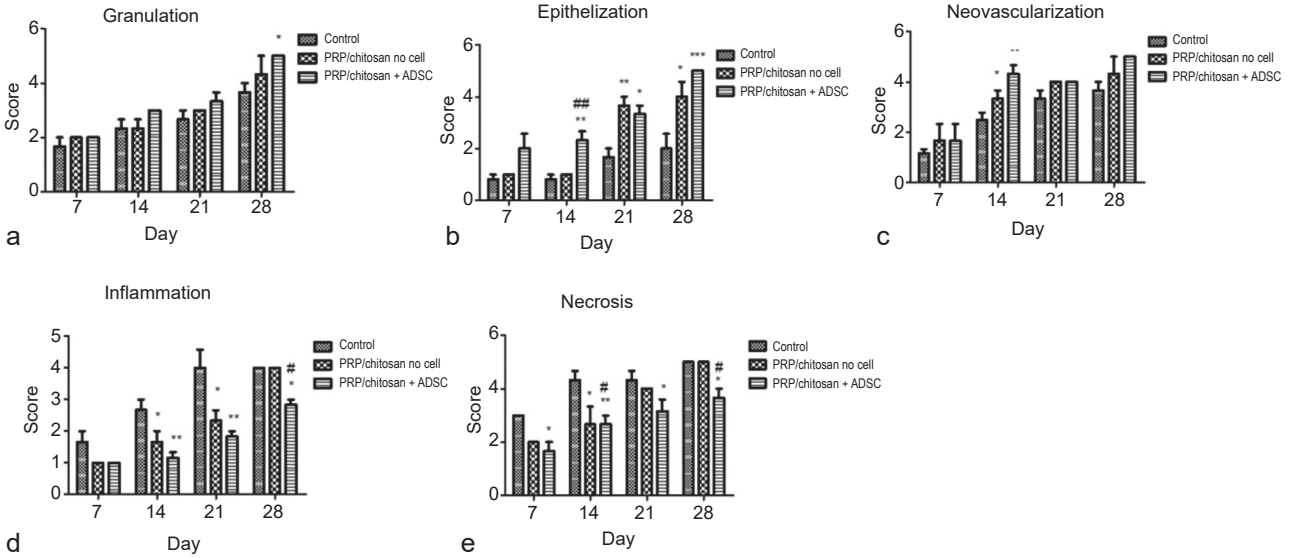

Figure 4. Analysis of granulation, epithelialization, neovascularization, inflammation, and necrosis. (a): Use of cell-based PRP/chitosan scaffolds leads to a significant increase in granulation compared to the control group. (b): Results of epithelialization analysis showed that the use of cell-based PRP/chitosan scaffolds leads to a significant increase in epithelialization compared to the control group. (c): The use of cell-based PRP/chitosan scaffolds leads to a significant increase in the rate of neovascularization compared to the control group. (d): The use of a cell-based PRP/chitosan scaffold leads to a significant reduction in inflammation compared to the control group. (e): Use of cell-based PRP/chitosan scaffold with cells leads to a significant reduction in necrosis compared with the control group. (*P < 0.05 **P < 0.01, ***P < 0, compared to the control group), (##P < 0.01 #P < 0.05 compared to the cell-less scaffolds group).

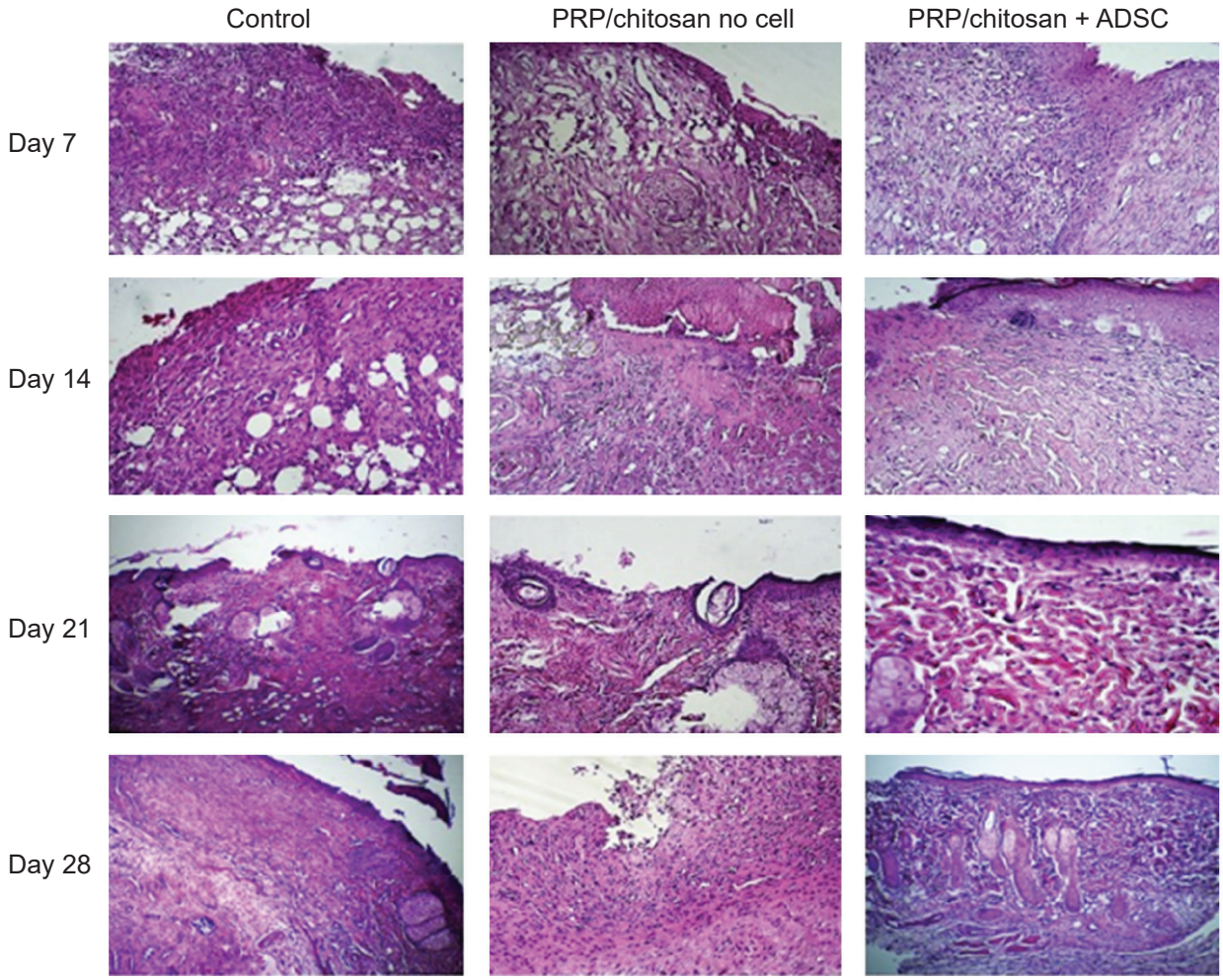

Figure 5. H&E staining assessments for wound tissues. epithelialization, granulation, tissue formation, and angiogenesis for wound samples of control, PRP/chitosan no cell, and PRP/chitosan + ADSC groups on days 7, 14, 21, and 28 post-treatment.

significant on days 14, 21, and 28 compared to the control group ($P < 0.05$, $P < 0.01$). On day 28, cell-based scaffolds significantly reduced inflammation compared to cell-free scaffolds ($P < 0.05$). In the event of necrosis, scaffolds with and without cells resulted in a statistically significant decrease in necrosis compared to the group receiving standard treatment ($P < 0.05$, $P < 0.01$). Utilizing a cell-based PRP/chitosan scaffold significantly reduced the rate of necrosis compared to the group receiving cell-free scaffolds ($P < 0.05$) (Figure 4E).

DISCUSSION

The effect of platelet-derived growth factors on the differentiation and proliferation of mature and chitosan scaffold-like stem cells could potentially significantly accelerate the healing process of burn wounds and reduce scarring at the site of the burn. The results of the present study indicated that the use of adipose-derived stem cells on a chitosan scaffold could have superior healing effects than using adipose stem cells, which indicated the potential effects of chitosan in wound healing. In the present study, platelet cryogel was used as one of the most essential factors in accelerating the wound healing process.

The wound healing process includes a set of mechanisms, including coagulation, inflammation, synthesis of the underlying material and matrix, angiogenesis, fibroplasia, epithelialization, wound contraction, and rearrangement. These physiological processes begin as soon as tissue damage occurs. Significant developments in stem cell technology have occurred over the last decade, and cell-based therapy has played an essential role in the treatment of a variety of diseases, including burns [21]. Adipose-derived stem cells, despite being autologous, could

have restorative and therapeutic effects in the treatment of wounds, burns, and skin diseases [22].

Active platelets are typically generated in the early stages of an ulcer. Platelets release inflammatory and mitogenic agents at the site of injury, which are involved in the entire wound-healing process. These cells provide growth factors to inflammatory cells, fibroblasts, and endothelial cells, guiding the processes involved in wound healing, such as chemotaxis for neutrophils, monocytes, and fibroblasts in wounds. One of the most essential molecules released during healing is PDGF, which stimulates neutrophils and macrophages, collagen synthesis, collagenase activity, and angiogenesis. PDGF activates TGF, which regulates cell proliferation and differentiation, as well as the synthesis of several extracellular matrix materials. This growth factor also stimulates fibroblast proliferation and chemotaxis, as well as collagen synthesis.

Over the last decade, platelet-releasing substances have been utilized on thousands of diabetic foot patients in the United States. According to Margolis *et al.*, platelet-releasing compounds were proven to have beneficial effects, and the treatment of diabetic wounds was more effective in patients with deeper wounds than in patients with superficial injuries [23]. Platelet gel therapy was successfully administered to patients suffering from mostly unhealed chronic wounds [24]. Lee *et al.* tested four levels of PRP in each of the 15 rats with four ulcers on the back, and the results showed that consuming a sufficient amount of platelet-rich plasma per day of full-thickness wounds on the rats' backs improved healing by reducing the rate of contraction while facilitating epithelial cell migration and vascular response [25].

Platelet adhesive is a platelet concentration that releases growth factors by stimulating platelets with thrombin and calcium. The most essential factors are transforming growth factor, platelet-derived growth factor, vascular endothelial growth factor, epithelial growth factor, and insulin-like growth factor, which trigger undifferentiated cells to the site of inflammation and initiate cell division [26]. Platelets also contain proteinase, which is involved in the release of proteolytic enzymes by other cells. These enzymes are effective in breaking down the basement membrane and extracellular matrix. Platelets are also involved in inhibiting the release of cytokines by macrophages. Various laboratory models indicated that cells involved in tissue repair are sensitive to platelet-derived growth factors, with TGF causing neutrophil and monocyte chemotaxis, PDGF causing migration and proliferation of fibroblasts to the wound site, and VEGF increasing vascular permeability [27].

In the present study, adipose was used to prepare stem cells. In most of the previous studies and with different scaffolds, these cells had positive results. Gu *et al.* found that combining adipose-derived stem cells with a polylactic acid-caprolactone scaffold significantly accelerated the wound healing of rats. In two other studies, the same cell produced similar effects with tropoalastin and carboxymethylcellulose scaffolds [28]. Finally, the findings of the present study demonstrated that the use of PRP/chitosan scaffolds could accelerate the wound healing process, which was consistent with the results of previous studies. It is suggested that in the continuation of the development of this work, PRP-cryogel combined with adipose-derived mesenchymal stem cells be utilized to treat small ulcers in patients, such as diabetic foot ulcers.

CONCLUSION

The present study showed that using PRP/chitosan scaffolds as wound dressings, based on the reported properties, could have beneficial effects in the treatment and acceleration of wound healing, which can be investigated further as a cell-based dressing. Autologous adipose capsules encapsulated in platelet cryogel and chitosan could be used in the treatment of burns. Since PRP preparations have inter-individual variability, this constraint should always be considered in prospective studies.

Authors' Contributions

Conception and design, acquisition of data, analysis, and interpretation of data were performed by LA, MB, RZ, and. drafting of the manuscript was done by SH.R, RZ. Critical revision of the manuscript for important intellectual content and statistical analysis was carried out by MB, SH.R, and LA. The final draft was approved by ES, SA, and MS. All authors read and approved the manuscript.

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Conflict of Interest: None declared.

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