



# The Effects of Fibroblast-Conditioned Medium Isolated from Human Neonatal Foreskin on Diabetic Ulcers in Rats

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## Abstract

**Background and Aim:** Diabetes is rising worldwide and late wound healing is one of its complications. The aim of this study was to investigate the effects of fibroblast-conditioned medium isolated from human neonatal foreskin on diabetic ulcer in rats.

**Methods:** In this experiment human neonatal foreskin was first isolated. Fibroblasts were then isolated from this tissue and characterized by immunohistochemically staining method. Streptozocin was used to induce diabetes in 10 male rats. 0.8-cm-diameter wounds were punched on the back of the diabetic rats. The rats were then divided into two groups: control group (normal saline injection) and experimental group (fibroblast-conditioned medium injection). After the treatment, wound healing was evaluated by photographic methods on days 7, 14 and 21.

**Results:** Cells isolated from neonatal foreskin expressed the Vimentin marker. Wound area was significantly reduced in the treatment group on day 7 compared to day 0, and on day 14 compared to day 7. In the control group, the wound area significantly decreased on day 7 compared to day 0, but did not change significantly on day 14 compared to day 7. On day 21, the wounds were healed in both groups.

**Conclusion:** Injecting fibroblast-conditioned medium isolated from human neonatal foreskin causes faster wound healing. Therefore, using these cells can be considered for diabetic wound healing.

**Keywords:** *Diabetic Ulcer, Fibroblast-Derived Conditioned Medium, Neonatal Foreskin, Rat.*

## Introduction

One of the causes of chronic wounds in diabetics is the lack of proper blood supply and appropriate nutrition for the cells in the wound area. The rate of collagen synthesis is also slower than in healthy people. Diabetic ulcers can lead to severe pain, nerve damage in the ulcer areas, and eventually amputation [1]. Stem cells, such as fibroblasts, can cause faster wound healing. They also lead to collagen synthesis and angiogenesis in the wound area, and eventually healing. Evidence from research suggests a positive effect of keratinocyte, endothelial and fibroblast cells on wounds [2]. Mesenchymal stem cells are derived from tissues such as bone marrow, umbilical cord, skin and adipose tissue. A study in 2016 showed that the effect of stem cells on wound healing at different stages is related to increased fibroblast cell proliferation and decreased inflammatory cells [3]. Although research shows that fibroblast cells play a role in wound healing and regeneration in diabetics, the mechanism of fibroblast cells is still unclear in wound healing, especially diabetic wounds, and it has sometimes been observed that these cells are not able to repair significantly [4]. The present study was performed due to the considerable prevalence of diabetic-derived disorders including diabetic ulcers in the world [5]), the limitations of previous studies, and also the limitations of pharmacological interventions in diabetic ulcers treatment. There was also lack of researches in application of fibroblasts isolated from neonatal foreskin. Therefore, this study investigates

the diabetic wounds healing and collagen changes following the injection of fibroblast cells in the skin wound area in rats and the findings can be used in the field of cellular therapy for diabetic wounds.

## Materials and Methods

Fibroblasts from human neonatal foreskin were isolated, and cultured by explant method. Cells from the third passage were flow cytometrically evaluated for survival. Immunohistochemically staining was also performed to identify these cells to examine the Vimentin marker. To prepare the fibroblast cell medium, the cells were cultured in DMED medium without phenol and finally the conditioned medium was concentrated up to 5 times by Master Flex. Male Wistar rats with an average body weight of 200 grams were purchased from the Pasteur Institute and kept in special cages. The room temperature was about  $22 \pm 2$  ° C. With 12:12 light–dark (LD) cycle during the study, all international animal rights were carried out in accordance with the standards. Rats were injected with 50 mg / kg streptozotocin. After three days, fasting blood glucose levels were measured using a glucometer. Rats with glucose levels greater than 246 mg / dL were considered as diabetic. Then, a wound was punctured on the back of the rats under anaesthesia. The rats were randomly divided into control and treatment groups. The treatment group was subcutaneously injected with 1 cc conditioned medium in the wound area and the treatment group was injected with 1 cc of normal saline. Wounds were imaged on days 0, 7, 14, and 21, and all wound areas were examined and compared.

## Results

The survival rate of cells was 90% according to the results of flow cytometry. The cell images showed high expression of Vimentin marker.

The results of examining the wound areas showed that the wound size decreased in the control group by days 7, 14 and 21 compared to day 0, and wound healing was evident. In addition, the wounds were completely healed on day 21 compared to day 14. Also, in the conditioned medium group, there was a significant decrease in the wound area on days 7, 14 and 21 compared to day 0, and the wound area was significantly decreased on day 7 compared to day 0. However, it was observed that wound healing was faster and earlier in the treatment group than in the control group, and the wound scars were completely disappeared in the treatment group on day 21.

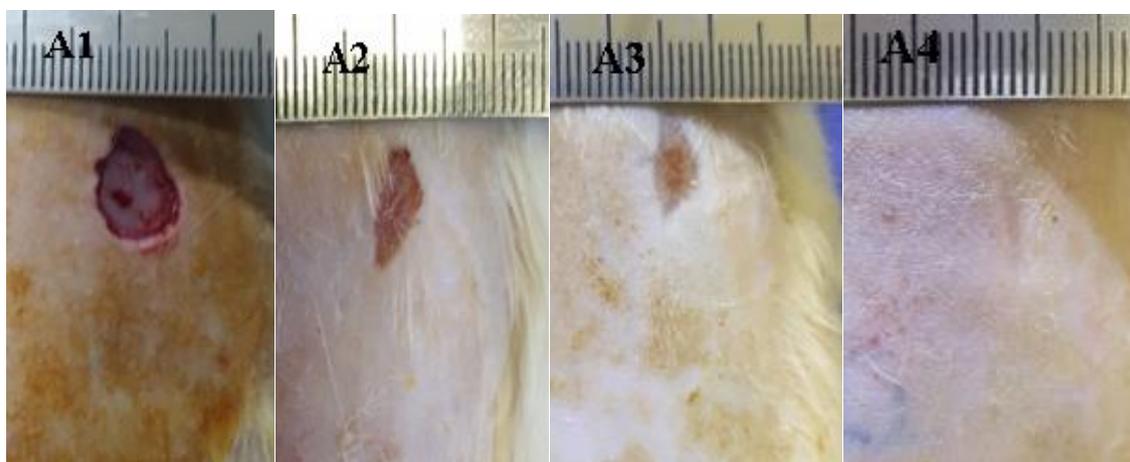




Figure 1. Wound surface images on days 0, 7, 14, and 21 from left to right, respectively. Wounds in the control group on day 0 (A1), day 7 (A2), day 14 (A3) and day 21 (A4). Wounds in the treatment group on day 0 (B1), day 7 (B2), day 14 (B3) and day 21 (B4).

## Discussion

The present study showed that the fibroblast-conditioned medium isolated from human neonatal foreskin had a significant effect on faster wound healing in diabetic rats and was also very effective in removing the scars. The changes in wound areas occurred faster and earlier in the treatment group than in the control group. These results were consistent with the findings of previous studies. For example, a study has shown that foreskin-derived stem cells have the ability to differentiate into tissues such as muscle and nerve [6]. Another study in 2017 also showed that the interaction between keratinocytes and fibroblasts accelerates the wound healing process [7]. In addition, mesenchymal stem cells stimulate the expression of vascular cells in wound areas. Conditioned medium stem cells also have the ability to heal wounds [8, 9]. A study in 2020 showed that culturing human skin-derived fibroblasts in a glucose-rich environment, and using their conditioned medium was very effective in the rapid diabetic wound healing in the animal models and reduced inflammation in granulation tissue [10]. Another study on stem cells showed that using a combination of umbilical cord stem cells with the conditioned medium from these cells had a healing effect on diabetic wounds [11]. One of the most important steps in healing skin wounds is granulation contraction, which is done by fibroblasts. Foreskin fibroblasts have great potential to stimulate wound contraction [12]. Another effective factor for wound healing is angiogenesis and blood supply to the wound areas and mesenchymal cells are able to stimulate angiogenesis [13]. In contrast, some findings have shown that mesenchymal stem cells do not have a significant effect on wound healing. [14]. If the stem cells are not successfully transplanted, the treatment process is not efficient through stem cells [15]. As a result, the current treatments for chronic and persistent wounds need to be replaced with the ones leading to faster healing and less complications. Among these, cellular therapies play a key role because stem cells are easily obtained and no side effects have been reported for them.

## Conclusion

We have shown that human foreskin is one of the major sources of fibroblast cells, and the fibroblast conditioned medium isolated from foreskin can improve wound healing in diabetic rats.



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