In Situ Bioprinting of the Skin for Burns

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Introduction

Burn injury is a common source of morbidity and mortality in the battlefield, comprising 10 to 30% of all casualties[1]. Most battlefield burns are massive injuries and require grafts for coverage and repair, since any loss of full-thickness skin of more than 4 cm in diameter will not heal by itself[2]. Unfortunately, current treatment options are unable to fully address the specific needs. Autografts and commercially available skin products are limited in size and some require a lengthy preparation time, making them unusable in severe cases that require prompt and aggressive measures to maintain the lives of wounded soldiers. Moreover, prompt management of burn wounds is pivotal to functional recovery and good cosmetic outcomes. Therefore, a new approach that permits immediate burn wound stabilization with functional recovery is necessary.

To address the present limitations, we propose a novel delivery system that would allow for on-site *in situ* repair of battlefield burn injuries using tissue-engineered skin grafts produced with a portable skin printing system. The ultimate goal of this project is to promptly respond to and manage burns on-site through delivery of allogeneic human skin products. A proposed portable skin printer is depicted in Figure 1.

To achieve this goal, the feasibility of printing different skin cells directly on a skin wound needs to be verified first. In this study, two different skin cells, human keratinocytes and fibroblasts were loaded into the skin printer we have developed previously and printed onto a athymic skin wound model.

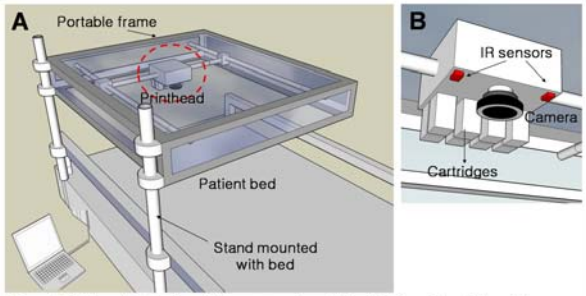

Figure 1. Schematic drawing of the proposed portable skin bio-printer (A) and close-up of the printhead (B).

Methods

In this study, a computer-controlled XYZ plotter and cell deposition system were used. The XYZ plotter is driven by step motors, and the printhead is equipped with a DC solenoid inkjet valve. A reservoir loaded with cell print suspension is connected to the inkjet valve. The printhead is mounted over a XYZ plotter platform to allow deposition of cells onto the wound area. Positioning the XYZ plotter with the printhead is controlled via a customized controller. The controller acquires the positioning information from software loaded on a computer. This software converts the image of the target to a special four byte protocol, which is used to activate specific inkjet valves and coordinate X-Y-Z position.

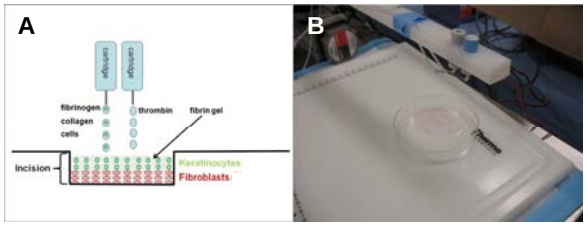

Figure 2. Diagram of the layout of primary experiment (A) and picture of current printer set-up (B).

As seen in Figure 2A, one valve ejects cells mixed with fibrinogen and type I collagen, and the other ejects thrombin. Fibrinogen reacts with thrombin to immediately form fibrin, a protein involved in the clotting of blood. A layer of fibroblasts is printed followed by a layer of keratinocytes. Figure 2B shows the current printer setup, which is equivalent to the diagram in Figure 2A.

The full thickness of skin was removed from the dorsa of female Nu/nu mice (L x W, 3 cm x 2.5 cm). Three experimental groups (cell printed, collagen and fibrin, and untreated) were examined at 1,2, and 3 weeks post surgery. Histology was performed at 3 weeks post surgery to examine the microscopic structure of the wound and scar.

Results

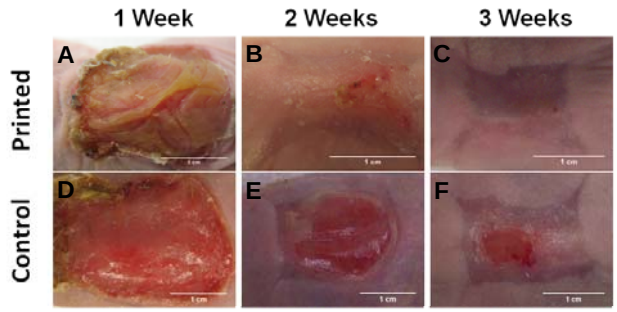

Figure 3. Gross examination of printed skin. Skin defect (L x W: 3 x 2.5 cm) was created on the dorsa of nude mice. (A) Cell printed mouse at 1 week shows membrane formation over the wound. (B) Cell printed mouse at 2 weeks shows scar formation and has a much smaller open wound than control mice. (C) Cell printed mouse at 3 weeks shows complete coverage of the wound by scar tissue. (D) Untreated mouse at 1 week shows an open wound. (E) Untreated mouse at 2 weeks shows scar formation. (F) Untreated mouse at 3 weeks shows scar formation and open wound.

Gross evaluation of the skin defect over the course of three weeks showed a striking difference in the ability of the three different treatments to cover the wounded area (Figure 3). At one week post-surgery the cell-treated mouse showed a membrane covering the entire wound area (Fig. 3A) which progressed to a scar at two 2 weeks (Fig. 3B). The untreated controls did not show coverage of the wound at one week and while they did form scars at two weeks, their open wounds were 400% larger than the printed skin.

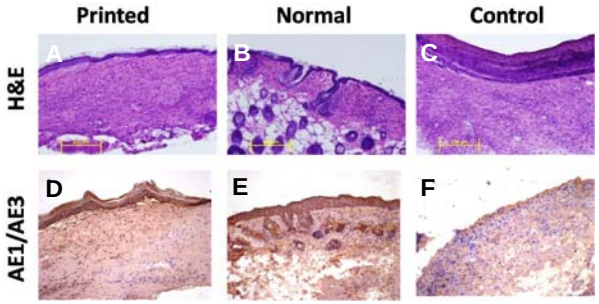

Figure 4. Assessment of bioprinted skin. (A) H&E stain of printed skin shows organization of dermal and epidermal tissue of similar makeup and thickness to normal skin. (B) Normal skin shown on H&E. (C) H&E stain of untreated control shows scarring and inflammatory tissue. (D) AE1/AE3 immunohistochemistry for cytokeratin demonstrates formation of epidermal tissue of similar composition to normal skin. (E) AE1/AE3 stain of normal skin. (F) AE1/AE3 stain of untreated control shows no epidermal regeneration. Magnification: 100x

At three weeks the untreated and gel-treated controls had scars but still showed an open wound (Fig. 3F) while the printed skin showed complete coverage of the wound area with a scar (Fig. 3C). The mice also showed differences in their ability to cope with the wound. Untreated mice were lethargic and began losing weight immediately. In contrast, the cell-printed mice were robust after recovering from the initial surgery.

Microscopic evaluation of the skin defect at three weeks post-surgery showed interesting characteristics of the skin printing (Fig. 4). Untreated mice show inflammation and little discernable restoration of normal skin structure (Fig. 4C, F). The printed skin is seen to organize into structures similar to that of normal skin (Fig. 4A, D). Fibroblasts take on a dermal structure near identical to the dermal structure of normal murine skin. AE1/AE3 immunohistochemistry for cytokeratin demonstrates epidermis in the printed construct and the thickness of the printed epidermis is similar to the thickness of the normal epidermis. The printed cells were human and formed skin similar to murine skin. These results suggest that murine cells at the edges of the wound are responsible for organization of the printed skin into a murine structure. Further studies will characterize this formation of skin with additional stains including type III collagen, type I collagen, human and murine cell proliferation, and cell survival.

Conclusion and Future Plans

This study shows that two different skin cells could be directly delivered onto a wound in a nude mouse model. The printed cells could survive through the *in situ* printing and *in vivo* implantation. Moreover, the wound printed with the skin cells exhibits fast recovery.

The mouse *in vivo* study is ongoing and will include 1, 3, and 6 week time points, evaluating scar formation as well as remodeling of scar tissue. This work serves as the basis for further studies in large animals.

References

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(2) MacNeil S. Progress and opportunities for tissue-engineered skin. Nature 2007;445:874-880.