SKIN PRINTER: MICROFLUIDIC APPROACH FOR SKIN REGENERATION AND WOUND DRESSINGS

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ABSTRACT

We developed a microfluidics-based approach for the in-flow formation and *in vivo* application of cell-populated wound dressings, with the ability to reproduce key features of human skin. Human fibroblasts are incorporated within a continuously extruded biopolymer sheet with precise spatio-temporal control over the cell location and cell seeding density. The platform enables the continuous formation of cell-populated single-layer, bilayer, and vascularized sheets. The resulting wound dressings were implanted into murine wound models and the improved wound healing and keratinization were observed.

KEYWORDS: 3D, scaffold, skin, microfluidics

INTRODUCTION

Skin is an important organ that forms a protective barrier against the external environment. Once broken, the process of wound healing is immediately set in motion [1]. However, in conditions associated with severe skin loss (up to $1m^2$ in severe burn), normal wound healing cannot reconstitute the barrier, leading to high mortality (fig.1a). To mitigate this, different wound dressings are routinely employed in surgical practice. However (a) more cost-effective dressings that offer shorter recovery times and (b) dressings that better resemble important physiological features of skin with minimal morbidities are needed. Although current skin grafts are cell-free, minimizing rejection, they require long recovery times and are costly (over 50,000$ to cover 40% burn area on an average male [1]). Moreover, they are fragile and lack in reproducing important physiological characteristics such as the layered architecture of skin, vascularization, and the presence of sweat glands and melanocytes (fig.1b).

A number of microfluidic approaches have been suggested for drug screening in microscale organ-mimetic platforms [2], as well as for the continuous formation, assembly and *in vivo* application of cell populated microfibers [3]. Function of skin cells and wound-healing behavior have also been studied using microfluidic platforms [4], and bioprinting efforts are beginning to make their way towards the repair of burn wounds and cartilages [5].

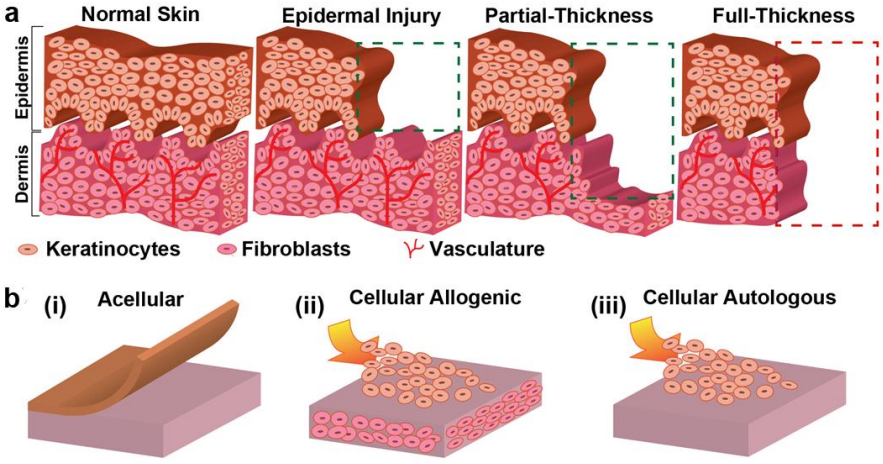

Figure 1: Schematic illustration of (a) pathophysiological characteristics of human skin as related to three levels of burns. (b) Clinically employed skin substitutes[1]: (i) acellular dermis requiring thin split skin graft from patient,(ii) allogenic fibroblasts cultured for 3 weeks followed by seeding of allogenic keratinocytes on surface, (iii) autologous keratinocytes obtained from patient biopsy and cultured on support dressing to allow for handling.

EXPERIMENTAL

The skin printer consists of a microfabricated cartridge that enables the continuous formation of wound dressings from mosaic hydrogel sheets, with the controlled incorporation of viable human fibroblasts, as well as the ability to define multilayered and vascularized (i.e.perfusable) hydrogel sheets (fig.2) [6].

In order to print cell-populated sheets with materials promoting both cell viability and proliferation while easy to handle, two materials are used: (1) providing stiffness to the sheet, (2) for cell printing.

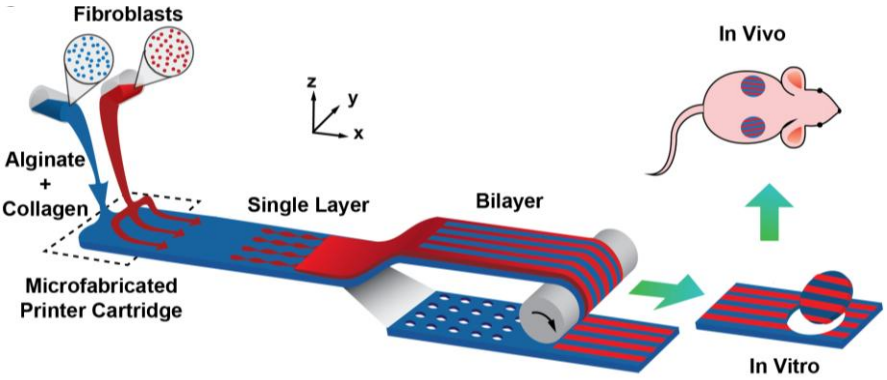

Figure 2: Illustration of proposed skin printer for formation of cell-populated skin substitute in a microfabricated printer cartridge and application in vivo.

RESULTS AND DISCUSSION

Precise control over the combination of these two materials is critical, as the printed patterns size and pitch will directly impact on the sheet stiffness. Different patterns were produced by varying the valve actuation time and inlet gas pressure (fig.3a-c). Pattern in fig.3a was repeated on a bilayer system to illustrate the ability to create a structure that would enable the co-localization of keratinocytes (top) and fibroblasts (bottom) (fig.3d). Blue (main sheet), green (top patterns), and red (bottom patterns) fluorescent microbeads (0.1µm diameter) were loaded into the solutions at a density of 1%v.v. to distinguish the different layers and patterns.

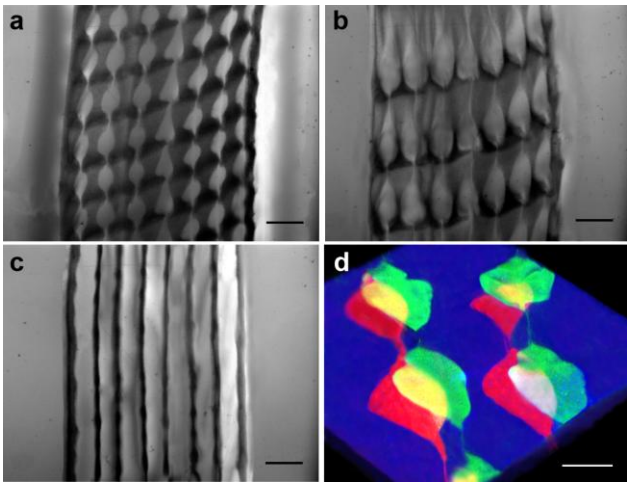

Figure 3: (a-c) Phase contrast images of patterned sheets illustrating a variety of printed features. Obtained with following valve actuation and gas pressure: (a) 50ms open – 500ms closed, 1.5psi, (b) 100ms open – 500ms closed, 1.5psi, (c) fully open, 2psi. Distinction between the patterning solution and base material solution was achieved by incorporating $BaSO_4$ at a concentration of 1%w.t. into the base material. (d) 3D Confocal scan of a bilayer sheet patterned using actuation in (a). Scale bars 1mm (a-c),500µm (d).

These patterns can either be hollow to promote vascularization while providing relevant thicknesses (fig.4), or cell-populated with local control over cell seeding density. As a case study, we printed biopolymer sheets where human fibroblasts were seeded into various shapes such as spots and parallel stripes. The incorporated viable cells were printed at a density of 1.94 million cells/mL and were shown to proliferate and attach *in vitro* (fig.5).

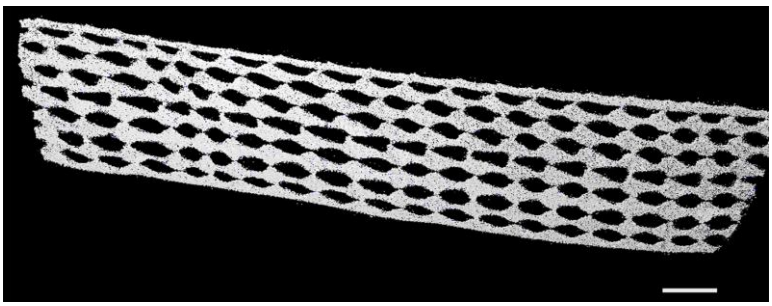

Figure 4: Microcomputed tomographic scan of patterned planar sheet containing a hole array. Contrast obtained by incorporating 1%w.t. $BaSO_4$ into the biopolymer sheet solution. Scale bar 1mm.

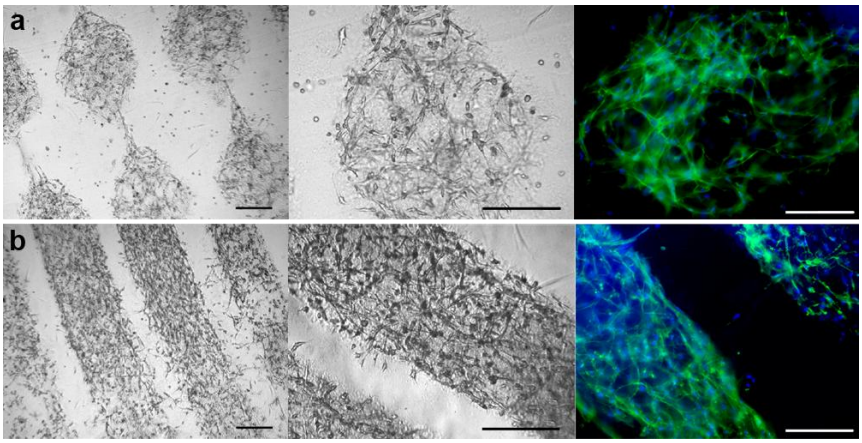

Figure 5: (a, b) ***Printed skin substitute in vitro.*** *Phase contrast and fluorescence images of human fibroblasts-patterned sheet. Day 10. Scale bars 100µm.*

By performing excisional skin biopsy on immunodeficient mice, we replaced excised skin with biopolymer sheets patterned with fibroblasts at a density of 1.94 million cells/mL (fig.6a). Trichrome (fig.6b,c) and keratin 14 (fig.6d,e) staining of wounded skin suggests that our printed biopolymer sheets led to a better wound healing and keratinization, suggesting an improved skin regeneration as compared to our control.

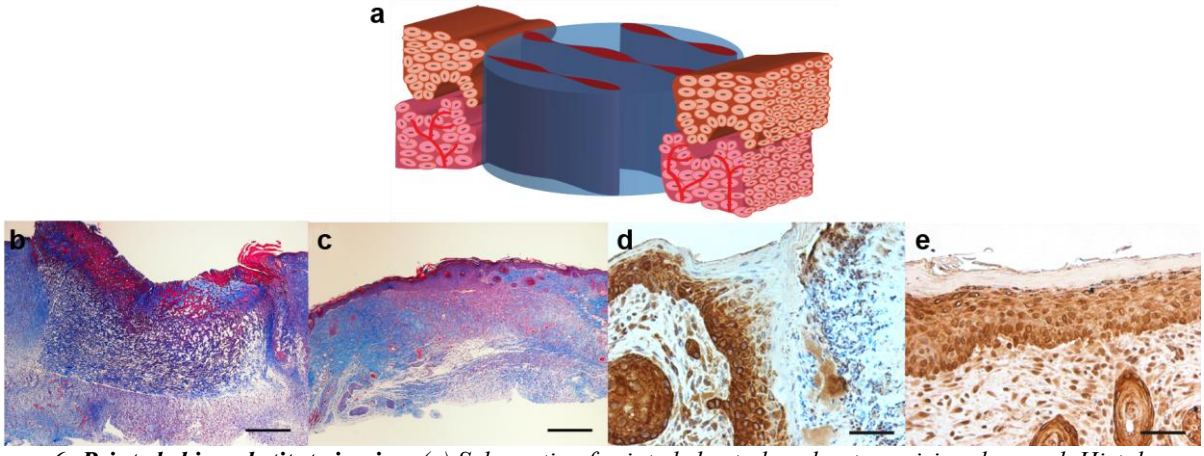

Figure 6: ***Printed skin substitute in vivo.*** *(a) Schematic of printed sheet placed onto excisional wound. Histology sections of (b,d) control 4%w.t. alginate without cells, (c,e) 4%w.t. alginate patterned with human fibroblasts. Trichrome staining (b,c), keratin 14 staining (d,e). Scale bars 1mm (b, c), 100µm (d, e).*

CONCLUSION

The presented technology enables the formation of up to 35mm wide sheets at rates of up to 10mm/s, equivalent to $1m^2$ in 48min. We believe the skin printer provides a scalable approach and uniquely addresses the need to provide skin substitutes at affordable prices that are easy to handle and apply, while potentially reducing wound recovery times along with improving clinical outcomes.

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