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Original paper

The Healing Effects of Conditioned Medium Derived from Human Neonatal Fibroblast Cells on Diabetic Wound

Arthur Wdowiak[1], Shokouh Kamalzadeh[2]*, Sona Zare[3]

[1] Department of Obstetrics and Gynecology, Faculty of Health Sciences, Medical University of Lublin, Poland
[2] Department of Genetics, Faculty of Advanced Science and Technology, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran
[3] Skin and Stem Cell Research Center, Tehran University of Medical Sciences, Tehran, Iran

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Abstract

Background and aim: Although many studies suggest that conditioned medium derived from stem cells can be effective in wound healing, the results are still challenging. Accelerating the wound healing in diabetic patients is one the most important topics currently being discussed. The aim of the research is to investigate the effects of conditioned medium derived from neonatal fibroblast cells on diabetic wound healing in male rats.

Materials and methods: Neonatal fibroblast cells were isolated from foreskin and cultured in DMEM culture medium. Conditioned medium was harvested. Male rats were divide into control (without treatment) and experiment (treatment with conditioned medium) groups (n=5 in each group). Diabetes was induced in animals by intraperitoneal injection of streptozotocin (150 mg/kg), and a full-thickness skin defect model was generated. Wound healing was evaluated 7, 14 and 21 days after treatment using Image J software.

Results: Significant decrease in wound area was observed only at 21 days after treatment in control group; However, wound area was significantly decreased 14 and 21 days after treatment compared to the day 0.

Conclusion: Our findings show that the conditioned medium derived from neonatal fibroblast cells isolated for foreskin has accelerating effects on diabetic wound healing in animal model.

Keywords: *Conditioned medium, Wound healing, Diabetic rat*

***Corresponding author:** Shokouh Kamalzadeh, Department of Genetics, Faculty of Advanced Science and Technology, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran.
E-mail address: shokouh.kamalzadeh@gmail.com

Introduction

Diabetes is a type of chronic metabolic disease that is increasing in the world, the cause of which is insufficient secretion of insulin or the inability of the body to use insulin. Deficiency and deficiency of insulin secretion and hyperglycemia are caused by the destruction of pancreatic beta cells by an autoimmune reaction. It causes type 1 diabetes. Type 2 diabetes, which is the most common type of diabetes, is diagnosed by a decrease in the response of body cells to insulin (insulin resistance) and a decrease in insulin production by beta cells, resulting in an increase in serum glucose [1]. Diabetes has become a growing epidemic in the last century, exacerbated by the exponential increase in obesity in recent decades, and has become one of the leading causes of death worldwide [2]. In 2017, 425 million people worldwide had diabetes. This is due to the global increase in the prevalence of obesity, inactivity, and unhealthy behaviors, including improper diet and physical activity. The high and rapidly growing prevalence of diabetes poses significant challenges to the health care system. According to the report of the International Diabetes Foundation, in 2021, about 537 million people were living with diabetes, and the number of patients is expected to reach 783 million by 2045. 2021, and is expected to reach 1054 billion dollars by 2045 [3]. Diabetes leads to clinical complications over time including kidney failure, retinopathy, arteriosclerosis, peripheral vascular disease, loss of peripheral sensory nerve function (neuropathy), and impairment of the ability to heal wounds [4], [5]. Chronic skin ulcers caused by diabetes, are considered to be the most common complications of diabetes [6]. Skin ulcers occur as a result of microvascular complications of diabetes, impaired wound healing, and other unknown mechanisms. Diabetic ulcers occur in almost two-thirds of patients with type 1 and 2 diabetes, and 15% of people with diabetes have lower limb ulcers [7], [8], [9]. High blood glucose leads to the abnormal continuation of the inflammation stage, prevention of cell proliferation, high level of matrix metalloproteinases, and increasing of inflammatory cytokines which is followed by wound healing delay. This problem causes diabetic patients to face complications such as wound infection, limb amputation, high treatment costs, and even death [10]. Diabetic foot ulcers are one of the major complications in people with diabetes, which is the main cause of hospitalization and nontraumatic amputation of the lower limb, which is associated with impaired functional status and quality of life in these patients. Diabetic foot is estimated to be 15-25% among patients with diabetes during life [11]. Diabetic foot ulcers are a serious medical condition that can lead to amputation if not treated quickly. Annually, untreated diabetic foot ulcers account for more than 100,000 amputations. The prevalence of diabetic foot ulcer problems in the US population is increasing due to aging and obesity [12]. A study conducted in 2021 showed that fibroblast cells with conditioned medium isolated from human foreskin had a significant effect on faster wound healing in diabetic mice and were also very effective in removing scars. Changes in wound areas occurred faster and earlier in the treatment group than in the control group [13]. The results of another study regarding the effects of stem cells in wound healing in rats indicated that stem cells alone or together with scaffolds can improve wound healing [14]. A study has shown that stem cells derived from the foreskin have the ability to differentiate into tissues such as muscle and nerves [15]. Also, in a study conducted in 2018 to investigate the effect of conditioned medium (CM) derived from mesenchymal stem cells (MSC) on skin wounds in rats, it was found that MSC-CM significantly accelerated wound closure and improved the quality of healing. MSC-CM secretes various factors to stimulate fibroblasts and keratinocyte proliferation, migration, and differentiation to promote damaged skin regeneration and functional recovery. It has been shown that MSC-CM causes cell proliferation, regeneration of cells such as sebaceous glands, and angiogenesis [16]. Another study on stem cells showed that using the combination of umbilical

cord stem cells with conditioned medium has a therapeutic effect on diabetic wounds [17]. A meta-analysis has shown that the use of stem cells heals skin wounds, which is mainly through angiogenesis and anti-inflammatory actions [18]. Another study in 2017 showed that the effect of stem cells on wound healing at different stages is related to increased proliferation of fibroblast cells and decreased inflammatory cells [19]. It was also found that conditioned medium along with a pluripotent human adult progenitor cell environment improves wound healing by modulating inflammation and angiogenesis in mice [20]. In a study conducted in 2021, it was found that the conditioned medium with mesenchymal stromal cells (MSC) provides the treatment of skin disease [21]. Contrary to research findings that show that fibroblast cells play a role in wound healing and regeneration in diabetic patients, the mechanism of fibroblast cells in wound healing, especially diabetic wounds, is still unclear, and in some cases, it has been observed that these cells are unable to repair significantly [22]. Also, it is believed that weak grafting and retention of transplanted stem cells in the wound site are important obstacles to the therapeutic efficiency of these cells in wound regeneration [23]. The results of a study regarding the effects of stem cells in wound healing in rats indicate that stem cells alone or together with scaffolds cannot improve wound healing [14]. Considering the significant prevalence and extent of clinical complications caused by diabetes, especially diabetic ulcers, and also considering the important clinical, psychological, social and economic complications resulting from diabetic ulcers in sufferers and few effective treatments for diabetic ulcers, the present study aims to investigate the healing effect of conditioned medium obtained from fibroblast cells of human foreskin on the diabetic wound in male rats.

Material and Methods

Preparation of Conditioned Medium

Human newborn foreskin tissue was obtained following routine circumcisions and cut to small pieces. The sample was digested enzymatically with 1 mg/mL collagenase type 1 (Sigma, C0130) for 1 h at 37 °C and 5% CO2. Cells were collected and centrifuged to remove collagenase. The fibroblast cells were cultured in DMEM medium supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin, and 25 µg/mL amphotericin B in a humidified atmosphere at 37 °C and 5% CO2. When the cultured cells reached 80% confluence state, they were subcultured using 0.25% trypsin-EDTA solution. After that, supernatants were collected, pooled and centrifuged at 1000 g and filtered with a 0.22-µm filter. Finally, the condition medium was lyophilized, stored at −80°C.

Animal Experiments

Rats were divide into control (without treatment) and experiment (treated with conditioned medium) groups (n=5 in each group). Diabetes was induced in animals by intraperitoneal injection of streptozotocin (150 mg/kg), and 6 mm diameter wounds were created on dorsal skin of Wistar rats. Conditioned medium was applied as a dressing and wound area was measured by Image j software 7, 14 and 21 days after treatment.

Statistical Analysis

Data is expressed as mean ± SE. Analysis of variance was used to analyze the data. The difference between groups was significant when $P < 0.05$.

Results

Figure 1 shows observable morphological changes in wound area in control and experimental rats at the day 0 and 7, 14 and 21 days after treatment:

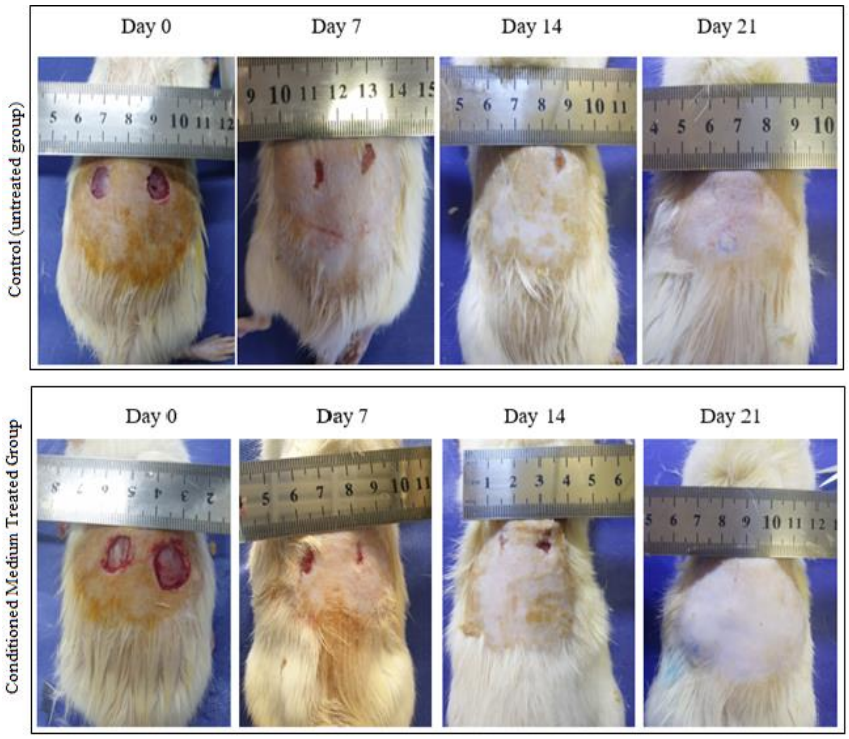

Figure 1. Wound area morphology in control rats and rats treated with conditioned medium at the day 0 and 7, 14 and 21 days after treatment.

Wound area did not significantly change in control and conditioned medium treated animals 7 days after treatment compared to the day 0. However, wound area significantly decreased 21 days after treatment in control and treated groups compared with the day 0 ($p<0.01$). Although the wound area did not significantly change in control group, it significantly decreased in conditioned medium treated animals compared to the day 0 ($p<0.05$) (Figure 2).

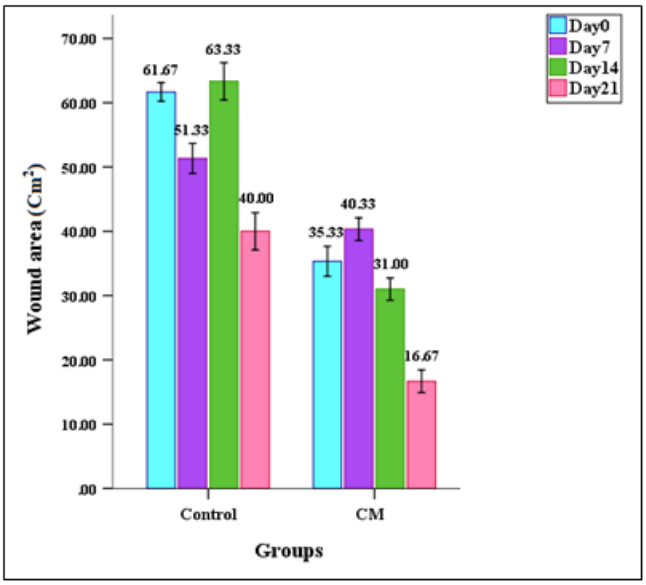

Figure 2. Wound area at the day 0 and 7, 14 and 21 days after treatment in the control rats and animals treated with conditioned medium.

Discussion

Many studies have shown that the effects of conditioned medium obtained from fibroblast cells are of significant importance in the field of healing diabetic wounds, while in the normal ways of healing diabetic wounds, the treatment process is often associated with failure or takes a long time. The results of the present study showed that fibroblast stem cells derived from the foreskin, along with conditioned medium, can accelerate the healing of diabetic wounds in male rats. In agreement with this finding, other research in 2020 showed that the cultivation of fibroblasts derived from human skin was very effective in the rapid healing of diabetic wounds in animal models and in reducing inflammation in connective tissue. Gene expression analysis showed that treatment by fibroblasts leads to upregulation of epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) genes [24]. It was reported that the conditioned medium together with the human pluripotent progenitor cell accelerates wound healing in mice by modulating inflammation and angiogenesis [20]. The results of the experiments conducted in 2021 showed that the conditioned medium with mesenchymal stromal cells provides treatment for skin diseases [21]. However, some research findings have shown that mesenchymal stem cells do not have a significant effect on healing wounds. In this regard, the results of the study on the effects of stem cells in wound healing in rats indicated that stem cells alone or together with scaffolds cannot improve wound healing [14]. Some findings have shown that if the stem cells are not successfully transplanted, the stem cell therapy process would not be efficient [23].

Regarding the mechanism of the effects of conditioned medium from human newborn fibroblast cells on diabetic wounds, it seems that these it causes the skin tissue in the wound area to regenerate faster [25]. The conditioned medium with stem cells can improve interstitial matrix, and as a result, the epithelial cells have a high ability to migrate to the granulation tissue and can block the opening of the wound in a shorter period of time and cause accelerated healing process [26].

In general, fibroblasts derived conditioned medium can increase angiogenesis and wound contraction, and stimulate fibroblast and macrophage cells, digest fibrin clot, reduce the inflammatory phase and promote the cell proliferation phase in the wound healing process [20]. The conditioned medium in the dressing, creates a suitable substrate for the growth and differentiation of fibroblast cells and increases the elasticity, causes the wound to heal faster [24], [27].

Conclusion

Our findings show that the conditioned medium derived from neonatal fibroblast cells isolated for foreskin can accelerate the healing diabetic skin wound process in animal model indicating its potential to repair the skin wounds.

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Conflict of interests

Authors declare that there is no conflict of interests regarding the publication of this paper.

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