Wound Medicine 10–11 (2015) 1–8

Contents lists available at ScienceDirect

Wound Medicine

journal homepage: www.elsevier.com/locate/wndm

Original Research Article

# Effect of adipose derived nucleated cell fractions with chitosan biodegradable film on wound healing in rats

Moein Mehrtash [a], Rahim Mohammadi [a,*], Rahim Hobbenaghi [b]

[a] Department of Surgery and Diagnostic Imaging, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
[b] Department of Pathobiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran

ARTICLE INFO

*Article history:*
Received 21 August 2015
Accepted 20 September 2015
Available online 25 September 2015

*Keywords:*
Chitosan
ADNCs
Wound healing
Full-thickness wounds

ABSTRACT

Wound healing is the interaction of a complex event in cellular and biochemical actions ending up the reestablishment of structural and functional integrity of injured tissues. In this study the effect of combination of adipose derived nucleated cell fractions (ADNCs) and chitosan film was evaluated on full thickness wounds. For excisional wound healing model twenty-four male white Wistar rats were randomized into four groups of 6 rats each: control group (CG) including creation of wounds and no treatment, ADNC group including creation of wounds and application of 1 mL ADNCs, chitosan group (CHIT) including dressing the wound with chitosan and CHIT/ADNC group including application of 1 mL ADNCs and dressing the wound with chitosan. Wound size was measured on 6, 9, 12, 15, 18 and 21 days after surgery. For incisional wound healing model twenty-four healthy male Wistar rats were randomized into four groups of 6 rats each the same way in excisional wound model. Reduction in wound area, hydroxyproline contents and biomechanical parameters indicated that there was significant difference ($P > 0.05$) between CHIT/ADNC group and other groups. Biomechanical testing was performed 9 days after surgery in incisional model. Quantitative histological studies and mean rank of the qualitative studies demonstrated that there was significant difference ($P > 0.05$) between CHIT/ADNC group and other groups. ADNCs can provide a readily accessible source of cells in large quantities that contribute to wound healing in the emerging field of regenerative medicine especially where a traumatic injury is dealt with. ADNCs with chitosan have beneficial effects on wounds repair and could be suggested for treating various types of wounds in animals and human being.



## 1. Introduction

Wound healing is the interaction of a complex cascade of cellular and biochemical actions leading to the restoration of structural and functional integrity with regain of strength of injured tissues [1].

There are advantages and disadvantages for conventional repair method and the development of advanced wound healing technology has triggered the use of cells to overcome limitations of the conventional methods. Cell therapy has a potential to improve wound healing conditions without major surgical procedures and donor-site morbidity. Cell therapy can be applied for both acute and chronic wounds [2].

Cell therapy can increase wound healing rate, reduce scar contracture, and minimize donor-site morbidity in treatment of acute wound [2]. In the treatment of chronic wounds, attempts are made to convert the wound bed into the environment where maximum wound healing can be achieved by transplanting cells with an excellent wound healing capacity to the wound bed [2].

The original and classical view of adipose tissue as a rather specialized passive storage organ has been changed dramatically [3]. The adipose tissue bears several properties that are advantageous for neuronal sprouting and direction and has been used in different areas of surgery in recent decades [4,5]. Apart from adipocytes and pre-adipocytes, the adipose tissue contains microvascular endothelial cells, smooth muscle cells, resident monocytes, lymphocytes and stem cells [6].

In recent years, it has been identified that adipose tissue possesses a population of multi-potent stem cells which can be of benefit for treatment of wound injuries of various kinds [7–12].

Utilizing cultured cells for clinical purposes require FDA-approved facilities and techniques and a lengthy culture period.

* Corresponding author at: Department of Surgery and Diagnostic Imaging, Faculty of Veterinary Medicine, Urmia University, Nazloo Road, Urmia 57153 1177, Iran.
*E-mail address:* r.mohammadi@urmia.ac.ir (R. Mohammadi).

http://dx.doi.org/10.1016/j.wndm.2015.09.001
2213-9095/

Adipose-derived SVF cells can consider favorable alternative cultured cells [13]. Extensive clinical cases of the tissue-engineered dermis have been reported using the ADNC cells for the coverage of defects on the face, hand, and foot [14,9–11].

The ADNC cells can be also applied for chronic wounds. An in vitro study has been performed to determine the effect of cell therapy using uncultured ADNC cells on cell proliferation and collagen synthesis of diabetic fibroblasts, which are the major contributing factors in wound healing [12]. In addition, a synergic effect of the ADNC cell autograft was observed possibly due to mutual stimulation of the diabetic fibroblasts and ADNC cells by growth factors secreted from them. A clinical trial study also has demonstrated that ADNC cell therapy accelerated diabetic wound healing [12].

In cases of severe distortion of the tissue architecture, the healing process may not lead to morphofunctional normality, however, result in the formation of disoriented connective tissue with a fibrous appearance [15,16]. This abnormal tissue architecture reduces the mechanical strength and leads to scar formation. Biomaterials can assist the proper physiological reconstruction of the skin and reduce or prevent scar tissue formation. Chitin, chitosan and their oligomers have been found to promote wound healing, especially in the phases of proliferation and matrix formation [17]. Chitosan and its oligomers are well known for their interesting biological properties, which have led to various applications. Lysozyme slowly hydrolyzes chitosan membrane and produces chito-oligomers that stimulate correct deposition, assembly and orientation of collagen fibrils in extracellular matrix components [18]. Moreover, it has been indicated that chitosan membrane stimulates the migration of inflammatory cells and promotes cellular organization [19,20].

To the best knowledge of the authors, literature is poor regarding the effect of ADNCs and chitosan combination on full thickness wound healing. The aim of the present study was to evaluate the wound healing activity of chitosan and ADNCs combination on full thickness wounds in a rat model. Assessment of the healing process was based on excision, incision, hydroxyproline estimation and biomechanical studies.

## 2. Material and methods

Our study protocol was reviewed and approved by Urmia University ethical committee. All animals received humane care in accordance with the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication No. 85-23, revised 1985).

### 2.1. Preparation of chitosan

Chitosan solution was prepared by dissolving a medium molecular weight crab shell chitosan (~400 kDa, 85% deacetylated) (Fluka, Sigma–Aldrich, St. Louis, MO, USA) in an aqueous solution (1%, v/v) of glacial acetic acid (Merck, Darmstadt, Germany) to a concentration of 2% (w/v) while stirring on a magnetic stirrer-hot plate. The solution was stirred with low heat (at 50 °C) for 3 h. The resultant chitosan solution was filtered through a Whatman No. 3 filter paper. Then it was vacuum filtered to remove any un-dissolved particles. To overcome the fragility of chitosan, glycerol (Sigma Chemical Co., St. Louis, MO, USA) was added as 30% (w/w) of the total solid weight in solution [21]. Chitosan (2%, w/v) in acetic acid was freeze-dried, and cross-linked with 5% (w/v) tripolyphosphate and freeze-dried again to produce a sponge-like matrix. Chitosan sheets were prepared and dressed on created wounds.

### 2.2. Collection of adipose tissue and isolation of ADNCs

Four donor rats were also assigned to ADNCs isolation and preparation. The entire abdomen was prepared aseptically and after ventral midline incision approximately 4–5 g omentum were harvested from donor animals. The donor animals were then euthanized by over dose of the anesthetics. The technique of ADNCs isolation is described elsewhere [22–26]. In brief, the way the harvested omentum was rinsed with HANKS-buffered saline (HBS), trimmed, minced with two scalpels into very small pieces, and aspirated into a 10-mL pipette; then the tissue was transferred into a 50-mL Erlenmayer flask containing 1500 U/mL collagenase type II (Sigma Chemical Co.). The ratio was 1 g of omental tissue to 2 mL of collagenase. Then warm 0.1% collagenase type I was added into the bottles containing the washed adipose tissue sample and was placed in a shaking water bath at 37 °C at 75 rpm for 60 min. Samples were then centrifuged for 10 min at $600 \times g$ at room temperature. The digested tissue was homogenized by repetitive pipeting, transferred into a 15-mL tube, and centrifuged twice at $100 \times g$ for 5 min. The supernatant contained mainly adipocytes and the collagenase solution. The cell pellet was resuspended in 10 mL phosphate-buffered saline (PBS), filtered through a 150-μm pore-size mesh to remove non-digested large tissue fragments, and then washed two times with HBS. The ADNCs pellet was resuspended in sterile PBS solution as 10-μL aliquots ($2 \times 10^7$ cells/mL), each loaded into sterile syringes. The cellular population count was performed using a hemocytometer and $2 \times 10^7$ nucleated cells per milliliter of adipose tissue were counted. The syringes containing PBS solution and ADNCs were shipped chilled to the investigators for immediate administration.

### 2.3. Excision wound model and planimetric studies

For excisional wound healing model 24 healthy male Wistar rats weighing approximately 160–180 g and seven weeks of age were randomized into four groups of 6 rats each: Sham surgery group (SHAM) including creation of wounds and no treatment, ADNCs treated group (ADNC) including creation of wounds and application of 1 mL adipose derived nucleated cell fractions ($2 \times 10^7$ cells/mL), chitosan group (CHIT) including dressing the wound with chitosan and CHIT/ADNC group including application of 1 mL adipose derived nucleated cell fractions and dressing the wound with chitosan. After induction of anesthesia with Xylazine HCL 2% (5 mg/kg/IP, Alfasan International, Woerden, Holland) and ketamine HCL 10% (60 mg/kg/IP, Alfasan International, Woerden, Holland) rats were fixed in a ventral posture on a surgery table. Following shaving and aseptic preparation, a circular excision wound was made by cutting away approximately 300 $mm^2$ full thickness of predetermined area on the anterior-dorsal side of each rat. All the test formulations were applied for 10 days starting from the day of wounding. Wound-healing property was evaluated by wound contraction percentage and wound closure time. Photographs were taken immediately after wounding and on days 6, 9, 12, 15, 18 and 21 post-operation by a digital camera while a ruler was placed near the wounds (Fig. 1). The wound areas were analyzed by Measuring Tool of Adobe Acrobat 9 Pro Extended software (Adobe Systems Inc., San Jose, CA, USA) and wound contraction percentage was calculated using the following formula:

$$\text{Percentage of wound contraction} = \left(\frac{A_0 - A_t}{A_0}\right) \times 100.$$

where $A_0$ is the original wound area and $A_t$ is the wound area at the time of imaging [27]. The animals were left in separate cages for four days at room conditions for acclimatization. Animal houses were in standard environmental conditions of temperature