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Evaluation of the efficacy of cell and micrograft transplantation for full-thickness wound healing

Carla R. Kruse, MD,[a,b] Dharaniya Sakthivel, MS,[a] Indranil Sinha, MD,[a] Douglas Helm, MD,[a] Jens A. Sørensen, MD, PhD,[b] Elof Eriksson, MD, PhD,[c] and Kristo Nuutila, MSc, PhD[a,*]

[a] Division of Plastic Surgery, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts
[b] Department of Plastic and Reconstructive Surgery, Odense University Hospital, Denmark
[c] Harvard Medical School, Boston, Massachusetts

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ABSTRACT

Background: Skin grafting is the current standard of care in the treatment of full-thickness burns and other wounds. It is sometimes associated with substantial problems, such as poor quality of the healed skin, scarring, and lack of donor-site skin in large burns. To overcome these problems, alternative techniques that could provide larger expansion of a skin graft have been introduced over the years. Particularly, different cell therapies and methods to further expand skin grafts to minimize the need for donor skin have been attempted. The purpose of this study was to objectively evaluate the efficacy of cell and micrograft transplantation in the healing of full-thickness wounds.

Materials and methods: Allogeneic cultured keratinocytes and fibroblasts, separately and together, as well as autologous and allogeneic skin micrografts were transplanted to full-thickness rat wounds, and healing was studied over time. In addition, wound fluid was collected, and the level of various cytokines and growth factors in the wound after transplantation was measured.

Results: Our results showed that both autologous and allogeneic micrografts were efficient treatment modalities for full-thickness wound healing. Allogeneic skin cell transplantation did not result in wound closure, and no viable cells were found in the wound 10 d after transplantation.

Conclusions: Our study demonstrated that allogeneic micrografting is a possible treatment modality for full-thickness wound healing. The allografts stayed viable in the wound and contributed to both re-epithelialization and formation of dermis, whereas allogeneic skin cell transplantation did not result in wound closure.

Introduction

Full-thickness wounds after trauma and large burns pose a major threat to the organism by exposing the body to external pathogens,[1] disrupting thermoregulation, and increasing the water loss.[2,3] Rapid wound closure with optimal skin quality after healing can shorten the length of hospitalization and increase the quality of life of the patient.[4]

Skin grafting is the current standard of care in the treatment of large burns and wounds. It is an efficient treatment

* Corresponding author. Brigham and Women's Hospital, Harvard Medical School, Division of Plastic and Reconstructive Surgery, 75 Francis Street, Boston, MA 02115. Tel.: +001 617 732 4836; fax: +1 6177326387.
E-mail address: knuutila@bwh.harvard.edu (K. Nuutila).
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technique, but it is associated with substantial problems, such as poor quality of the healed skin, scarring, and lack of donor sites in large burns.[5,6] To overcome limited donor-site availability and increase the expansion ratio of the graft, the split-thickness skin grafts (STSGs) are generally meshed and expanded with a ratio ranging from 1:2 to 1:6.[7] Meshing also improves wound drainage and the malleability of the graft. However, it compromises both the esthetic and functional outcomes. Furthermore, in the case of a very large burn, the same donor sites are used multiple times, increasing the risk of infection and donor-site scar.[8]

Therefore, multiple attempts have been made to overcome the limitations of the STSG technique. Various cell therapies have been introduced since Rheinwald and Green (1975) isolated and cultured patients' own keratinocytes *in vitro*,[9] developing cultured epithelial autografts (CEA) which have been used to treat large burns with varying success.[10] In addition, techniques where noncultured skin cells are sprayed on the burn wound have been used.[11] The most recognized commercial cell spray product is ReCell.[12] These techniques, although promising, are cumbersome and expensive, and their efficacy has not been as good as that with STSG.

Besides cell therapies, other methods to further expand skin grafts and minimize the need for donor skin have been attempted. One of these techniques is micrografting where an STSG is minced to micrografts (0.8 × 0.8 mm) and subsequently transplanted on the injured area with large expansion ratios. Meek described a technique for mincing an STSG into micrografts, allowing a skin expansion of up to 10-fold.[13,14] Despite several modifications, the Meek micrografting technique never gained widespread clinical application, in part, because the micrografts had to be placed with the dermal side down to ensure survival. The orientation requirement was impractical, especially with very small graft fragments. Subsequently, it was discovered that in a moist environment, the orientation of transplanted micrografts does not matter.[15] Using the moist environment, many preclinical studies have been conducted. In a porcine model, Hackl *et al.*[16] showed that the micrografts can provide a 100-fold expansion ratio of a skin graft. In another porcine study, Singh *et al.*[17] demonstrated that micrografts can be minced to even smaller pixel-size grafts (0.3 mm × 0.3 mm) and stay viable and re-epithelialize a full-thickness wound. In addition, Nuutila *et al.*[18] have further shown that also dermis can be minced to micrografts and contribute to wound healing.

The purpose of this study was to objectively evaluate the efficacy of cell therapy and micrografting on wound healing and compare its efficacy to that of current standard of care. In our previous study, we have investigated the efficacy of cultured autologous keratinocytes for full-thickness wound healing and demonstrated that the autologous keratinocyte-transplanted wounds were 27% and 97% re-epithelialized on days 6 and 12, respectively.[19] Therefore, the efficacy of allogeneic cells was investigated in this study. Cultured allogeneic keratinocytes and fibroblasts, alone and together, as well as autologous and allogeneic micrografts were transplanted into full-thickness rat wounds.

Re-epithelialization and regeneration of dermis was observed over time. A novel titanium chamber wound-healing model was used in this study to assure the objective evaluation of the efficacy of the transplanted material on wound healing.[20] In addition, the wound microenvironment after transplantation was studied by measuring the concentrations of multiple growth factors and cytokines from the wound fluid after transplantation.

Materials and methods

Animals

All the animal experiments were performed according to protocol 2016N000386 by the Brigham and Women's Hospital Standing Committee on Animals and conformed to regulations and federal status related to animal use. Female Wistar rats (200-250 g) (Charles River, Wilmington, MA) were used in this study. Wistar rat line was used in the study because it is an outbred line. Rats in the outbred lines have interindividual genetic variation meaning that each animal is genetically different.[21] For all the procedures, animals were anesthetized by delivering 1%-2% isoflurane (Piramal, Andhra Pradesh, India) via snout mask throughout the surgery. After the procedure, 0.005 mg/kg of buprenorphine was administered subcutaneously for pain control during the first 24 h.

Titanium wound chamber model

The wounds were created as described in our previous communication.[20] Briefly, four circular concentric punch wounds, 10 mm in diameter, were created on the dorsum of each rat, and customized titanium wound chambers, with a squared base, were implanted transdermally (Harvard School of Engineering, Cambridge, MA). The flange of each chamber was placed under the skin edge of the 10-mm circular incision (Fig. 1A), completely separating the interior space of the chamber from the surrounding wound edges. To secure the chamber to the underlying muscle fascia, two 3-0 prolene sutures (Ethicon Inc, Somerville, NJ) were placed in diametrically opposite holes in the base of the chamber. In addition, to create a watertight seal between the chamber and surrounding skin edge, a purse-string suture was placed in the skin surrounding the circular rim of the chamber using 4-0 Vicryl (Ethicon Inc). After the full procedure, a custom-made titanium screw lid was used to seal the top of the chamber (Fig. 1A).

Experimental design

In total, 24 rats (4/treatment group) with four wounds each were used in this study. The animals were divided into six treatment groups: (1) STSG, (2) autologous micrograft (1:4 expansion ratio), (3) allogeneic micrograft (1:4), (4) allogeneic cultured keratinocytes (500,000 cells), (5) allogeneic cultured fibroblast (500,000 cells), and (6) allogeneic cultured keratinocytes (250,000 cells) and fibroblasts together (250,000 cells). Autologous cultured keratinocytes had been used in a previous study which provided the autologous cell control.[19] The number of cells (500,000) was chosen for transplantation because it was calculated that it corresponds to the number of cells that can be isolated from the 1:4 transplanted