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## The Healing Effects of Human Acellular Amniotic Membrane on Skin Wound in Diabetic Animals

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### Abstract

**Background and aim:** Human amniotic membrane has been used as a versatile skin alternative in dermatology as an ideal biological dressing material. It has been reported to have wound healing effects, however, the findings still are challenging. The aim of this study was to investigate the healing effects of acellular human amniotic membrane on skin wound healing in diabetic rats.

**Materials and methods:** Acellular amnion membrane was prepared using DMSO and characterized by electron microscopy. Rats were divided into control (without treatment) and experiment (treatment with acellular amniotic membrane) 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 days after treatment using Image J software.

**Results:** Significant decrease in wound area was observed 7, 14 and 21 days after treatment in experiment rats compared with control animals. There was also significant difference between wound area at 7, 14 and 21 days after treatment.

**Conclusion:** Our findings indicated that acellular human amniotic membrane has a significant wound healing potential, accelerating the wound healing process in diabetic subjects.

**Keywords:** *Human acellular amniotic membrane, Wound healing, Diabetic rat*

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## Introduction

Diabetes is a group of metabolic disorders that affected 463 million people in the world (8.8% of the adult population) by 2019, this rate is similar in men and women, and the number of affected people is still increasing. The disease doubles the risk of early death in people and is the seventh leading cause of death worldwide. Diabetes is caused by the insufficient production of insulin by the pancreas (loss of beta cells) or by the body's cells not responding properly to the insulin produced (insulin resistance) and as a result, the blood sugar level rises over a long period of time. Urination, increased thirst and increased appetite are its symptoms, and diabetic ketoacidosis, hyperosmolar state, hyperglycemia or death are its acute complications. Its long-term effects include cardiovascular disease, stroke, chronic kidney disease, leg ulcers, nerve damage, and eye damage. And cognitive impairment [1], [2]. Diabetic ulcer is one of the major complications of diabetes. Some disorders disrupt the wound healing process, and diabetes mellitus is one of these metabolic disorders [3], [4]. Studies show that some tissues, such as the amnion tissue of the human placenta, are effective in healing skin wounds. Human cell-free amniotic membrane is a cell-free matrix substance that is extracellular and has various bioactive factors [5].

Studies have shown that placenta tissue can have positive effects on skin wounds. The placenta supports the growth and development of the fetus and is therefore rich in amino acids, vitamins, growth factors and nutrients. Placental therapy is used in various clinical cases because it has properties such as antioxidant, anti-inflammatory, cell proliferation, tissue regeneration and wound healing properties. Research results indicate that there is a significant relationship between the use of placenta tissue and the healing of skin wounds. In the past, placenta extract was used in the treatment of chronic wounds, burn injuries, post-surgery dressings, and the treatment of varicose veins. It has been used as a traditional method. Due to the presence of growth factors (EGF, TGF- $\beta$ , IL-1 and FGF), the placenta regenerates the skin and heals wounds quickly. The placenta membrane can be used as a dressing for wounds. Studies show that placental tissue can increase the speed of healing and treatment of diabetic wounds [6]. In fact, amniotic membrane cells with their high flexibility are very useful for tissue regeneration based on cell therapy [7]. Research findings show the significant effects of amniotic membrane on the treatment of skin wounds. Frozen human placental membrane grafts (vCHPM) are effective in the treatment of diabetic foot ulcers and venous leg ulcers [8]. Laboratory research has shown that the amniotic membrane can be effective in the healing and treatment of diabetic ulcers. Dried human amnion and chorion allograft have a significant healing role in the treatment of diabetic foot ulcers [9].

Considering the widespread prevalence of diabetes and diabetic ulcers in the world [10], the increasing use of amnion tissue in the repair and healing of skin wounds in many societies [7], [11], widespread clinical, social and economic complications resulting from diabetes [1], [2], and also considering the numerous and useful effects of human acellular amniotic membrane on the healing and treatment of skin wounds, especially diabetic wounds [5] and few research on the therapeutic effects of human acellular amniotic membrane on skin wounds caused by diabetes, the present study investigates the therapeutic effects of human acellular amniotic membranes on the skin wounds in diabetic animals.

## Material and Methods

### *Preparation of Acellular Human Amniotic Membrane*

Human amniotic membranes were obtained from healthy women undergoing a cesarean section with their consent. The amnion was separated from the chorion and subsequently frozen at  $-80^{\circ}\text{C}$  for 48 h and then thawed prior to decellularization. To prepare the acellular human amniotic

membrane, the amnion was thawed then rinsed 3 times with PBS (Gibco, USA) and was then put in 0.03% (w/v) solution of sodium dodecyl sulphate (SDS) (Merck, Germany) in tris-buffered saline (TBS) (Sigma, USA) and saken at room temperature for 24 hours. In the next step, the amnion was washed in PBS. Decellularized amnion was characterized by electron microscopy.

#### *Animal Experiments*

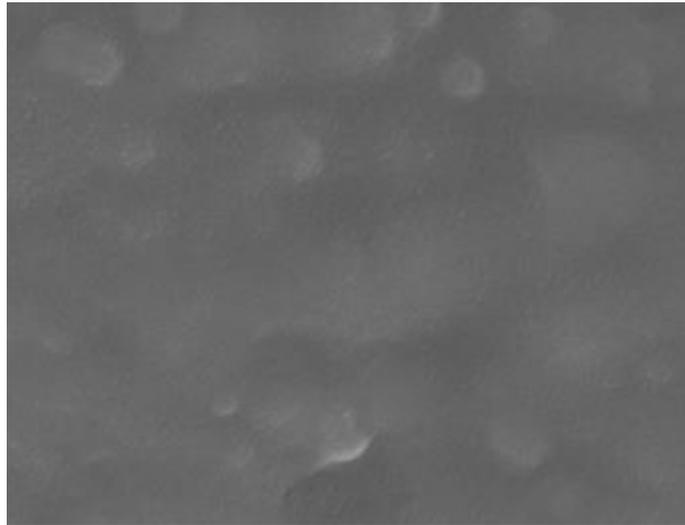
Rats were divide into control (without treatment) and experiment (treatment with acellular amniotic membrane) groups (n=5 in each group). Diabetes was induced in animals by intraperitoneal injection of streptozotocin (150 mg/kg), and 6 mm diameter wounds were created on dorsal skin of Wistar rats. Acellular human amniotic membrane 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  $\pm$  SE. Analysis of variance was used to analyze the data. The difference between groups was significant when  $p < 0.05$ .

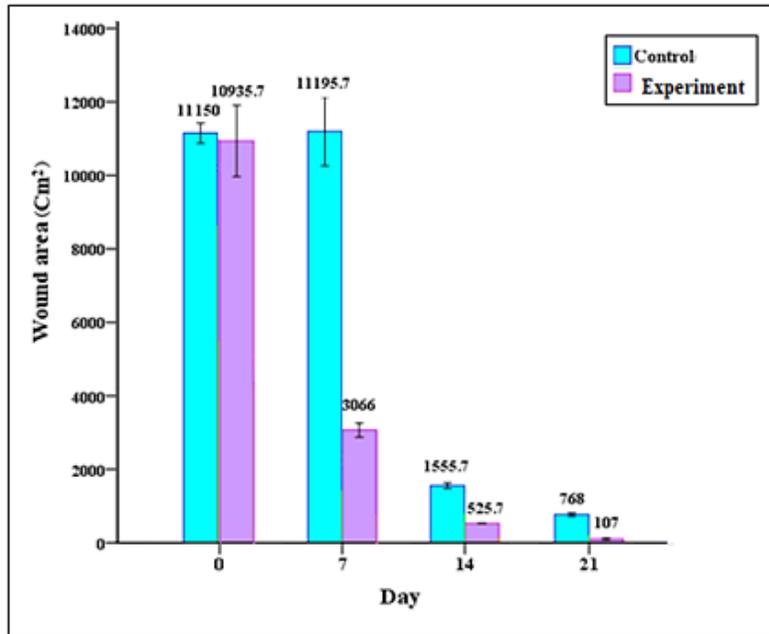
## **Results**

Electron microscopy images confirmed the process of decellularization (Figure 1):



**Figure 1.** Electron microscopy image of acellular amniotic membrane.

Significant decrease in wound area was observed 7 ( $p < 0.05$ ), 14 ( $p < 0.01$ ) and 21 ( $p < 0.01$ ) days after treatment in experiment rats compared with control animals. However, wound area significantly decreased in control group 14 and 21 days after treatment compared to the day 7 ( $p < 0.01$  and  $p < 0.001$ , respectively). There was also significant decrease in wound area in experiment group 14 days after treatment compared to 7 days after treatment ( $p < 0.01$ ) and 21 days after treatment compared with 14 days after treatment ( $p < 0.01$ ).



**Figure 2.** Wound area at the day 0 and 7, 14 and 21 days after treatment in the control rats and animals treated with acellular amniotic membrane.

## Discussion

Many studies have shown that amniotic tissue is of significant importance in the field of treating skin wounds. The results of this study have shown that the decellularized amniotic membrane can heal diabetic skin wounds. In line with this finding, other studies have also shown that amniotic derivatives can have positive effects in the healing process of diabetic wounds, and in this regard, the results of the study on the potential of *in vitro* healing effect of the human placenta for regeneration of damaged tissues [11], [12], [13]. As we know, wound healing requires the interaction of cells, growth factors and extracellular matrix proteins [14], [15], [16]. Various studies show the positive and significant therapeutic effect of placental tissue not only on diabetic wounds but also on the healing of wounds as a biological dressing [17] including burn injuries [18], [19] and human corneal wounds [20], [21], [22]. What is clear from the studies is that the placental membrane has anti-inflammatory, antimicrobial and angiogenic properties, which support the natural process of wound healing [23], [24], [25]. Since the placenta is rich in materials and factors necessary for the growth and development of tissues [6], [26] it can play a significant role in wound healing process.

## Conclusion

Acellular human amniotic membrane showed a great potential in promoting skin wound healing in diabetic animals, providing strong evidence for the possible future clinical applications in wound healing in diabetic human subjects.

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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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