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Cell-based skin substitutes accelerate regeneration of extensive burn wounds in rats

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ABSTRACT

Background: This study investigated the effects of amniotic membrane combined with adipose-derived stem cells or fetal fibroblasts on regenerating extensive burns in rats.
Methods: Third degree burns of 1100–1800 mm^2 were induced on 32 Sprague-Dawley rats. Burned sites were excised and randomly covered with Vaseline gauze (control), human amniotic membrane (HAM), human fetal fibroblasts seeded on HAM (HAM-FF), or human adipose-derived stem cells seeded on HAM (HAM-ASC), and followed by wound closure and histological assessments.
Results: Wound closure rates of HAM-FF, HAM-ASC, HAM and control groups at seven and 14 days after the treatment were 42.2% and 81.9%, 41.9% and 81.7%, 33.5% and 74.2%, and 16.5% and 69.7%, respectively. Wounds of HAM-FF, HAM-ASC, HAM and control groups were closed on 40, 40, 50 and 60 days after the treatment, respectively ($P < 0.05$). Histological assessments revealed lower inflammatory cell infiltration in HAM-ASC and HAM-FF groups.
Conclusions: Cell-based engineered skin substitutes seem to accelerate wound regeneration, especially within the first 14 days.

1. Introduction

Understanding the pathophysiology of burn wound healing has expanded in recent years, leading to new treatment modalities.[1] Normal wound healing is a chain of dynamic and harmonized cellular and molecular events.[2,3] Burn severity defines the amount of local and systemic responses to the injury.[4] Severe burns are usually accompanied by intense dehydration, inflammatory cytokines' high secretion, micro-organisms' colonization, loss of vascularization and extensive cell destruction that interrupts the healing process.[5,6]

Currently, early excision of necrotic tissue and then instant autografting is the standard management of severe burns. However, the donor tissue limitation harms the treatment success in extensive burns.[7,8] In the past decades, several covering materials have been applied such as allogeneic substitutes, synthetic membranes and permanent materials including cultured epidermal or dermal cells.[5]

Recently, application of stem cells and tissue engineering has become a novel therapeutic modality for regenerating damaged skin. It has been demonstrated that many growth factors and cytokines accelerate wound regeneration, while stem cells are good

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Abbreviations

ASC	adipose-derived stem cell
bFGF	basic fibroblast growth factor
EGF	epidermal growth factor
GM-CSF	granulocyte-macrophage colony-stimulating factor
HGF	hepatocyte growth factor
KGF	keratinocyte growth factor
TGF-β	transforming growth factor beta
VEGF	vascular endothelial growth factor
HAM	human amniotic membrane
HAM-ASC	human adipose-derived stem cell seeded on human amniotic membrane
HAM-FF	human fetal fibroblasts seeded on human amniotic membrane

sources.[9] In addition to direct differentiation of stem cells to endothelial and epithelial cells, the stem cells have paracrine effects through secretion of soluble growth factors and cytokines such as basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), keratinocyte growth factor (KGF), hepatocyte growth factor (HGF) and transforming growth factor beta (TGF-β).[1,5,8,10]

The stem cells research main focus in wound healing has been on bone-marrow derived mesenchymal stem cells. However, adipose-derived stem cells (ASCs) have recently come to spotlight because of higher proliferation, hematopoiesis and wound regeneration with minimal harvesting risk.[1,11,12] Besides, the anti-inflammatory and anti-fibrotic characteristics of fetal fibroblasts have encouraged the investigators to apply them on difficult-to-heal wounds.[13,14] Concurrent application of bioscafolds and stem cells has also been studied recently. They are probably associated with more host cell infiltration and implanted cell engraftment.[5,15,16]

Amniotic membrane, the innermost layer of fetal membrane, is the first biomaterial scaffold that has been widely used in wounds since 1910. Several studies have demonstrated the effectiveness of amniotic membrane on wound healing due to its biological and mechanical characteristics. There is evidence that amniotic membrane provides a proper environment for cell proliferation and migration, granulation tissue formation and extracellular matrix remodeling, thanks to secreting high levels of epidermal growth factor (EGF), KGF, bFGF, TGF-β, VEGF, angiogenin, and IL-8.[15]

In spite of promising results of applying stem cells in burn wound healing, few studies have investigated their effects on large and deep burns. This study has compared the effects of amniotic membrane combined with ASCs or fetal fibroblasts on regenerating extensive burns in rats.

2. Materials and methods

This study was done between July 2014 and March 2015 and was approved by the ethics committee of Shahid Beheshti University of Medical Sciences in Tehran. All the rats received humane care according to the instructions of Institute of Laboratory Animal Resources.

2.1. Surgical procedure and animal model

A number of 32 male Sprague-Dawley rats (280–320 g) were selected from a hospital animal lab in Tehran. They were trained by a single investigator before being recruited for the study to reduce stress and mortality. The rats were kept in sterile and separate cages during the study. From six days before the surgery, the rats received 10 mg/kg diluted Cyclosporine subcutaneously and then 20 mg/kg the day before it until 21 days after the surgery. 5 mg/kg Ciprofloxacin was injected subcutaneously before and after the surgery. General anesthesia was done by Halothane loading dosage of 1.5–2% and maintenance dosage of 0.8–1%. Then the procedure started.

First, the hair on the dorsal skin was cut and a 3rd degree burn injury was induced by prepared bar of a standard comb model that was originally described by Regas and Ehrlich.[17] The bar was kept in boiling water for 5 min and then laid on the dorsal site for 30 s. The bar size was 20 × 55 mm (each column 10 × 20 mm with 5 mm intervals) and around 5–10% of a rat's total body surface area was burned. After the operation, the rats were treated with 5 ml subcutaneous normal saline injections for one week.

The day after burning, burned sites underwent approximately 1100–1800 mm^2 full thickness excision (Fig. 1). The rats were randomized into four groups: Vaseline gauze (control), human amniotic membrane (HAM), human fetal fibroblasts seeded on HAM (HAM-FF), human ASCs seeded on HAM (HAM-ASC). The wounds were covered randomly. We gently put the skin substitutes on the burn wound sites and then sutured them to the wounds' marginal skin. Finally, the skin substitutes were covered by Vaseline gauze. The dressings remain intact until seven days. The Vaseline gauzes were changed every seven days until complete re-epithelialization.

2.2. Cell and HAM preparation

All the biologic dressings were prepared in Royan Institute and were transferred to the hospital animal lab.

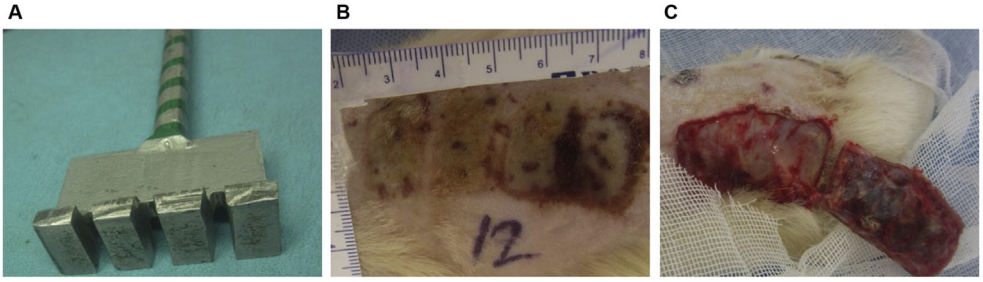

Fig. 1. Animal model. (A) Standard comb model. The bar size was 20 × 55 mm. Each column was 10 × 20 mm with 5 mm intervals. (B) The bar was kept in boiling water for 5 min and then laid on the dorsal site of rats for 30 s. Third degree burns of 1100–1800 mm^2 were induced. (C) Burned sites were excised and randomly covered with Vaseline gauze (control), human amniotic membrane (HAM), human fetal fibroblasts seeded on HAM (HAM-FF), or human adipose-derived stem cells seeded on HAM (HAM-ASC).