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Opinion

Skin Bioprinting: Impending Reality or Fantasy?

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Bioprinting provides a fully automated and advanced platform that facilitates the simultaneous and highly specific deposition of multiple types of skin cells and biomaterials, a process that is lacking in conventional skin tissue-engineering approaches. Here, we provide a realistic, current overview of skin bioprinting, distinguishing facts from myths. We present an in-depth analysis of both current skin bioprinting works and the cellular and matrix components of native human skin. We also highlight current limitations and achievements, followed by design considerations and a future outlook for skin bioprinting. The potential of bioprinting with converging opportunities in biology, material, and computational design will eventually facilitate the fabrication of improved tissue-engineered (TE) skin constructs, making bioprinting skin an impending reality.

Trends

Significant progress has been achieved in TE skin constructs for clinical applications over the past three decades.

Despite its partial clinical success, there are increasing demands from both patients and clinicians to address some of the existing limitations in current TE skin constructs.

The potential outlooks of skin bioprinting are presented, and we suggest that its potential has yet to be realized.

The Tissue Engineering of Skin

TE skin constructs alleviated the problem of limited donor skin and induced a paradigm shift in wound management from using passive wound dressings to using bioactive cell-impregnated skin constructs [1]. A **scaffold**-based (see Glossary) approach [2,3] relies on temporary scaffolds to facilitate cellular attachment and proliferation, followed by cellular secretion of **extracellular matrix (ECM)** to remodel the surrounding environment. However, these commercially available skin constructs are mass produced with fixed dimensions either in a cellular or acellular form to provide off-the-shelf availability. Although conventional TE skin constructs have provided considerable benefits to patients with burns or chronic wounds, the existing bioengineered constructs are still far from ideal in terms of pigmentation, vascularization, missing hair follicles, and so on. Despite the immense challenges of fabricating completely functional skin constructs, the **bioprinting** approach facilitates the highly automated fabrication of complex bioengineered constructs, comprising additional cell types and biomaterials to enhance the homology to native skin and improve functional outcomes.

Bioprinting is an advanced manufacturing platform that enables the predefined deposition of living cells, biomaterials, and growth factors using computer-aided design (CAD) to fabricate customizable constructs via a layer-by-layer printing process with a high degree of flexibility and repeatability (Figure 1) [4]. Bioprinting technology has the potential to directly create graded macroscale architectures to better mimic the natural ECM, thereby augmenting the attachment and proliferation of multiple types of cell concurrently. Moreover, microfeatures, such as ridges and modulated surfaces, can be incorporated using multiple material platforms with various dispensing mechanisms, to provide mechanical and biochemical cues at microscale levels that guide and enhance cellular alignment and differentiation [5]. Hence, bioprinting facilitates concurrent engineering design that spans the micro- and macroscales, thus enabling the fabrication of constructs that can better satisfy the various requirements of a natural niche for skin cells.

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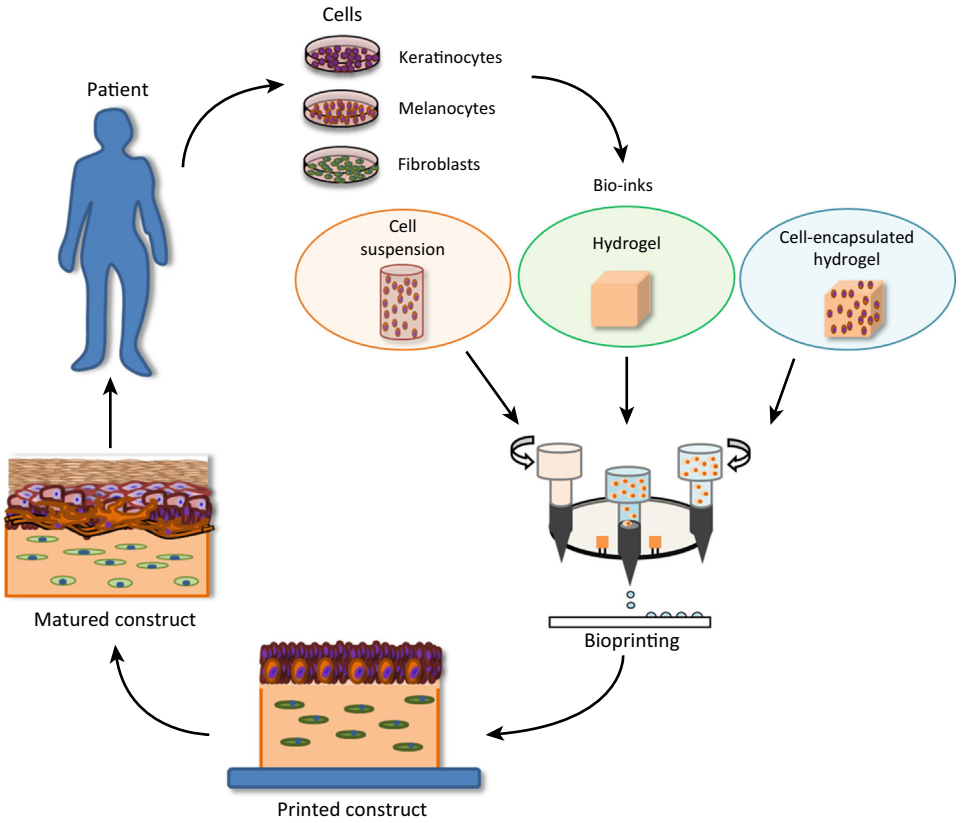

Figure 1. Schematic of Bioprinting Technology. A skin biopsy is collected from the patient and cultured *in vitro* to obtain a sufficient amount of cells. Bio-inks in the form of cell suspensions, hydrogels, or cell-encapsulated hydrogels are used to fabricate bioprinted skin constructs. The printed constructs are cultured under submerged conditions followed by an air–liquid interface to obtain matured skin constructs suitable for tissue transplantation.

Progress in 3D Skin Bioprinting

Various biomaterials have been evaluated for bioprinting and tissue-engineering applications, including polymers [6,7], metals [8–10], hydrogels [11–14], and ceramics [15]. The bioprinting approaches used for the deposition and patterning of biological components are extrusion, inkjet, laser, and microvalve-based systems [16]. To date, laser-based (Box 1) and microvalve-based (Box 2) printing techniques have been used to fabricate multilayered skin constructs and the use of collagen-based **bio-ink** is prevalent in skin bioprinting (Figure 2A) [17–21].

Laser-Based Bioprinting

A recent *in vitro* study demonstrated the deposition of 20 layers of fibroblasts (mouse NIH-3T3) and subsequently 20 layers of keratinocytes (human HaCaT) embedded in collagen gel onto a sheet of Matriderm® (decellularized dermal matrix) (Figure 2B) [17]. Evaluation of the printed skin constructs after 10 days of cultivation showed the presence of **cadherins** and **connexin 43** (Cx43) in the epidermis, which are fundamental for tissue **morphogenesis** and cohesion. Furthermore, bioprinted 3D grafts comprising adipose-derived stem cells expressed adipogenic markers resembling those found in native adipose tissue [22].

In another study, the *in vivo* transplantation of a printed skin construct in the dorsal skin fold chamber of nude mice demonstrated good graft take with the surrounding tissue, and ingrowth of some blood vessels from the wound bed was observed after 11 days of transplantation [18].

Glossary

Angiogenesis: the development of new blood vessels.
Anisotropic: the property of being directionally dependent.
Bio-ink: a printable cell-supporting material.
Bioprinting: the process of generating spatially controlled cell patterns using 3D printing technologies, where cell function and viability are preserved within the printed construct.
Cadherins: a class of transmembrane proteins that are dependent on calcium (Ca^{2+}) ions to form adherens junctions to bind different cells within tissues together.
Connexins: a family of transmembrane proteins that form gap junctions.
Extracellular matrix (ECM): extracellular molecules that are secreted by cells that provide structural and biochemical support to the surrounding cells.
Functionally graded: a functionally graded scaffold fulfills the biological and mechanical requirements of the target tissue
Melanosome: specific organelles produced by melanocytes in which melanin pigment is synthesized and deposited.
Morphogenesis: a biological process that causes an organism to develop its shape.
Neogenesis: regeneration of biological tissues.
Scaffold: a temporary supporting structure that facilitates cellular attachment and proliferation.
Vasculogenesis: the differentiation of precursor cells into endothelial cells to facilitate *de novo* formation of a primitive vascular network.