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A Brief Review on 3D Bioprinted Skin Substitutes

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Abstract

The global escalating cases of skin donor shortage for patients with severe wounds warn the vital need for alternatives to skin allografts. Over the last three decades, research in the skin regeneration area has addressed the unmet need for artificial skin substitutes. 3D bioprinting is a promising innovative technology to accurately fabricate skin constructs based on natural or synthetic bioinks, whether loaded or not loaded with native skin cells (i.e., keratinocytes and fibroblasts) or stem cells in the prescribed 3D hierarchal structure to create artificial multilayer and single cell-laden construct. In this paper, the recent developments in 3D bioprinting for skin regeneration are reviewed to discuss different aspects of skin bioprinted substitutes, including 3D printing technology, bioink composition, and cell-laden constructs. The impact of 3D printing parameters on functionality of the skin substitute and cell viability is reviewed to provide insight into controlled fabrication as the critical component of advanced wound healing. We highlight the recent and ongoing research in skin bioprinting to address the progress in the translation of advanced wound healing from lab to clinic.

Keywords: 3D bioprinting; skin; wound healing; tissue regeneration; bioink

1. Introduction

The skin as the outmost layer of human body, is particularly dealing with traumas and injuries. Wound healing refers to the normal physiologic response to injury which is critical for patient survival. There is a variety of wound healing products available in the market, the majority of which are biosynthetic wound dressings [1]. Despite the good clinical outcomes, there are challenges for treatment of different types of wound. For example, burn wounds as a major wound type are highly dehydrated and require a moist environment. Furthermore, most wound dressings cannot support the scarless wound healing with skin appendages, such as hair follicle, sweat glands and native pigment, which are very important for the normal appearance and functionality of the skin [2-4]. Therefore, research on wound healing has been focusing on developing modern wound dressings to enable scarless healing and to reduce both physiological and psychological inconvenience for patients [5].

Tissue/organ printing, known as 3D bioprinting, enables accurate construction based on biomolecules, synthetic/natural hydrogel and cells. These 3D printed structures have the potential to repair skin and

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accelerate wound healing, as well as skin related pharmacology, toxicology and drug development [6].

The first research on skin bioprinting was published in 2009, where primary human dermal fibroblasts were added to a collagen hydrogel [7]. Currently, skin bioprinting is more focused on selecting high-performance materials to achieve precise printability and faster wound healing simultaneously [8-10]. Besides the biofunctionality and mechanical properties (e.g., viscoelasticity) of bioink, the printing technology plays a crucial role in controlling the macrostructure of the built construct by using engineering paradigms [11].

Comparing to the conventional skin regeneration approaches, 3D bioprinted skin substitutes are taking advantage of the automation and standardization for clinical application and precision in deposition of cells. Additionally, by employing the 3D bioprinted technology, the production time for large size skin substitutes decreases significantly. Cells can be added to the bioink prior to printing, otherwise cell-free bioinks can be printed to develop acellular skin regeneration products. Both of the scaffolds take advantage of the increased surface area after printing, which results in more reactivity and faster healing [12].

According to the literature, different synthetic or natural hydrogels such as alginate, collagen, and cellulose have been investigated in terms of printability, mechanical integrity, and biological response. Physical and chemical properties including glass transition point, viscoelastic behavior, rheology, chemical reactivity, and molecular weight play an important role in the quality of printed construct [10, 12, 13].

In order to promote the skin regeneration, stem cells [14-16] and/or terminally differentiated cells [17-21] have been widely used in different biofabrication methods including 3D bioprinting. Many researchers added keratinocyte, fibroblast, or mesenchymal stem cells to the bioink to address the regenerative medicine approach. The printed cell-laden construct could be either applied on the wound immediately or after maturation. Some research used novel hybrid co-culture systems on a 3D layered structure to mimic the bilayer epidermal/dermal structure of the native skin [18, 19, 21].

The recent developments in 3D bioprinting for skin regeneration are reviewed to discuss different aspects of skin bioprinted substitutes, including 3D printing technology, bioink composition, and cell-laden constructs. The impact of 3D printing parameters on functionality of the skin substitute and cell viability is reviewed to provide insight into controlled fabrication as the critical component of advanced wound healing. We highlight the recent and ongoing research in skin bioprinting to address the progress in the translation of advanced wound healing from lab to clinic.

2. Printing Technology

In the recent years, 2D skin substitutes have been produced based on electrospinning, casting, freeze-drying, and 3D bioprinting. Despite the thin and flat geometry of skin, 3D skin substitutes have become more popular comparing to 2D substitutes, due to the enhanced cell viability and tissue regeneration they can provide [22, 23].

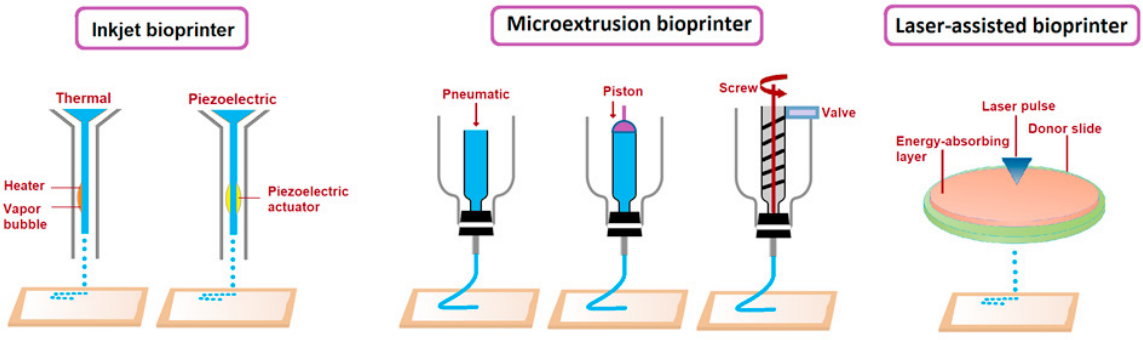

Figure 1- Schematic representation of various techniques of 3D bioprinting. Inkjet bioprinting: Thermal inkjet printers electrically heat the printhead to produce air-pressure pulses that force droplets; while acoustic printers use pulses formed by piezoelectric or ultrasound pressure. Microextrusion bioprinting: These printers use pneumatic or mechanical dispensing systems (piston or screw) to extrude continuous beads of a bioink. Laser-assisted bioprinting: These printers use lasers focused on an absorbing substrate to generate pressures that propel cell-containing materials onto a collector substrate. (Copyright 2020, Figure adapted from ref. [24].)

Table 1- Main skin bioprinting strategies, pros and cons [7-9, 11, 25-27]

Technology	Description	Advantages	Drawbacks
Inkjet bioprinting [15, 21, 28-30]	In these printers the bioink is sprayed by heater or piezoelectric actuator over a biomaterial or culture dish.	– Simple method and customizable – Low cost – High cell viability (80-90%) – High resolution – High speed – High reproducibility	– Low cell density – Limited to bioink viscosity – Risk of exposing cells and reagent to thermal and mechanical stress – Nozzle clogging
Laser-assisted bioprinting [21, 31-34]	These printers use focused laser pulses to create a high-pressure bubble that pushes the bioink into a laser-absorbing layer, where the scaffold is produced layer-by-layer.	– High cell viability (80-90%) – Variety of printable bioinks – Nozzle-free and non-contact	– Low cell density – Complexity – High cost – Low flow rate due to the rapid gelation
Microextrusion bioprinting [17, 20, 21, 35-43]	This printer uses pneumatic, piston or screw forces to dispense bioink through a nozzle that produces continuous flow of material.	– Printability of highly viscous bioinks – Printability of high cell density – Simple method	– Low cell viability – Low speed – Low resolution

Bioprinting refers to the 3D simultaneous deposition of cells and a bioink using a computer-controlled printer, to produce functional tissue equivalents. Currently, bioprinting has shown promising results in skin regeneration and wound healing. The main advantages of skin bioprinting compared to the traditional cell therapy systems, are reproducibility and technical flexibility, besides enabling production of patient-specific constructs. The applications of skin bioprinting are limited since there are few printable polymers and solvents [26, 44].

The three main bioprinting strategies for skin tissue engineering are inkjet-based, laser-assisted, and microextrusion-based. The printed constructs can be in the form of cell suspensions, cell-encapsulated hydrogels, or cell-free constructs [11, 22].

3. Bioink Composition

Regardless of the bioprinting technology, the functionality of the bioprinted skin substitute is highly dependent on the bioink composition and cell type, in terms of rheology, mechanical integrity, biocompatibility, biodegradation, and antimicrobial activity.

Hydrogels or 3D networks of hydrophilic polymer chains have the ability to absorb water up to ten times of their initial weight. Hydrogels show promising results on wound healing due to providing a moist environment and a cell carrier. According to the source of production, hydrogels used for skin bioprinting can be classified into two main groups, namely natural polymers (e.g., collagen, gelatin, alginate) and synthetic polymers (e.g., PCL (polycaprolactone), PLA (polylactic acid), ABS (acrylonitrile butadiene styrene)). Despite the good wound healing efficiency, natural hydrogels have poor printability with significantly longer recovery time [10, 34]. An ideal hydrogel for printing requires to stay in liquid form during printing and to become solid after printing to maintain the desired geometry in a repeatable manner [16]. Table 2 presents a list of hydrogels for skin bioprinting as the results of recent research on different bioprinted skin substitutes. In order to provide stable constructs through predefined shapes, 3D bioprinting technology requires crosslinking or rapid gelation.

In order to mimic the bilayer structure of the native skin, many researchers have conducted multiple-nozzle printing to take advantage of layered structure of bioprinting technology. One of the most common hydrogel combination for keratinocyte/fibroblast co-culture is an alginate/gelatin double-nozzle printing system. Despite the good printability of alginate, its application is limited by low cell viability [45]. Hence, many researchers combined gelatin with alginate to increase cellular response [15, 16, 38, 43].

Table 2 – Typical hydrogels for skin bioprinting

Hydrogel	Cell	Advantages	Drawbacks	Crosslinking
Collagen [17, 29, 30, 36, 38]	– Keratinocyte – Fibroblast – Neonatal Fibroblast –	– High porosity – Enhance cell attachment and proliferation – Absorbability – Low immunogenicity	– Poor solubility – Poor mechanical properties – Slow gelation time – Fibrotic tissue formation – Easily clog the nozzle	N/A
Gelatin and Gelatin-derived polymers, e.g. GelMa [15, 16, 21, 35-38, 41, 43]	– Keratinocyte – Fibroblast – Mammary – progenitor cells – Neonatal Fibroblast – Human amniotic epithelial cells	– Low cost – Biodegradability – Low antigenicity – Reversible	– Poor mechanical strength – Unstable to heat – Modification required	UV exposure (for GelMa) Mushroom tyrosinase (enzymatic cross-linking)
Alginate [15-18, 23, 38, 43]	– Keratinocyte – Fibroblast – Mammary – progenitor cells	– Easy and fast gelation – Low cost	– Low mechanical strength – Poor cell attachment	$CaCl_2$
Chitosan [19, 28, 41]	– Keratinocyte – Fibroblast	– Mild gelation conditions – Antibacterial	– Poor solubility – Weak mechanical strength – Slow gelation	Polyethylene glycol (PEG)
Silk [28, 37]	– Fibroblast	– High mechanical strength – Feasible structural modification – Controllable degradation – Low immunogenicity	– Poor solubility – Need to mixed with other polymers for optimal rheology and printability – Brittle – Easily clog the nozzle	N/A
Hyaluronic acid [17, 21, 42]	– Keratinocyte – Fibroblast	– Excellent moisture retention – Promotes proliferation –	– Viscous gel – Slow gelation rate, – Rapid degradation	Thiol (DTP) UV light (365 nm) for 30 s.
Poly urethane [21]	– Keratinocyte – Fibroblast	– Flexibility – Low antigenicity – Increases the printability of other polymers	– Low fluid controllability – Need to be mixed with other hydrogel – Unstable to heat	Thrombin

Koch et al. carried out the first research on bioprinted skin substitutes using a collagen bioink mixed with keratinocytes and fibroblasts separately and developed a bilayer construct based on collagen with keratinocyte and fibroblast on the surface layer and lower layers, respectively [7]. Further endeavors on developing multilayer skin constructs have utilized multiple nozzles to deposit the cell-laden layers more precisely [17-19, 21, 31]. Some researchers have equipped stem cell-laden bioinks to take advantage of the differentiation potential [46-48]. As shown in Table 2, bioprinted skin constructs could be divided into three groups in term of the nature of loaded cells, namely primary cell-laden constructs (e.g., keratinocyte, fibroblast, and skin appendages), stem cell-laden constructs (bone marrow derived, adipose derived, and umbilical stem cell), and cell-free constructs [17, 19, 38]. Figure 2 (A, B) shows the printability of cell-laden 3D bioprinted constructs based on alginate and gelatin. To investigate the cell viability of the 3D construct, the live/dead assay has been used in most research, as is exampled in Figure 2 (C) [3, 16, 23, 30, 34, 38, 41, 44].

To address the main challenges of skin regeneration, such as poor vascularization, lack of hair follicles and other skin appendages, some researchers focused on regenerating skin appendages, such as hair follicles, sweat glands, and melanocytes [15, 49-51]. Terminally differentiated cells need to be insulated and harvested prior to printing to mimic the natural niche

of skin. Moreover, in order to regenerate more functional and vascularized skin constructs, either biomolecules (e.g., growth factors, proteins, and nanoparticles), or physical signaling factors (e.g., mechanical/electrical stimuli, hollow microchannels and branched microstructure) have been focused in the ongoing research [4, 52, 53].

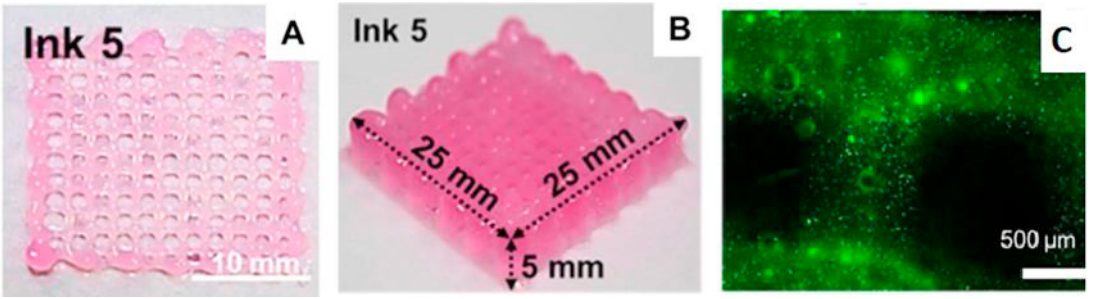

Figure 2. Printability of bioinks based on alginate and gelatin, Lattices printed with bioinks consisting of a 1:2 ratio of low to high molecular weight alginate in (A, B). Cell viability within these lattices after 7 days of culture, scale bar 500 µm (C). Copyright 2019, Reproduced with permission from Williams et al. [54]

4. Conclusion

The promising results in skin bioprinting 3D have shown that not only adding keratinocytes, fibroblasts and stem cells directly into different bioinks such as gelatin, alginate, and chitosan is possible, but also printing reproducible skin constructs with mechanical integrity and functional regeneration of skin tissue with minimal damage to printed cells leads to enhanced wound healing. Moreover, by controlling the printing parameters including temperature, extrusion rate, and geometry, as well as bioink chemical and physical properties (e.g., viscosity, cell density, and crosslinking), the functionality of the bioprinted construct has been optimized in terms of cell viability, wound healing and vascularization. Although the main application of skin bioprinting technology is wound healing, it has also been used in diagnostic applications such as toxicity, pharmaceutical, and cosmetic testing. However, reconstructive surgery and scarless wound healing, as the main clinical outcomes of skin bioprinting technology, are very promising research avenues to facilitate clinical translation.

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