3D-Bioprinting for Chronic Wound

Subjects: Dermatology | Medicine, Research & Experimental

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Skin substitutes can provide a temporary or permanent treatment option for chronic wounds. The selection of skin substitutes depends on several factors, including the type of wound and its severity. Full-thickness skin grafts (SGs) require a well-vascularised bed and sometimes will lead to contraction and scarring formation. Besides, donor sites for full-thickness skin grafts are very limited if the wound area is big, and it has been proven to have the lowest survival rate compared to thick- and thin-split thickness. Tissue engineering technology has introduced new advanced strategies since the last decades to fabricate the composite scaffold via the 3D-bioprinting approach as a tissue replacement strategy. Considering the current global donor shortage for autologous split-thickness skin graft (ASSG), skin 3D-bioprinting has emerged as a potential alternative to replace the ASSG treatment. The three-dimensional (3D)-bioprinting technique yields scaffold fabrication with the combination of biomaterials and cells to form bioinks.

3D-bioprinting cellular activity precision medicine bioinks wound healing biomaterials

1. Introduction

The skin substitution approach has been extensively accepted for clinical use to enhance wound closure and promote normal skin function [1]. Dry wound dressing, including gauze and bandages, are widely used in the early stage of wound healing [2]. The goal of wound dressings is to promote wound closure, enhance new tissue formation, and reduce scar formation. Clinically, the autologous split-thickness skin graft (ASSG) remains a gold standard for extensive wound treatments. It involves taking a specific thickness of healthy skin from other patients and reapplying the ASSG onto the injury site [1]. However, patients with severe burns may not receive adequate skin grafts and are at a greater risk of acquiring infections, including hepatitis B or C [3]. Besides, another traditional approach for chronic wound therapy is via fish skin acellular treatment [4]. This method is considered as one of the significant treatments due to its histological properties that promote cellular regulation and is rich with omega-3 fatty acids to supply to the local tissue [5][6].

1.1. Wound Healing

Wound healing is a dynamic and complex process that initiates the immune response for tissue repair [5]. Several types of wounds, including vascular ulcers, pressure ulcers, and diabetic ulcers, are primarily categorized as chronic wounds [6]. The abnormal pathological conditions of chronic wounds lead to a poor healing rate or excessive scar formation after recovery. Generally, the chronic wound is the most critical challenge related to skin

problems. The wound healing phases start immediately after wound formation, followed by the inflammatory phase begins after the hemostasis phase is completed [7]. The hemostasis phase involves the activation of the enzyme precursors, which results in platelet aggregation at the wound site. Thus, the production of a fibrin clot (fibronectin and factor XIII) will be activated to prevent excessive blood loss [8][9]. Besides, the secretion of extracellular proteins, including plasma fibrinogen and fibronectin, promotes wound closure by accelerating cell migration, proliferation, and function [10]. Overlapping the hemostasis process, the inflammation phase helps to recruit the inflammatory cells to the wound area. In this cascade, the inflammatory cells will eliminate pathogens from the wound site and prevent severe complications. Within two to ten days of post-injury, the proliferation phase will take place, where new tissue formation begins with cell proliferation and migration of keratinocytes towards the lesion [11]. Finally, the tissue remodeling begins after several weeks of the injurious event and may last over more than a year [11]. During this phase, all of the essential cellular responses that were stimulated during injury are downregulated and eventually terminated [8]. **Figure 1** shows the graphical abstract for wound healing phases, as discussed in the review paper of A.Przekora (2020) [12].

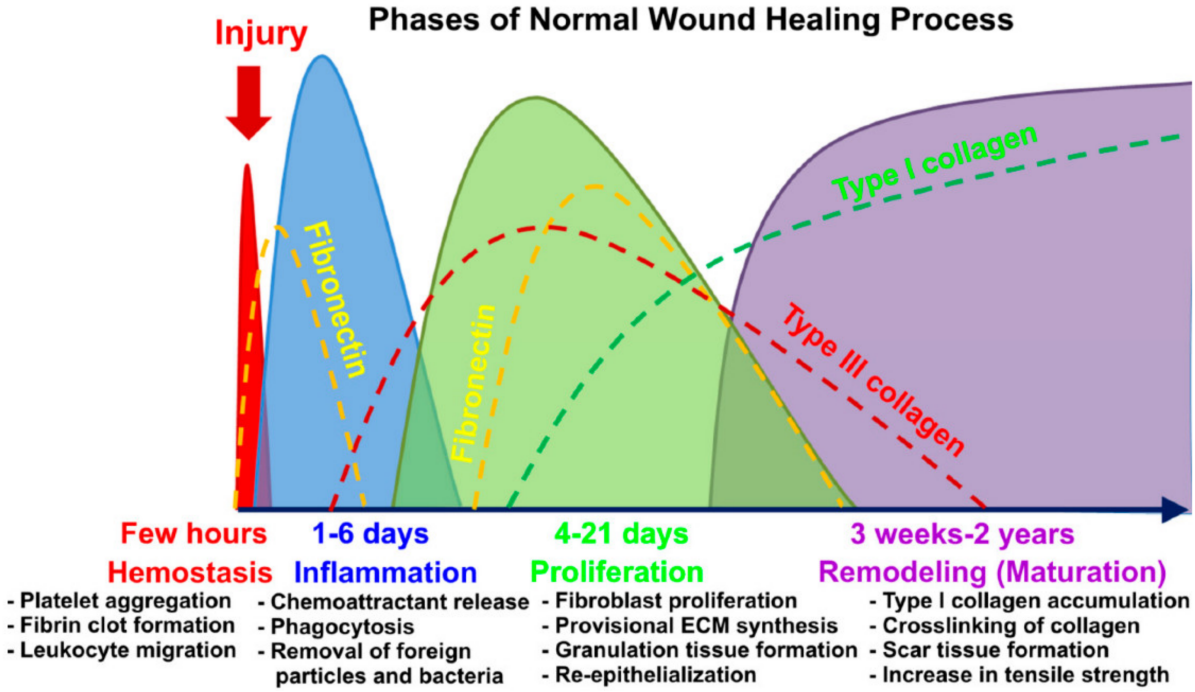

Figure 1. Graphical abstract for wound healing phases [12]. Used under the Creative Commons License (http://creativecommons.org/licenses/by/4.0/) accessed on 13 November 2021.

Chronic wounds are more likely to occur by sustained stimulation, such as hyperglycemia, chronic inflammatory responses, or persistent tissue injury [13]. Non-healing wounds fail to complete the entire wound healing stages and usually have prolonged inflammatory phases. Interruption of the normal healing phase may result in additional phases of a chronic condition, which may indirectly increase the patient's vulnerability to infection and, ultimately, damage the patient's quality of life [14]. Problematic wound healing can occur due to a wide range of health conditions and pathologic developments, including chronic inflammation, persistent infections, "open wounds", and cancerous wound transformation [15]. Diabetes mellitus (DM) has a serious complication that might result in

diabetic foot ulcers (DFU). DFU has been related to poor wound healing progress due to cytokines and poor cellular responses, infections, poor vascularisation, and diebetic neuropathies [16]. The primary goal of wound healing is to prevent the wound from being infected by the pathogens from the external environment [17]. Thus, the neutrophil influx is an early inflammatory response required for the clearing of pathogens and cellular debris during cutaneous wounds [18]. Hence, faster wound repair is vital for wound healing treatment. **Figure 2** shows the comparison of normal and chronic wound conditions.

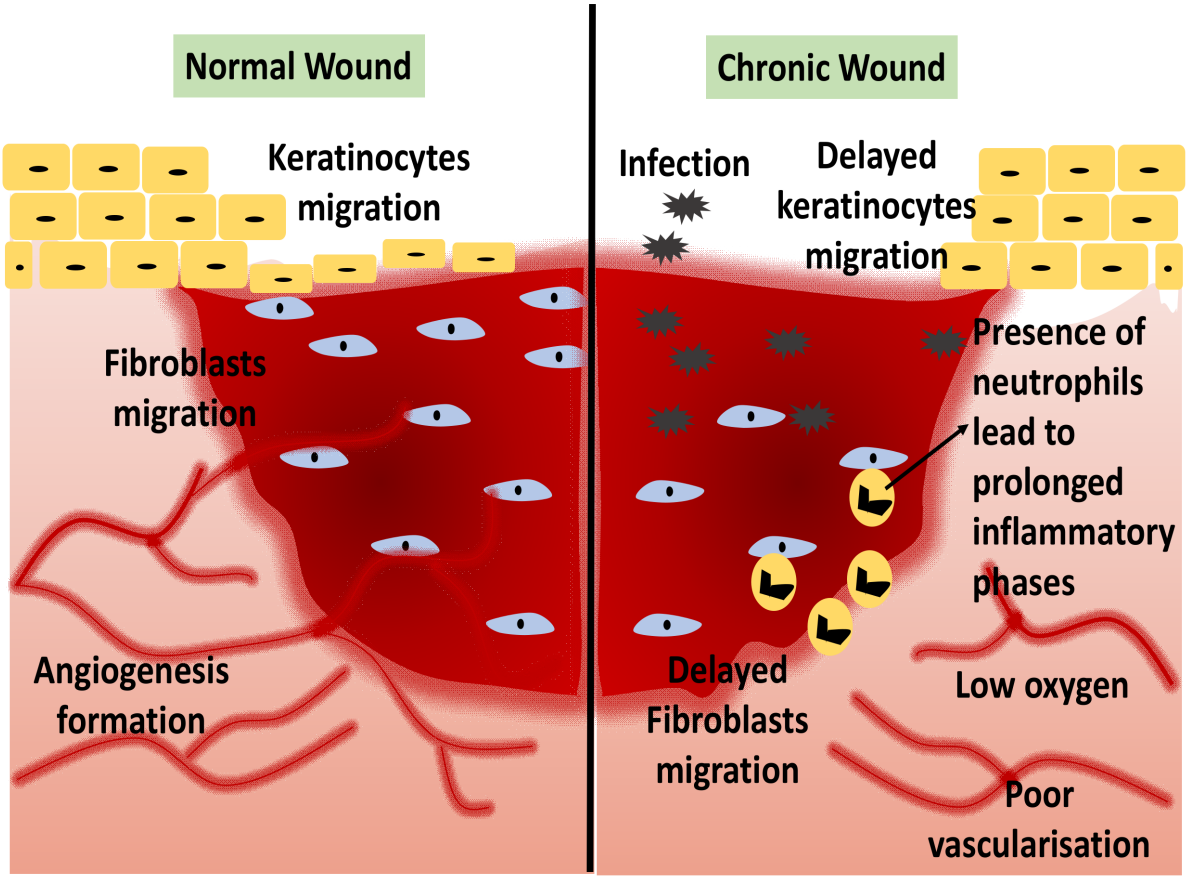

Figure 2. A comparison between normal and chronic wounds.

Tissue engineering has proposed a combination of cells, biomolecules, and biomaterials approach to replace the conventional skin graft. The complex structure of skin tissue requires a combination of several types of elements to form a biocompatible scaffold that mimics the native tissue. Thus, three-dimensional (3D) bioprinting is an innovative fabrication technique that combines selected cells with “inks” composed of biomaterials, crosslinkers, and growth factors to fabricate tissue-like structures for various applications. On the other hand, the use of 3D-bioprinted technology decreases the number of operations necessary for skin replacement. The 3D-shaped bioscaffolds open up new alternatives, such as broadening the range of structures accessible to treat injured skin tissues [19]. It allows for the precise placement of skin cells to replace damaged skin [20]. The bioscaffold has the potential to generate better properties for skin constructs with good elasticity, extensibility, and a high yield of skin reconstruction [1]. The network of blood vessels may be printed as well to ensure the long-term survivability of the skin tissue.

1.2. Current Trend of 3D-Bioprinting for Chronic Wound

Although skin has a highly complex structure, bioprinting techniques are the most reliable and convenient transfer of cells with accurate printing outputs and mimic native skin tissue [21]. In skin tissue engineering, 3D-bioprinting is continuously changing as researchers innovate and propel the field ahead. The recent trend in using the 3D-bioprinting approach for chronic wound healing treatment is still under study with several limitations. **Figure 3** shows the current trend of the publications for chronic wound healing treatment by using a 3D-bioprinting approach from the year 2010 until 2020. A comprehensive search strategy was followed to collect the digital publication records on Web of Science. The search was limited to articles published from the year 2010 until 2020. The search query consists of seven terms including “3D-bioprinting”, “bioinks”, “three-dimensional”, “tissue engineering”, “skin cells”, “skin regeneration”, and “wound healing”. The publication summary (**Figure 3**) indicates that the research for chronic wound healing treatment by using 3D-bioprinting was highest in the years 2018 and 2019 compared to the previous eight years. The researchers used different types of biomaterials as bioinks. However, most of the biomaterial entails certain limitations, and the bioinks used successfully met the skin cells’ ideal conditions, including dermal fibroblasts (DFs) and keratinocytes (KCs).

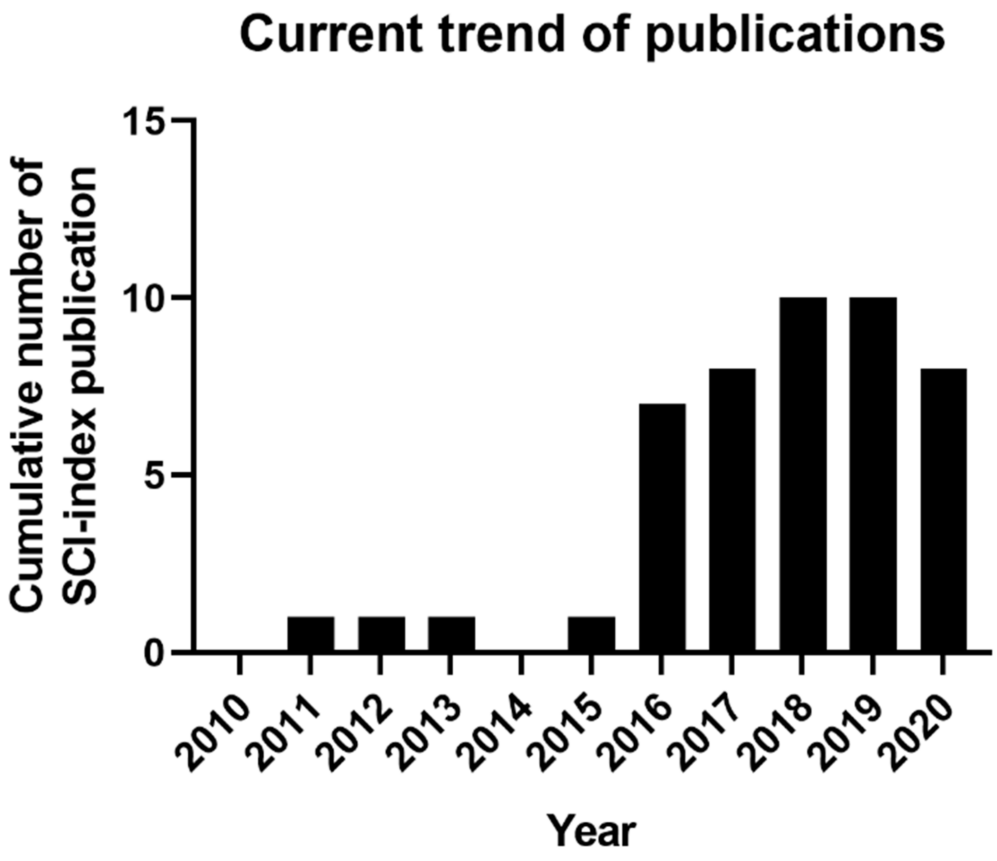

Figure 3. The current trend of SCI-indexed publications on Web of Science for chronic wound healing treatment by using a 3D-bioprinting approach.

2. Human Skin Structure

Skin is the largest organ of the human body with three different complex layers (epidermis, dermis, and hypodermis) and several other components, including the extracellular matrix (ECM), blood capillaries (veins and arteries), nerves, and hair follicles [12]. It is essential for maintaining skin integrity and stability for appropriate function in retaining body homeostasis [22]. **Figure 4** shows the illustration of the complexity of human skin structure.

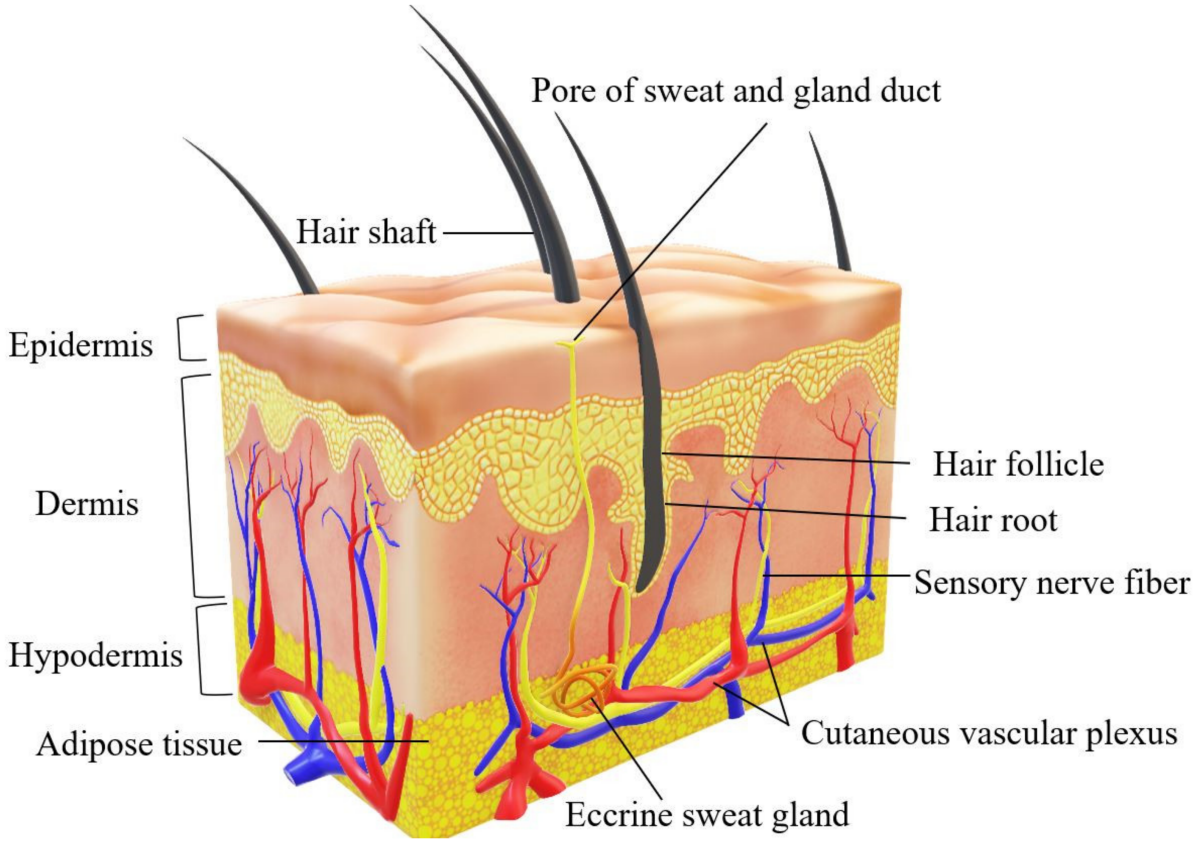

Figure 4. Complex human skin structure (epidermis, dermis, and hypodermis).

The epidermis layer is abundant with keratinocytes to protect the skin from external infections, whereas the dermis layer acts as the skin's appendages [23]. The dermis is made up of fewer cellular constituents, primarily fibroblasts [24]. The dermis layer lies within a complex connective tissue structure occupied with nerves, hair follicles, glands, and blood vessels for nutrient transportation [21]. Dermal fibroblasts (DFs) are the most abundant cells that occupy the dermis layer of the skin [25]. The dermis is composed of two connective tissues that interact to form an interconnected network of collagenous and elastin fibers produced by DFs [26]. The well-vascularisation inside the dermal layer will supply nutrients to the DFs. In the skin, DFs are responsible for the secretion of growth factors and extracellular matrix (ECM) for tissue regeneration [27]. The subcutaneous tissue, or hypodermis, is a fibrofatty layer that is loosely connected to the dermis layer of the skin [28]. The hypodermis is mainly composed of adipose tissue, which serves as an energy storage and insulation system for the body as well as a cushion for the skin. A muscle layer can be found adjacent to this layer, which overlies either bony prominences or interior tissues and organs. It is also the site of the formation of certain blood vessels that extend into the dermis [29].

3. 3D-Bioprinting for Chronic Wound

Nowadays, in parallel with the advance of technology, direct printing of living cells and biomaterials have opened up new possibilities for 3D tissue engineering and regenerative medicine [30]. The final product for the 3D-bioscaffolds is in the form of a hydrogel. Hydrogels are widely perceived as one of the excellent wound dressings [31]. The selection of bioinks must meet certain criteria, including printing resolution, gelation, viscoelasticity, mechanical properties, and biocompatibility to maintain the viability of the cells upon bioprinting [32]. The interaction of cells with the components of the bioinks needs to be considered for developing a harmoniously organized tissue [33]. Previously, the generation of autologous single-layer keratinocytes, single layer fibroblasts, and bilayer skin in prior work (MyDerm^{TM}) was successfully implanted in patients [34]. The success of this work has proven that tissue replacement can be accomplished by using the patient's cell with a combination of autologous biomaterial. Besides, this approach also eliminated the risks of immune rejection upon post-implantation failure. Consequently, it is preferable to use a biomaterial that maintains a homogenous solution of encapsulated cells with minimal cell sedimentation [31].

3.1. In Vitro Skin 3D-Bioprinting

In vitro skin bioprinting aims to improve the tissue maturation progress before transplantation to the wound site is performed [35]. As a result, this approach allows rapid wound healing progress and tissue regeneration. The usage of appropriate bioinks allows the composite scaffold to achieve adequate pore sizes, improve mechanical strength, and optimize the biodegradation rate for future clinical applications [36]. The bioinks optimization step is designed to provide a cell-friendly environment that promotes cell proliferation rate. However, the most challenging aspect of skin bioprinting is to combine various types of cells in the bioinks for skin tissue reconstruction. Dermal fibroblasts (DFs) and keratinocytes (KCs) are the major cells involved in skin model development [37]. **Figure 5** shows the in vitro 3D-bioprinting of the skin layer by using DFs and KCs at different layers.

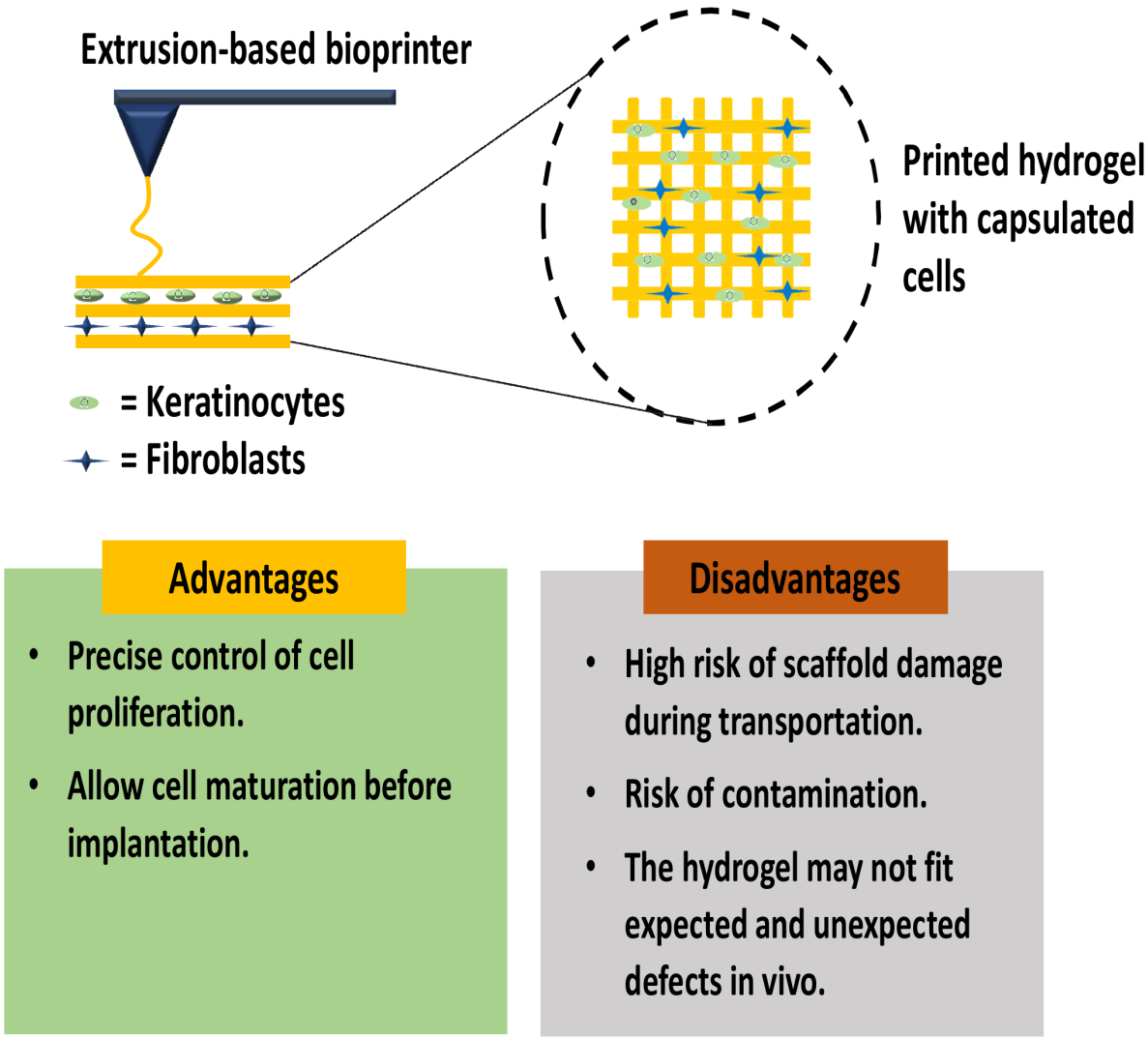

Figure 5. In vitro 3D-bioprinting using extrusion-based bioprinting.

The 3D microenvironment is required to facilitate cell development and maturation. The DFs and KCs easily isolated from any healthy skin biopsies samples using the standard operative procedure. Skin tissue promotes oxygen transportations and nutrients to all surrounding tissue; it is critical for developing a new tissue/organ with a vascularized structure. Fortunately, 3D-bioprinting opens up new possibilities for constructing adaptable skin models with vascularization and complex macrostructures [37]. Some researchers are susceptible to using in situ skin bioprinting against in vitro bioprinting due to several limitations during the handling and implantation procedure. An in vitro skin bioprinting study discovered that certain reconstructed 3D-skin models exhibited significant fragile micro and macro-structures. This may result in structural impairments such as swelling, contraction, or distortion upon transplantation. Furthermore, in vitro bioprinting is subject to a significant risk of contamination during transportation and manual implantation [38].

3.2. In Situ Skin 3D-Bioprinting

To date, significant progress in tissue engineering has been proved by introducing in situ bioprinting technique. The basic principle for in situ bioprinting is performing a bioprinting method of pre-cultured cells directly onto the skin

injury site and allowing for skin maturation at the wound area [35]. The in situ bioprinting approach provides a novel delivery bioinks approach for cell deposition at the injury site. **Figure 6** shows the deposition of bioinks in a mouse wound by using the inkjet-based bioprinting technique. In situ bioprinting of the skin construct directly on the wound site is dependent on the patient's body acting as a "bioreactor" for the functional maturation of the bioprinted tissue [39]. However, the wounds were scanned first to get accurate information on the wound topography, which was then used to direct the printing head to deposit the bioinks onto the injury site [40].

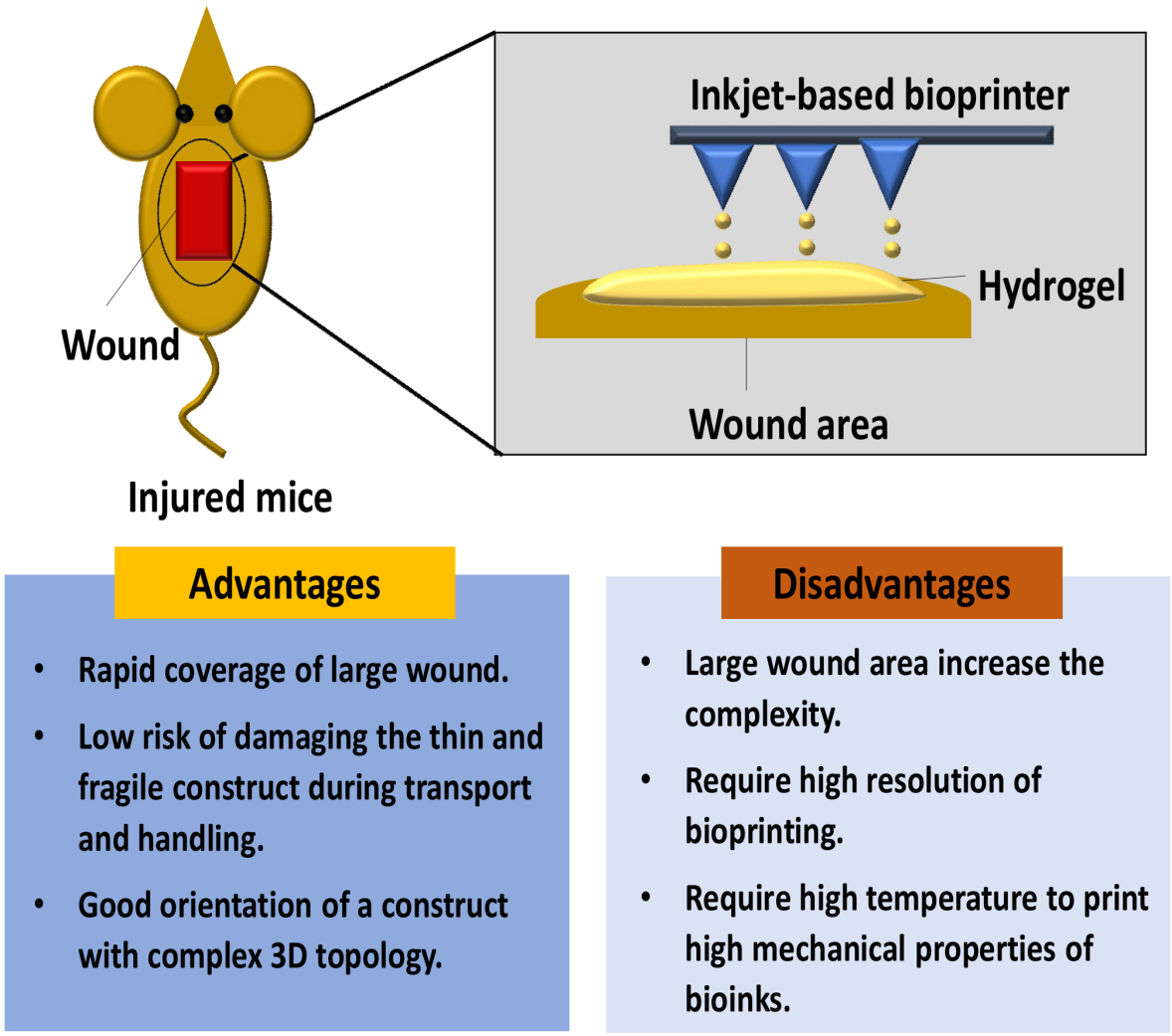

Figure 6. In situ bioprinting for the wound by using inkjet-based bioprinting technique.

Overall, the laser wound scanner aids in the creation of a precise shape/map of the lost skin, and the bioinks will be printed out to this region [35]. The major advantage of the in situ bioprinting technique is that it facilitates the removal of artificial microenvironment formation, which is essential in newly formed tissue. In situ bioprinting approach provide rapid coverage towards the larger wound area [39].

4. Natural Biomaterials

A desirable property of bioinks should enhance the physicochemical properties, including the rheological, mechanical, chemical, and biological properties of the fabricated scaffold to mimic the native tissues. A hydrogel that resembles the composition of the ECM has received much attention. Natural-based bioinks have become the most favored bioinks for tissue engineering applications due to their non-immunogenic, biocompatibility, biodegradability, and hydrophilicity properties [41]. **Table 1** summarizes the comparison of the biomaterial properties. On the other hand, synthetic-based bioinks provide better opportunities for tissue/organs construction [42]. The optimization of the bioinks should lead to an acceptable level of cellular activities, including cell migration, cell proliferation, cell viability, protein/gene expression, as in **Figure 7**.

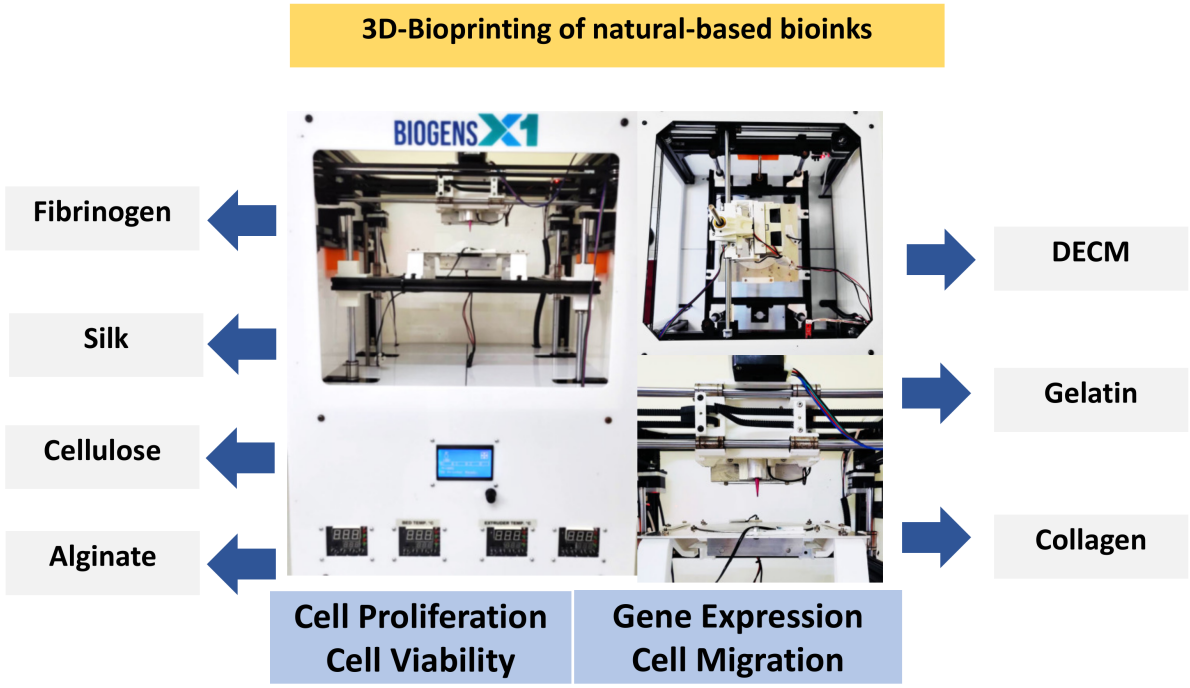

Figure 7. Cellular activities that the bioinks can influence.

Table 1. Properties of natural-based bioinks.

Type of Bioinks	Sources	Properties	References
DECM	Majority composed of ECM	dECM-based bioinks have viscoelastic behavior and rheological properties of dECMs, including shear viscosity and shear modulus that can preserve cells during printing. Besides, it is a biodegradable and low cytotoxicity biomaterials.	[43][44]
Collagen	Bovine, porcine, murine, and	Low viscosity, high shear stress, low viscosity, and weak mechanical strength.	[45][46][47]

Type of Bioinks	Sources	Properties	References
	marine		
Gelatin	Bovine, porcine	Has controllable mechanical properties depending on the concentrations, temperature-dependent, reversible state from solid to gel, and its challenging to optimize the temperature and its viscosity	[48][49]
Alginate	Algae	has high shear-thinning properties and a faster polymerization time after printing. However, alginate do not have cell adhesion sites	[50][51] [52]
Cellulose	Plant or bacterial ECM	Naturally occurring, biocompatible, biodegradable, and abundant biopolymer, high solubility in water and numerous carboxyl groups	[53][54]
Silk	Silkworms and spiders	low concentration and viscosity, slow biodegradation rate	[52][55] [56]
Fibrinogen	Plasma protein	Biocompatibility, biodegradability, adjustable mechanical properties, nanofibrous structural characteristics, and low viscosity properties	[57][58]
Chitosan	Chitin	Biocompatibility, antibacterial properties, thermosensitive, and low mechanical strength	[59][60] [61]

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