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Three dimensional (3D) printable gel-inks for skin tissue regeneration

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Abstract:

Recent and rapid progression in three-dimensional (3D) printing techniques has revolutionized conventional therapies in medicine; 3D printed constructs are gradually being recognized as common substitutes for the replacement of skin wounds. As gel-inks, large numbers of natural and synthetic (e.g., collagen and polyurethane, respectively) substances were used to be printed into different shapes and sizes for managing both acute and chronic skin wounds. The resultant 3D printed scaffolds not only provide physical support but also act as supporting niches for improving immunomodulation and vascularization and subsequent accelerated wound healing. Recently, the use of thermosensitive and pH-responsive gels has made it possible to prepare 3D printed constructs with the ability to facilitate in situ crosslinking within the biopolymer and with native wound edge tissue as well as to fill the exact shape of wound damage. In this chapter, we aim to introduce the current state of 3D printable gel-inks utilized for skin wound treatment and illustrate future prospects in this amazing area of science.

Keywords: Three dimensional (3D) printing; Additive manufacturing; Gel-inks; Skin tissue engineering; Wound healing

1. Introduction

The skin, the largest organ of the human body, forms nearly 15% of total body weight in human adults. Similar to other organs, the skin is composed of extracellular matrix (ECM) and various cell types, which exert structural and functional activities. In fact, the skin makes the outermost layer of the body (covering other tissues and organs) and plays pivotal roles in the protection (UV light absorption, mechanical support, and immune surveillance), perception (temperature, pain, and touch), and regulatory mechanisms (hemostasis, thermal, hydration, and excretory) of the body [1]. Human skin comprises two major layers, the epidermis and the dermis, and a third region called subcutaneous tissue. The major constituents of the epidermal layer are keratinocytes, which generate a stratified epithelium and undergo terminal differentiation to generate functional mature keratinocytes [2, 3]. This layer resides onto the basement membrane, separating the epidermis from the dermis. The dermis is made of ECM, containing mostly collagen synthesized by fibroblasts [4].

The skin is the outermost tissue of the body which is highly susceptible to environmental stresses, leading to a wide range of skin injuries generated by acute trauma, thermal, mechanical, chemical, microbial, and radiation issues. Furthermore, skin injuries can be caused by genetic disorders, surgical interventions, and chronic wounds [5, 6]. Depending on the extension and depth of skin damage, the epidermis or dermis may be affected, especially in third-degree burns and full-thickness wounds, which could lead to high morbidity and mortality [7].

Numerous skin substitutes and wound care products have been developed to be used in managing different types of skin injuries. Traditional therapeutics rely on utilizing the epidermal, dermal, or dermo-epidermal substitutes by processing auto-, allo-,, and xenografts that provide a highly resemble replacement for damaged tissue [8]. However, there are a few limitations with these

biological substitutes, including their time-consuming fabrication, lack of donor tissue, and the risk of immunological rejection and pathogen transmission. Therefore, advanced bioengineered constructs with high regenerative capacity have emerged as promising alternatives to the traditional substitutes. Regarding skin nature, the use of biocompatible polymers (natural and synthetic) resulted in the best clinical outcomes. Collagen, gelatin and alginate are among the widely used natural polymers for skin wound healing, whereas poly(ε-caprolactone) and polyurethane are extensively applied for managing dermal injuries. In order to take optimal results, the mentioned biopolymers should be utilized as three dimensional (3D) constructs; 3D printed polymeric scaffolds are excellent patient-specific substitutes for skin tissue engineering. A series of 3D printing methods (e.g., extrusion-, laser-assisted routes) was well-used for fabricating effective skin replacements. Apart from the method, the type of printable gel-inks is of utmost importance as to their critical roles in determining physico-chemical, mechanical, and biological properties of the final products. In this chapter, skin tissue is initially introduced structurally, and functionality and then different printable polymeric gel-inks are presented and discussed.

2. Skin: A histological overview

Understanding the anatomical and physiological functions of the skin is of great importance for researchers aiming to prepare tissue substitutes used in the repair and regeneration of skin wounds. The skin tissue has a very complex multi-layered structure containing ECM components, capillary networks, nerves, appendages (e.g., hair follicles and sweat glands), and various cells. It is well known that the ECM is the most constituent of the skin, contributing to the formation of an integrated tissue both structurally and functionally. In particular, the ECM components can provide a favorable substrate for cell attachment and migration, as well as

nutrient and metabolite exchanges. Furthermore, the polysaccharides, proteins, and water available in the ECM contribute to the tensile strength and elasticity of the skin as a result of generating a gel-like network [9].

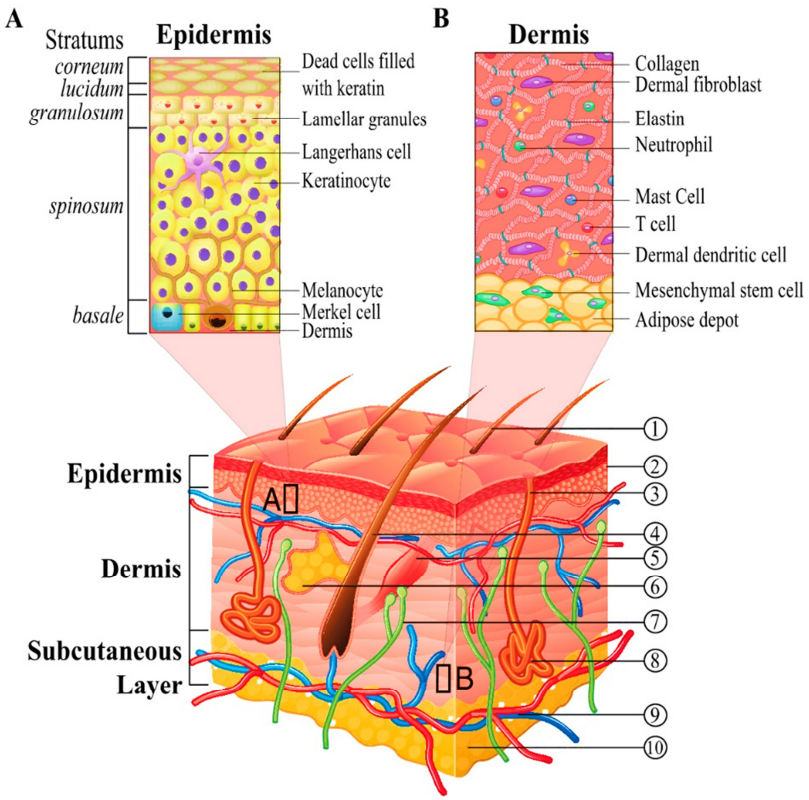

Figure 1. A schematic representation of microanatomy of the skin tissue with underlying cells in the epidermis and dermis layers. The epidermis consists of five distinct layers, including basal, spinous, granulosum, lucidum, and corneum layer (from the deep to the superficial). Keratinocytes are the major cell type of the epidermis that undergo terminal differentiation to generate the stratified skin. The dermis is mostly composed of fibroblasts, which are distributed in collagen fibers. Other components are discussed in detail in the text. Adapted from [10].

As shown in Figure 1, the skin is structurally made of three distinct layers, i.e., the epidermis, dermis, and hypodermis (also named subcutaneous tissue). The main constituents of these layers are summarized in Table 1 and will be discussed in the details in the following sections.

Table 1. The major components of the three layers of the skin [11].

Components	Function(s)
Epidermis	
Keratinocytes	Making a protective barrier against pathogens, UV radiation, heat, and water loss
Melanocytes	Producing the pigment melanin, which protects against UV-B light exposure
Merkel cells	Associated with tactile sensation
Langerhans cells	Antigen-presenting cells of the skin tissue
Dermis	
Collagens	Fibrous proteins responsible for mechanical support and elasticity of the skin
Fibroblasts	Synthesizing ECM ingredients and collagens and possessing a fundamental role in wound healing
Mast cells	Wound healing, angiogenesis, allergy response, and anaphylaxis
Hypodermis	
Fibroblasts	Synthesizing ECM ingredients and collagens and possessing a fundamental role in wound healing
Adipocytes	Fat formation with the aim of energy storage
Macrophages	Phagocytosis, wound healing, immune response

2.1. Epidermis

The epidermis is a stratified squamous epithelium with a high proliferation capacity which can regenerate itself routinely. The epidermis is mostly composed of keratinocytes (up to 95%) (Figure 1.A), while other cell types are found in this layer, including small populations of Langerhans cells, melanocytes, Merkel cells, and unmyelinated axons. Keratinocytes originate from the cells located in the stratum basal and migrate up toward the stratum corneum and progressively differentiate. According to the differentiation stage of keratinocytes, the epidermis

is divided into four functionally separate layers, including the stratum basal (basal layer), the stratum spinosum (spinous layer), the stratum granulosum, and the stratum corneum (cornified layer) (from deep to superficial) (Figure 2). The stratum basal is generally made of a monolayer of cells that are settled on the underlying basement membrane. The basal layer possesses a subpopulation of stem cells, which may have a critical role in the high regenerative potential of the skin tissue. Post-mitotic keratinocytes are located on the top of the stratum basal and migrate from the spinous layer (containing the youngest cells) to the oldest cornified layer during terminal differentiation. Keratinization is initiated in the stratum spinusom (8-10 layers of cells) when columnar basal cells differentiate into polygonal keratinocytes. These cell types then synthesize keratins, insoluble proteins, causing a hydrophobic and impermeable barrier. Thus, the cells in the spinous layer contain a high concentration of keratin and are attached to each other by desmosomes (intercellular junctions) [12]. The spinous cells transform into a more squamous shape and acquire keratohyaline granules and generate the stratum granulosum (1-3 layers of cells). Further differentiation of granular cells leads to losing their nucleus and organelles via lysosomic processes considered as dead cells and make up the stratum corneum (almost 10-15 layers of flattened cells). The turnover rate of the corneum cell layer is estimated at about 30 days. The resulted keratinization may lead to the generation of an impermeable barrier that avoids water loss and entry of pathogens detrimental molecules to the body. Furthermore, keratinocytes also produce various cytokines and growth factors (GFs), playing a role in the repair and regeneration process. These bioactive molecules include transforming growth factor (TGF-β), interleukins (IL-1, IL-6, IL-8), interferons (IFN-α and IFN-β), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), tumor necrosis factor (TNF-α), and granulocyte-macrophage colony-stimulating factor (GM-CSF) [13].

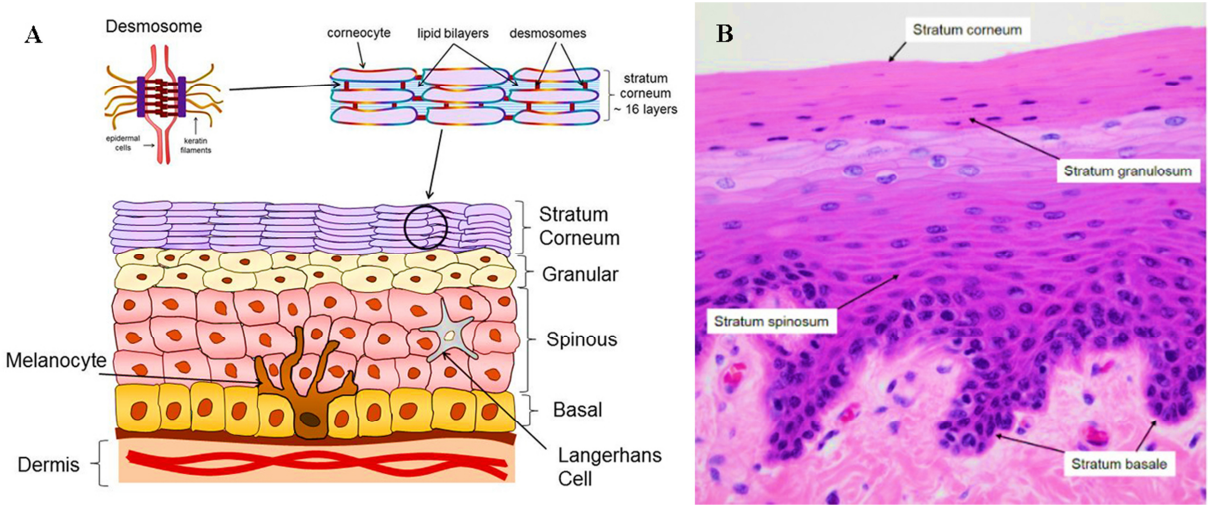

Figure 2. Various layers of the epidermis and its underlying cells. (A) Schematic representation of major cells constituting the epidermis, including basal, spinous, granular, and corneal layers. Keratinocytes are major cellular components of the epidermal layer in which desmosomes (intercellular connecting proteins) are highlighted. Other cell types include Langerhans cells and melanocytes. (B) Histological demonstration of epidermal layer stained with hematoxylin and eosin (H & E). Adapted from [14].

In addition to keratinocytes, the epidermal layer contains melanocytes that are randomly distributed in the basal layer. Melanocytes are primarily responsible for the skin color via producing the pigment melanin (UV-protective pigment). Melanin is encapsulated within organelles named melanosomes, followed by transitioning to the adjacent keratinocytes [15]. Once the pigments have reached the keratinocyte cytoplasm, they arrange in a well-orchestrated manner to protect the nucleus from detrimental UV irradiation. Scientific evidence shows that UV induces melanization via promoting the p53 pathway through synthesizing melanin stimulating hormone (MSH) by keratinocytes. In addition, the p53 pathway would initiate apoptosis of keratinocytes possessing inadequate pigmentation to prevent side effects of UV exposure (e.g., defective and premalignant mutations) [16]. Langerhans cells are mainly recognized as epidermal dendritic cells and participate in the immune response. They mostly

reside in the spinous layer and constitute up to 2-4% of epidermal cells. Another important cell type in the epidermis is Merkel cells located in the basal layer. These cells are possibly responsible for the sensation by generating synaptic junctions with peripheral nerve endings [17].

2.2. Basement membrane

The basement membrane (BM), also called the basal lamina, immediately lies beneath the epidermal layer and serves as a boundary between the epidermis and the underlying connective tissues. Although BM is mostly composed of collagen □, it has a very complex molecular structure. The cells residing in the stratum basal could communicate with the components of BM through hemidesmosomes (anchoring plaques containing collagen □□). BM could be separated into two specific layers, including lamina densa and lamina Lucida. The first one is the superficial portion just beneath the epidermis and mostly made of collage IV, while the latter is considered the deep part and constructed from laminin and other glycoproteins. The lamina densa is connected to the dermal layer via epidermal-dermal anchoring proteins (mainly collagen □) [17].

2.2.3. Dermis

The dermis is an intricate and dynamic microenvironment that conveys blood vessels, nerves, hair follicles, sweat glands, and sebaceous glands. The dermis plays a vital role in maintaining the elasticity and integrity of the skin; providing mechanical and structural support to the epidermis; immunosurveillance; cutaneous nutrition; sensory perception; and regulating the body temperature [17]. The dermal layer is divided into two functionally and structurally distinct

layers, i.e., the papillary dermis and the reticular dermis. The papillary dermis forms the superficial layer and contains loosely woven fibers, including collagen-□, -□, anchoring fibrils of collagen-□, and elastin fibers. On the other hand, the reticular dermis makes the deep layer that is composed of compacted fibers of collagen-□ (diameter of 100 μm) arranged in parallel to the skin surface [18].

In normal physiological conditions, the dermis contains a broad range of specialized cells, including fibroblasts, endothelial cells (ECs), monocyte/macrophage, dermal DCs, mast cells, lymphocytes, Schwan cells, pericytes, and mesenchymal stem cells (MSCs). These cells are harbored within a complex and acellular matrix mainly composed of collagen and glycosaminoglycans (GAGs).

Fibroblasts are the major cell type of the dermal layer, which synthesize dermis' ECM components, including collagen (the most abundant fibers) and elastic fibers, GAGs, and proteoglycans that are incorporated within the ground substance. It has been indicated that the papillary dermis is prevailed by loosely woven collagen-□, while the reticular dermis is dominated by dense and compacted bundles of collagen-□ (Figure 3, A). Altogether, the ratio of collagen-□ to collagen-□ is 4:1 in normal physiological conditions. In addition to structural proteins like collagens, the ground substance is mainly composed of GAGs such as hyaluronic acid. These polysaccharide molecules are bound to the peptide chains to generate high molecular weight combinations named proteoglycans (Figure 3, B). Table 2 summarizes a collection of peptide- and saccharide-based components of skin components.

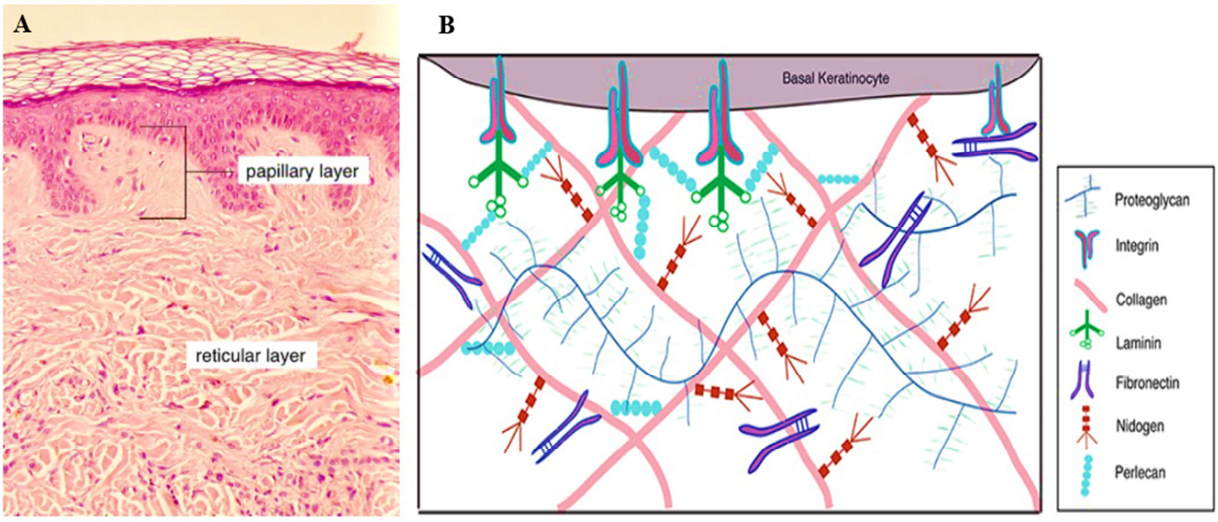

Figure 3. (A) Histological illustration of the papillary and reticular dermis. (B) A schematic representation of major components of skin ECM including proteins (e.g., collagen), glycoproteins (like fibronectin), and proteoglycans.

Table 2. Some major constituents of skin ECM.

Constituent	Function(s)	Ref(s)
Proteins		
Collagen	The main structural constituent of the dermis Promotes tensile strength of the skin	[19]
Elastin	Provides elasticity of the skin	[20]
Glycosaminoglycans (GAGs)		
Hyaluronic acid	High water absorption capacity which leads to greater compression resistance and proangiogenic potential	[21]
Heparin sulfate	Promotes mechanical strength of the skin Contributing to cell adhesion, proliferation, and migration, collagen fiber formation, granulation tissue formation, and basement membrane regeneration in connection with wound healing	[22]
Glycoproteins		
Fibrillin	Providing integrity and elasticity of the skin	[23]
Fibronectin	Modulating the interaction between cells and ECM components and promoting angiogenesis	[24, 25]
Laminin	Providing stable attachment of epidermis and dermis Facilitating the assembly of basement membrane leading to	[26, 27]

	promoted wound healing Promoting re-epithelialization, angiogenesis, and cell migration in the wound healing process	

Epidermal and dermal cells supply their nutrition and metabolite exchanges through the blood vessels located in the dermis. The lumen of dermal microvessels are lined by ECs which express vascular markers, such as CD31, CD34, and CD144 [28]. The dermal vascular microenvironment is surrounded by a collection of immune cells, e.g., macrophages, DCs, and mast cells. Generally, these cells participate in initiating the immune response and triggering inflammation and hemostasis. Dendritic cells can serve as antigen-presenting cells (APCs) and play a phagocytic role. These cells express a class of epitopes like CD34 (hematopoietic progenitor marker) and factor □a (activated fibrin stabilizing factor), which may have a vital role in the early stages of wound healing.

3. Skin wound healing: What we know and need to know

In the normal physiological condition, the skin shows self-healing property with a dynamic and well-orchestrated cascade of wound healing signaling pathways leading to advance repair and/or regeneration. It is well known that efficient wound healing results in the restoration of skin both structurally and functionally. Although the skin regeneration process reconstitutes an identical copy of the injured tissue without scarring in some species (e.g., fish, salamanders, and spiny mice) [29], it usually causes fibrosis and scarring in adult humans [30]. This process contains a complex interaction of cells and bioactive molecules that can be classified into four overlapping stages: (1) hemostasis (clotting), (2) inflammation, (3) proliferation, and (4) tissue remodeling

[30]. Some of the major bioactive molecules involved in the wound healing process have summarized in Table 3. All these stages are comprehensively discussed in the following section.

Table 3. Major GFs and cytokines involved in the skin wound healing process.

Bioactive molecule	Primary function	Ref(s)
Epidermal growth factor (EGF)	Proliferation of keratinocytes and fibroblasts	[31]
FGF-1, -2, and -4	Promoting angiogenesis and fibroblast proliferation	[32]
PDGF	Recruitment of macrophages and fibroblasts, macrophage activation, fibroblast proliferation, and ECM synthesis	[33]
Insulin-like growth factor (IGF-1)	Fibroblast and EC proliferation	[34]
Vascular endothelial growth factor (VEGF)	Promotes angiogenesis, granulation tissue formation, and re-epithelialization	[35]
Hepatocyte growth factor (HGF)	Migration, differentiation, and maturation of keratinocytes Scarless wound healing	[36-38]
TGF-β	Keratinocyte migration, recruitment of macrophages and fibroblasts Scarless wound healing	[39, 40]
IL-1	Activating GF expression in keratinocytes, fibroblasts, and macrophages	[41]
IL-4	Migration and differentiation of fibroblasts ECM synthesis	[42, 43]
IL-10	Modulating fibroblast and endothelial progenitor cell (EPC) in differentiation Modifying inflammatory response	[44-46]
IL-12	Early inflammatory response and angiogenesis Modulating GF synthesis	[47]
TNF-α	Activating GF expression in keratinocytes, fibroblasts, and macrophages	[41]

Hemostasis is a phenomenon happening immediately after happening the injury to the skin; it occurs in a few hours and causes coagulation and formation of a fibrin network. This clot suppresses hemorrhaging and provide a temporary scaffold (mainly composed of fibrin, fibronectin, and collagen) for cellular adhesion and migration. From a molecular mechanism point of view, clotting is initiated by the attachment of the von Willebrand factor to the subject tissue resulting in the aggregation of platelets along the damaged endothelium. The platelets

contribute to clot formation by producing thrombin and releasing pro-inflammatory factors, including PDGF and TGF- β [30, 48]. PDGF initiates the recruitment of neutrophils, macrophages, fibroblasts, and endothelial cells (ECs), playing vital roles in the following steps. TGF-β contributes to macrophage infiltration into the wound site and secretion of FGF, IL-1 (acute inflammatory response), TNF-α (acute inflammatory response), and more PDGF from macrophages. In addition, TGF-β also promotes the recruitment of fibroblasts and ECs [30].

The inflammatory phase initiates with the infiltration of neutrophils into the wound bed and lasts for about 2-5 days in a normal condition [30]. Neutrophils release cytokines such as TNF-α, IL-1β, and IL-6 to exert the first line of defense against pathogens and strengthen the immune response. After approximately three days, monocytes migrate to the wound site and differentiate into macrophages in order to phagocyte pathogens and cellular debris. Moreover, macrophages release bioactive molecules including IL-1, PDGF, TGF-β, TGF-α, FGF, IGF-1, and VEGF, which play pivotal roles in the recruitment of fibroblasts and transitioning from inflammation to proliferation phase [49].

The proliferation phase is recognized by main features, including re-epithelialization, angiogenesis, and granulation tissue formation. The re-epithelialization begins within the first hours after the injury and provide a covering onto the wound surface [50]. Residing cells at the wound edge secret various GFs, including EGF, keratinocyte growth factor (KGF), and TGF- β, to evoke keratinocytes and fibroblasts to migrate into the wound site. In fact, keratinocytes stimulate fibroblasts to produce and secrete GFs, which in turn trigger keratinocyte proliferation. Later, the proliferation of fibroblasts promotes the production of GFs in fibroblasts in a synergistic manner [51]. Furthermore, stem cells residing in the hair follicle bulge can differentiate into the epidermal progenitor lineages and facilitate the restoration of the epidermis

[52, 53]. Angiogenesis, also known as neovascularization, provide nutrients and metabolites exchanges for the newly formed tissue. Sprouting of existing blood vessels occurs by the attachment of VEGF, PDGF, bFGF, and thrombin to the receptors on the ECs. Matrix metalloproteinase (MMPs) secreted by vascular ECs triggers the migration of vascular branches toward the wound site that consequently differentiate to new mature vessels [54]. Finally, the granulation tissue is formed about four days after the injury. At this stage, secreted PDGF, TGF-β, and FGF stimulate fibroblasts to convert the provisional fibrin matrix with newly formed collagen type □ and ECM components such as fibronectin, GAGs, and proteoglycans [48]. In addition, some fibroblasts could be differentiated into myofibroblasts (i.e., contractile cells) leading to better wound healing via improved mechanical strength [55, 56]. This stage of healing can be followed by both macroscopically and microscopically, i.e., via wound closure rate and monitoring re-epithelialization, granulation tissue, density of dermal collagen fibers, leukocyte infiltration, respectively.

The remodeling phase is considered as the last phase of wound healing; this stage commonly initiates during 2-3 weeks after the injury and may last for several months. This includes remodeling of the granulation tissue into a mature scar via MMPs and other collagenases secreted by fibroblasts and macrophages. In addition, the remaining fibroblasts begin to differentiate into myofibroblasts [48]. It is worth noting that collagen type □ found in the granulation tissue is replaced by bundles of collagen type □ and further cross-linked by lysyl oxidase with a parallel orientation with the skin surface. This substitution of collagens increases the tensile strength of ECM from 25% to 80% [57].

Based on the etiology, the process of wound healing could be affected by the type of wound, i.e., acute or chronic. In acute conditions (e.g., burns), excessive contraction of myofibroblasts occurs

as a result of the poor apoptotic rate at the remodeling phase and lead to ECM degradation and fibrotic scar formation [58]. While in chronic wounds (e.g., diabetic ulcers), the healing process remains in a prolonged inflammation phase that leads to the overactivation of proteases (e.g., MMPs, elastase, plasmin, and thrombin) as well as reactive oxygen species (ROS) degrading the ECM [59-61]. One of the marked differences between chronic wounds and acute wounds is the epidermis diameter. In chronic wounds, the epidermal layer is thicker and highly keratinized, and often it is detached. Furthermore, the newly formed sin tissue in chronic wounds possesses less vasculature [62].

4. Bioengineered skin substitutes

Although many traditional and modern wound dressings have been found effective substances for managing various skin wounds, the use of tissue-engineered (TE) skin substitutes has gained much attention over the last years [63]. In general, an ideal skin substitute should have specific features including the ability to immediately replace injured epidermis and dermis, the capability of preventing and suppressing infection, avoiding water loss, reducing inflammation, enduring the shear forces, the lack of antigenicity, affordability, long-term stability, and availability [64]. Here, we summarized a collection of epidermal and dermal substitutes as well as advanced bioengineered grafts, including electrospun meshes and 3D bio-printed grafts.

4.1. Epidermal substitutes

Epidermal substitutes are utilized for managing superficial skin injuries to repair and/or regenerate the epidermal layer. They were initially reported in 1981 for large full-thickness burns. Generally, a skin biopsy with an extension of 2-5 cm^2 is taken from the patient, which is

called a skin autograft, followed by the separation of the epidermis from the dermis. After that, keratinocytes are isolated and cultured in the presence of fibroblasts that serve as a feeder layer. Since this process usually takes three weeks, the wounds are initially covered and treated with a provisional dressing to protect the wound bed and stimulate the healing [5, 65].

Despite several advantages, such as the lack of allogenic rejection, epidermal substitutes have some limitations, including long-term fabrication time, variable engraftment rates, expensive to use, and laborious handling due to their thin and fragile nature [65]. Direct cell spraying to the lesion can be considered as an alternative to the cultured keratinocytes as it shorts the fabrication time of the construct. In this strategy, obtained epidermal cells from a biopsy are locally sprayed at the wound site and consequently facilitate the epithelialization. ReCell® (Avita Medical, Perth, Australia) and Spray®XP (Graco, MN, USA) are two examples of available cell spray products for autologous re-epithelization [66, 67].

4.2. Dermal substitutes

Dermal constructs provide suitable substitutes for full-thickness wounds in which both the epidermis and dermis are affected. The main advantages of dermal constructs could be summarized as good mechanical properties, availability in various thicknesses and compositions, and the lack of wound contraction [68]. Dermal substitutes are made of either natural or synthetic materials [69] and are covered by a permanent epidermal graft [68]. Consequently, the substitutes undergo colonization and neovascularization, resulting in the formation of an autologous new dermis [65]. Dermagraft® (Advanced BioHealing, LaJolla, CA, USA) is a good example of a synthetic commercial dermal substitute, which is composed of a bioabsorbable

polyglactin mesh seeded with allogeneic neonatal fibroblasts. Dermagraft® is utilized as a provisional or temporary coverage for burns, chronic wounds, and diabetic ulcers [70, 71].

4.3. Dermo-epidermal substitutes

Comparing the epidermal and dermal substitutes, dermo-epidermal substitutes are the most skin-imitating constructs and made of keratinocytes and fibroblasts in underlying ECM to generate a temporary dressing [5]. In spite of a close resemblance to the skin construction, dermo-epidermal substitutes possess high production costs and may result in a lack of permanent wound closure due to the risk of allogenic cell rejection by the host [5]. Apligraft® (Organogenesis, Inc., Canton, MA, USA, and Novartis Pharmaceuticals Corp., East Hanover, NJ, USA) is a well-known artificial bilayered skin equivalent, which is made of allogenic keratinocytes and neonatal fibroblasts in a type □ bovine collagen matrix. This product should be freshly applied and has a shelf-life of 5 days at room temperature [71, 72]. The major application of Apligraft® is to heal partial to full-thickness burns, chronic wounds, diabetic ulcers, and Epidermolysis Bullosa [70].

With respect to major limitations of available skin substitutes (e.g., high production cost, poor engraftment rate, long fabrication time, rejection possibility of allogeneic cells, etc.), advanced bioengineering strategies have offered permanent and affordable alternatives to the existing ones. The following sections are focused on some of these advanced strategies, and their advantages and disadvantages are further discussed.

5. Advanced strategies for skin repair and regeneration

Advanced skin regeneration strategies propose an efficient and viable alternative to overcome the major obstacles of currently available skin substitutes (mainly allografts). These strategies incorporate biomaterials, cells, bioactive molecules, and novel fabrication techniques to generate a highly biomimetic skin construct. Two primary strategies could be noted regarding top-down or bottom-up approaches [73-76]. The top-down or scaffold-based approaches rely on the utilization of provisional scaffolds that provide a temporary environment for underlying cells to facilitate the attachment, proliferation, and secretion of their own ECM, leading to the promoted new tissue formation. In addition, the temporary scaffold provides physical support to guide and organize the formation of new skin tissue [73, 77, 78]. In contrast, the bottom-up approaches are considered as scaffold-free strategies and rely on the use of cells or cell-aggregates to generate a tissue-engineered construct [79, 80]. Accordingly, tissue-engineered constructs can be fabricated by self-assembled aggregation, fabrication of cell sheets, microfabrication of cell-laden hydrogels, or direct bio-printing [81].

5.1. Top-down approaches for skin regeneration

Top-down approaches are performed based on the fabrication of porous, biocompatible, and biodegradable scaffolds containing mammalian cells in the presence or absence of bioactive molecules (like GFs). The assembled 3D constructs are further maturated in a bioreactor [80, 82]. Scaffolds are generally made of natural, synthetic, or combination biomaterials to imitate the natural skin ECM both structurally and functionally [83, 84]. In fact, the skin ECM is comprised of structural proteins (e.g., collagen and elastin), specialized proteins (e.g., fibronectin and laminin), and proteoglycans (e.g., hyaluronic acid (HA) and heparin sulfate), which are well-orchestrated in desired skin layers [85]. For instance, the dermal layer is constituted of a 3D

fibrillary network, mostly composed of collagen fibers, with dimensions of submicron to nanoscale ranges to provide mechanical strength and structural integrity to the skin tissue [86, 87]. In order to regenerate a skin tissue similar to a healthy counterpart, either structurally and mechanically or functionally, the bioengineered constructs should be capable of resembling the microscale and nanoscale organization of the natural components of the skin as well as providing an ideal ambient for cell attachment, proliferation, and differentiation [88]. In this regard, several strategies have been developed to fabricate such constructs, such as electrospinning, self-assembly techniques, template synthesis, and phase separation [88, 89]. Among the mentioned approaches, electrospun nanofibers have emerged as promising scaffolds capable of resembling microscale and nanoscale organization of natural skin ECM, providing a desirable substrate for cell adhesion, proliferation, and differentiation. Furthermore, electrospun nanofibers can be used as delivery vehicles for a wide range of bioactive molecules, including GFs, cytokines, and adhesive peptides [90-92]. An electrospinning apparatus is fundamentally composed of a capillary tube containing polymeric solution, a high voltage supply, a grounded collector, and a syringe pump for a controlled jet of solution. In brief, the electrospinning process is initiated by the charged jet of a polymeric solution as a consequence of applied high voltage. The solvent is then evaporated, and nanofibers are finally deposited on the collector [93, 94]. It is well known that the morphology of nanofibers could be affected by a number of parameters, including molecular weight and viscosity of the polymeric solution, the applied voltage, capillary tip to collector distance, and capillary diameter [95]. A wide range of natural polymers (e.g., collagen, gelatin, and chitosan) [96, 97] and synthetic ones (e.g., poly(lactic-co-glycolic) acid (PLGA), poly(lactic acid) (PLA), and poly(ethylene glycol) (PEG)) [98, 99] and combinations of both natural and synthetic polymers [100, 101] have been utilized to fabricate electrospun mats for

promoted skin regeneration. These constructs benefit from a high surface to volume ratio and make an appropriate environment for cellular interaction and promoted angiogenesis [102]. In addition, the desired porosity of electrospun mats allows oxygen and nutrition exchange, which is necessary for prohibiting necrosis and failed skin wound healing [87]. Most importantly, the fiber dimensions of nanofibrous mats are in the range of natural ECM components and is mentioned a key parameter in accelerating the wound healing processes [88, 103]. Moreover, electrospun nanofibers can be utilized as drug delivery systems for sequential and controlled release of bioactive molecules (e.g., GFs, natural chemicals, and small molecules) at the wound site [104-107]. As an illustration, bFGF-loaded nanofibrous mats composed of PEG-PLA revealed a sustained release of bFGF for 4 weeks facilitating fibroblast cell adhesion, proliferation, and ECM synthesis. When implanted into diabetic wounds of rat, the constructs promoted re-epithelialization and maturation of skin appendages (hair and sebaceous glands) [105]. Still, poor mechanical strength and poor integrity to the body, and non-uniform thickness distribution are stated as the major drawbacks of electrospun constructs for skin regeneration applications [92].

In addition to electrospun fibrous scaffolds, experimental studies emphasize the use of hydrogels in managing a broad range of skin wounds as regards their capability of absorbing large amounts of liquids at the injured sites. Hydrogels can be described as 3D networks of hydrophilic polymers possessing hydrophilic chains allowing them to swell extensively. Accordingly, hydrogels offer a class of permanent or temporary dressings for regenerating the epidermis and/ or dermis in damaged skin [108]. Moreover, it is feasible to load different cell types, growth factors, and other therapeutic agents to hydrogels for boosting the healing process [109]. Hydrogels could be made of both natural (e.g., gelatin and alginate) and synthetic (e.g., PVA)

polymers, as well as their composites [110]. They are commonly categorized based on their crosslinking nature, i.e., chemical or physical hydrogels. Chemical hydrogels entail the formation of covalent bonds between the polymer chains, whereas physical hydrogels involve physical interactions between polymer chains (e.g., ionic interactions, hydrogen bonds, and molecular entanglement) [111, 112]. Major advantages of hydrogels as wound dressing materials include: (1) promoting wound debridement and absorbing wound exudates, (2) providing an optimum moist environment to accelerate the healing process, (3) permeable to oxygen and other soluble factors, (4) inhibiting bacterial infection, and (5) poor adhesion to the wound site which prevent trauma formation after its detachment from the wound bed [8]. Hydrogels can be loaded with both keratinocytes and fibroblasts [113, 114]. Despite their ease of fabrication, affordability, and good control over the scaffold properties, they lack sufficient mechanical strength, and they are unable to load individual cells at specific regions throughout the scaffold [115]. Hence, they are being improved as advanced biomaterials capable of encapsulating various cells and bioactive molecules, which are called gel bioinks.

5.2. Bottom-up approaches for skin regeneration

Bottom-up approaches utilize cells or cell-aggregates to generate tissue-engineered constructs without administration of scaffolds as supporting substrates [116]. These approaches generally entail three fundamental components: (I) a bioink containing the cell suspensions to be printed, (II) a biopaper, providing a temporary substrate for the deposited bio-inks, and (III) a bioprinter. The 3D bioprinting strategy, an advanced branch of the 3D printing technique, has been widely used in tissue engineering and regenerative medicine for fabricating substitutes with a maximum resemblance to human tissues and organs [117]. It is fairly well accepted that 3D constructs often

provide more accurate physiological situations than two dimensional (2D) counterparts since many functions naturally happen in the 3D condition of the human body.

Based on the main principles of casting, current 3D bioprinting strategies basically include laser-assisted, drop-based (inkjet), extrusion, stereolithography, electrohydrodynamic, and microfluidic bioprinting techniques [118-120]. Although all these methods are used as the fabrication route, it should be emphasized that printing a construct with a well-controlled and precise geometry is of utmost importance for efficient living cells and reconstruction of human tissues and organs in the laboratory [121]. In the following sections, the above-mentioned methods are briefly introduced, and then suitable gel-inks for fabricating 3D skin replacements will be presented.

5.1. Laser-assisted 3D bioprinting

Two separate main approaches of laser-induced forward transfer (LIFT) and laser-guided direct writing have been validated in laser-based bioprinting. To align and focus the laser, the device consists of a focusing system, a pulsed laser beam to induce the transfer of bioink, a ribbon as the absorbing layer, and a substrate for the bioink layer [122]. A laser source is used in this bioprinting technique, which is based on the LIFT model to irradiate high energy focused laser pulses at high precision onto thin substrates coated with a layer of laser absorbing material. The resulted bioink produces a high-pressure bubble to remove the cells and biomaterial from the substrate and deposit them onto the platform [123], where the scaffold is formed layer by layer [124]. Two layers are normally involved in this process: the energy absorbing layer (upper glass slide), which receives the pulsed laser, and the cell-containing layer of biomaterials (on the

bottom). After ejecting the cell-containing hydrogel precursor toward the platform, the final 3D structure will be shaped through the movement of the platform [125]. Some advantages have been reported for this method, including high cell viability (>90%), variety of printable bioinks with high viscosity, and nozzle-free and non-contact (between the bioink and processing device), which provides a mechanical stress-free medium for normal cellular activity [126]. On the other hand, the drawbacks of low cell density, complexity, low repeatability of the resulted droplet, time-consuming, high cost, and relatively low flow rate of crosslinking due to the fast gelation, which is essential for attaining a highly precise shape but can limit the applications of laser bioprinting approach, have addressed the researchers' focus on optimizing this method, which needs further study [127].

5.2 Drop-based bioprinting

As a highly versatile, rapid, simple, and cost-effective technique introduced in the early 2000s [128], drop-based bioprinting is compatibly capable of depositing picoliter droplets of various low viscous biological material inks (3.5-12 mPa/s) to avoid clogging in a precisely controlled way with high resolution and no contact between the substrate and the nozzle. Similar to laser-based bioprinting, this method faces some limitations, including non-uniformity of the droplets and inconsistent encapsulation of cells, as well as a restricted structural and mechanical integrity in bioprinted concepts. On the other hand, since it is difficult to control the porosity and vascularization, the size of the constructs can be restricted by cross-contamination of bioinks [129, 130]. Drop-based bioprinting has been classified into three main types of acoustic, micro-valve, and layer-by-layer inkjet bioprinting. The acoustic-droplet method produces the droplets from the bioinks through acoustic waves in an open pool without applying any heat, high voltage, or pressure. Micro-valve bioprinters generate droplets under pneumatic pressure through

the opening and closing of a microvalve. Moreover, the inkjet technique, as the most common system of drop-based bioprinting, includes electrodynamic, drop-on-demand, and continuous-inkjet bioprinting. These systems use mechanical, thermal and piezoelectric pulses to produce small (picoliter-volume) bioink droplets which can affect the cell viability in inkjet bioprinted microstructures [131]. By controlling the parameters affecting the ultrasound, including time, pulse, and amplitude, the inkjet technique as the first organ printing approach is capable of adjusting the desired size of ejected droplets, which is known as the primary advantage of this method [132]. Furthermore, the possibility of using multiple printing heads on the device allows different cells to be printed faster (1-104 drops/s) at the same time. However, possible thermal damage to cells and weak mechanical stability of the 3D-bioprinted structures, as well as the challenge of the drying process of droplets on the substrate, are the main issues to be solved. Additionally, this technique often leads to the construction of weak skin structures since high concentrations of cells and high viscous biomaterials cannot be used due to the low driving pressure of the nozzles, which should be considered in future studies [133].

5.3. Extrusion-based bioprinting

Extrusion bioprinting technique is evolved from inkjet technology and uses physical forces of pneumatic pressure, metal screw, or piston systems to selectively dispense the biomaterials with high geometric complexity through a mechanically driven nozzle where the extrusion head moves in three directions of x, y, and z to form 3D architectures of biomaterial on the substrate platforms. This technique has been branded as the most suitable for fabricating soft tissues among different bioprinting approaches [134]. In spite of its lower accuracy compared to laser-based and inkjet techniques, this technique allows the extrusion of different biomaterials,

including hydrogels, cell-spheroids, cell-laden bioink, and high viscous polymeric thermoplastics with various viscosity ranges of 6-30×10^7 mPa/s and the resolution of 100 µm-millimeter [135]. The ability to be installed in a multi-head system is the key benefit of applying this bioprinting technique, which provides printing one or more biomaterials simultaneously. These complex and quickly manufactured 3D tissue structures could mimic the human body both biologically and morphologically, verifying the extrusion technique as one of the most promising available clinical approaches [136]. While pneumatic systems use compressed gases to provide a continuous extrusion pressure to dispense bioinks, two other systems of piston and screw dispense bioinks through a pump using mechanical forces without any gases. With the help of the simple and low-cost extrusion bioprinting device, it is possible to fabricate a wide range of biostructures similar to skin tissues. However, bioink cells can potentially be damaged due to exposure upon external mechanical forces, which needs to be reduced as much as possible [137]. The use of extrusion-based 3D bioprinting in skin wound healing has been validated in recent studies [138, 139].

5.4. Stereolithography-based bioprinting

Photolithography techniques use photons/light to transfer the geometric shapes of a mask to a light-sensitive surface and are being effectively employed for constructing 3D scaffolds for tissue engineering applications. These techniques are generally divided into three main methods, including mask-based photolithography, multiphoton lithography, and stereolithography. As a notable 3D bioprinting technique, stereolithography uses a projected light source of the laser, infrared radiation, or an ultraviolet bulb to photolytically crosslink bioinks selectively in a layer-by-layer process to form highly precise 3D structures (commonly acrylics and epoxies) [140].

Stereolithography bioprinting systems consist of a light source, a digital mirror device, an elevator system, and a photopolymer reservoir with the biocompatible liquid photocurable resin as well as a print head which has to move only in one direction through an up and down movement [141]. While this method has been traditionally used to fabricate cell scaffolds [142], currently, it is applied in 3D printing of bioink with cells with high efficiency [143]. Compared to other 3D bioprinting techniques, stereolithography provides the advantages of high cell viabilities (>90%), high printing accuracy and resolution (<100 μm), short printing time (<1 h), as well as being simple and easy to control device [144]. However, this technique suffers from some drawbacks, including a high cost for system installation, the lack of available and useable photosensitive resins, and cytotoxicity of the photocurable resins, which can reduce the viability of embedded cells [145].

5.5. Electrohydrodynamic-based bioprinting

As a newly emerging 3D bioprinting technique, electrohydrodynamic printing has been applied in the controlled fabrication of 3D micro/nano-scale constructions [146, 147]. Combining the principles of electrohydrodynamics and layer-by-layer additive production makes this technique mainly appropriate for fabrication and biomimetic structural organization of artificial tissue models on a similar scale to that of living cells or native extracellular matrix, which proves its great potential to precisely regulate tissue regeneration [148] and control cellular behaviors [149].

5.6. Microfluidic-based bioprinting

Microfluidic 3D bioprinters employ a micro-printing apparatus based on microfluidic technology. They are different from traditional bioprinters (laser, inkjet, extrusion, and stereolithography) as to their capability of artificial printing tissues, for example, the skin, in a shorter period of time [150]. Microfluidic print heads use the combination with bioprinter to enable precise patterning of a biomaterial and cells in 3D. In addition, choosing the right bioink and design for your print is essential to create a functional 3D tissue. Bioink selection is of great importance since a bioink incorporates both cells and biomaterials. So, software (e.g., ASPECT®-Studio) is being used to specifically design a 3D structure [151]. One of the main benefits of microfluidic 3D bioprinting is the ability to pattern tissues on the microscale. Moreover, it is feasible to encapsulate different types of cells and materials as core-shell and concentrically multilayered fibers mimicking tissue interfaces. The size of microarchitectures and features may finally be controlled by microfluidics.

Although this system cannot entirely model all features of human skin, including hair follicles and pigmentation, it is capable of stimulating wound regeneration by printing a large amount of artificial transplantable skin in a fairly short time [152].

6. Natural 3D printable gel-inks for skin regeneration

In general, 3D printable gel-inks could be originally categorized as natural and synthetic polymers. It should be pointed out that their combinations have also been reported as a reasonable strategy for having an enhanced biological and mechanical property [153]. In spite of their shortage of mechanical stability, naturally-derived polymers are the main source of around 90% of polymeric substrates employed in 3D bioprinting applications [154]. The reason for the high usage of natural polymers is related to their inherent benefits, including high similarity to

human extracellular matrix (ECM) composition, which mimics cell native microenvironment and subsequently facilitates cells' attachment, proliferation, migration, and differentiation [155, 156]. Among a broad range of natural polymers, alginate, collagen, decellularized ECM (dECM), and gelatin inks have been extensively applied in skin tissue engineering. Natural protein-based inks, collagen, dECM, and gelatin-based polymers have shown remarkable potential in the regeneration of the epithelial layer of skin tissue [157].

6.1. Alginate

Alginate (known as alginic acid) is an anionic polysaccharide found in the cell walls of brown algae. This biopolymer has been widely utilized in various 3D bioprinting applications due to its rapid gelation post-printing and high shear-thinning [158]. Hydrated alginate can form a viscous gel as to its hydrophilic property, meeting the needs of physicochemical features suitable for 3D bioprinting. Due to its good biocompatibility and structural similarity to native ECM, alginate has been widely using as a wound dressing material [159]. Additionally, being directly polymerized by multivalent cations (e.g., Ca^{2+} and Ba^{2+}), alginate can generate a proper cell-compatible hydrogel ink for the bioprinting of human skin [160]. However, some limitations, including crosslinking delay, can reduce the shape fidelity of the alginate-based bioprinted constructs as it may adversely affect cell viability. Therefore, biomedical scientists have considered new ways to enhance alginate viscosity or extrude it using chemical crosslinkers (e.g., Ca^{+2}) to control the low shape fidelity of simple alginate solutions [161]. On the other hand, in order to increase cell viability without affecting alginate printability, researchers have conducted attempts to reduce alginate viscosity by using honey [162]. Future studies are also

required to enhance alginate cell adhesion due to their poor cell adhesion properties without altering its suitable physicochemical characteristics for 3D bioprinting applications.

6.2. Collagen

Collagen is another natural printable polymer which extensively utilized for skin wound healing applications. This biopolymer is known as the most plentiful protein in the human body and contains proline and glycine residues in its structure with a triple-helix polypeptide arrangement [163]. Among various types of collagen available in connective tissues (e.g., skin)[164, 165], collagen type I is the most abundant and also the most commonly used in 3D bioprinting applications [166]. Although collagen hydrogels are found to be printed in a desirable biodegradability level without using any chemical crosslinkers, the collagen direct 3D bioprinting approach is still restricted to collagen solutions. Collagen has revealed exceptional microstructures of macropores as well as a desirable shape consistency at 37 $^{\circ}$C to stimulate cellular attachment and proliferation [167]. However, incorporating cells or tissue spheroids may cause a reduced printability as well as a significantly longer recovery time [165]. These issues were further solved by a series of interventions including (I) adding chitosan [168], fibrinogen and thrombin [169], and fibrillar collagen [167] to collage; (II) using low concentrations (2-4%) of collagen [170]; and (III) controlling cell suspensions and densities [171] instead of using chemical crosslinking agents. In the case of thin structures, the protein gelation in a matrix of collagen could be controlled by temperature, pH, or both, while gelled and non-gelled regions are observed in thick structures (1 to 3 mm) as a result of diffusion and thermal conveyance limitations. Moreover, high levels of temperature and pH may severely harm cells' viability [171].

Collagen-based bioinks have been considered as extremely promising biomimetic materials due to their ability to increase cell attachment and proliferation using asparagine-glycine-aspartic acid residues as well as their low toxicity and immunological reactions [172]. However, the key benefit of applying collagen-based bioinks is implanting living cells within biochemical materials and ECM components. On the other hand, due to their crosslinking property, it is essential to use a temperature-dependent gelation process to form 3D concepts. Moreover, in order to improve mechanical properties and printability, collagen is combined with biocomposite including agarose [173], alginate [174], chitosan [175], and fibrin [176] to control the viscosity of the collagen contents.

6.3. Gelatin

Gelatin is a partially hydrolyzed form of native collagen with a broad range of applications in tissue engineering and regenerative medicine. Regarding its cost economy, gelatin is being used in experiments rather than collagen. Gelatin shows proper compatibility with living systems (human tissues and organs) and supports cell adhesion, growth, and proliferation due to its RGD sequence with abundant integrin-binding motifs [177]. In addition, this biopolymer displays good water solubility, low immunogenicity, adhesiveness, and safe degradation in the body.

The capability of gelatin in forming transparent gels under specific conditions makes it a suitable candidate for additive manufacturing of tissue substitutes. In spite of high rheological properties, gelatin hydrogels possess zero viscosity at temperatures above 27 ± 1 $^{\circ}$C and weak mechanical strength [178], encouraging the use of different crosslinking agents for optimal outcomes [161, 179-182]. Although gelatin keeps its thermo-sensitive properties by dissolving in water, it forms a reversible low viscous soluble phase at human body temperature [183]. All the drawbacks

mentioned above indicate that the pure gelatin does not seem a suitable substrate for 3D bioprinting; its composites with alginate [184], chitosan hydrogel [185], fibrin [186], hyaluronic acid [187], and silk [188, 189] have been developed to overcome low formability. Also, to enable photocrosslinkable properties of polymers and modify structural stabilization after bioprinting, researchers have widely used gelatin methacrylate as a potential advanced wound healing bioink. Indeed, gelatin methacrylate shows exceptional biological features, including improved biodegradability, enhanced cell adhesion and migration, as well as high thermal sensitivity and photo-crosslinking capability. For instance, the mixture of gelatin methacrylate and 2-hydroxy-1-(4-(hydroxyethoxy) phenyl)-2-methyl-1-propanone (Irgacure 2959) as the photoinitiator to form an applicable combined material which leads to a fast crosslinking after extrusion under UV light (360-480 nm) to improve suitable rheological properties with the desired quality [190]. Furthermore, gelatin methacrylate has been used to induce its high mechanical stability and shape fidelity after UV crosslinking in natural-based bioinks, such as silk sericin [191] and cellulose nanofibrils [179].

6.4. Chitosan

Chitosan (CS) is the main derivative of chitin, a polysaccharide usually derived from shells of aquatic animals (e.g., crabs and shrimps). CS is actually a polycationic polymer owning free acetamide groups and hydroxyl functions linked to the glucopyranose rings, make it susceptible to react through a nucleophilic attack. Therefore, a broad range of CS functionalizations could be carried out via selective modifications of the free amino groups. CS has no mutagenic effects and is recognized as a biocompatible material for biomedical applications. This biodegradable polymer is being widely employed in different areas of science (drug and cell delivery as well as

tissue engineering) thanks to its excellent properties, including antibacterial and mucoadhesive activities. For example, CS is currently being administrated for dermal tissue engineering regarding its capability of making hemostasis, which may provide a suitable condition for inducing collagen formation and subsequent tissue regeneration. In addition, CS could promote polymorphonuclear neutrophils (PMNs) migration and improve the granulation process by inducing dermal fibroblasts' proliferation. In general, CS has introduced an effective remedy playing positive roles in all the wound repair stages [192].

Up to now, various CS-based constructs (gels, films, and scaffolds) were successfully prepared via different production methods and showed great promises in wound healing. Currently, the use of CS as a suitable ink for additive manufacturing has attracted much attention as regards the possibility of making CS-based 3D scaffolds having the precise adjustment of porosity size and shape, fiber size, suitable interconnectivity of pores [193]. Extrusion-based 3D printing, FDM, single-arm robotic printing, two-photon-induced micro stereolithography, the 3D printer with jet dispenser have been used to print CS and its composites for preparing suitable constructs in tissue engineering [193]. For example, porous 3D-printed scaffolds with the film of CS at the base were successfully fabricated by the FDM-3D printing technique, which could support cell attachment and spreading in vitro and improve wound healing in rat models of diabetes [194]. Blending CS with other polymers (e.g., gelatin) was also examined for preparing gel-inks, and outcomes indicated an improvement in printability of gels through the extrusion-based process as well as cellular enhancement behaviors (adhesion, growth, and proliferation [195, 196]. In order to prepare potential bioadhesive dressing aiming wound healing purposes, 3D printed films based on CS were also reported in which genipin (GE) and glycerol (GLY) or polyethylene glycol 600 (PEG) were used as crosslinker with either as a plasticizer, respectively [197].

6.5. Silk fibroin

Silk fibroin (SF) is a natural polymeric protein made by a variety of insects, mostly derived from tame *Bombyx mori*. This polymer displays fascinating biological properties (high biocompatibility and biodegradability) and is being employed as an FDA approved material for imaging, drug delivery, and reconstructive applications [198]. From the tissue engineering point of view, SF may provide a suitable substrate for cell adhesion and growth as well as causing minimal immune responses due to its natural biopolymeric features [199]. SF-based biomaterials have shown excellent thermal and mechanical stability and validated cell adhesion and good fibroblast proliferation along with enhanced neovascularization leading to tissue healing and complete regeneration of wounds [200]. Up to now, a series of fabrication methods, including electrospinning, solvent casting, and gas foaming, were applied to make SF-based 3D scaffolds with the ability to use in managing skin injuries [201, 202]. However, lack of the desired fiber orientation, predefined internal architecture, and pore size are unsolved drawbacks of the mentioned traditional techniques.

Due to its fascinating properties like tunable biodegradability, biocompatibility, elasticity, and mechanical robustness, SF is being considered a promising bioink material for 3D printer machines [203]. SF exhibits high printability, proper mechanical strength, shear-thinning, and cytocompatible gelation behaviors, which verifying its usability for bioprinting application [204, 205]. Moreover, SF, as a polymeric protein bioink, possesses the ability to physically self-crosslinking through hydrophobic interactions without any additional chemical reactions or additives to stabilize the materials [206]. Still, the slow degradation rate of SF is considered as

the main concern; the incorporation of other rapid-degrading materials (e.g., gelatin) during the initial process of the scaffold fabrication is suggested as a reasonable and effective strategy. SF has been successfully printed by extrusion-, inkjet-, and laser-based 3D bioprinting [207].

Hydrogel bioinks are an excellent type of matrix for 3D bioprinting as a highly influential technology in tissue engineering to obtain a fast and precise 3D pattern of growth factors, cells, and biomaterials. In order to get rapid printing time and high spatial resolution of compound stereolithographic bioprinting by using digital light processing (DLP) has recently been applied to develop a novel SF-based bioink that has been widely used in biomedical fields due to its positive biological and biochemical properties as biomaterials [208]. Although regenerated SF (RSF) has revealed high-strength mechanical properties and excellent biocompatibility, it can hardly be applicable in 3D bioprinting in the traditional form to fabricate artificial implants. A printable 3D RSF hydrogel with remarkably improved mechanical properties has been recently reported, which is formed by a weak, two-step chemically crosslinking method, including a ripening process. With a maximum compressive modulus of 2.5 MPa, this RSF hydrogel reached the same order of magnitude as natural elastomers such as cartilage. The gelation mechanism exposed that this chemically cross-linked network could form a dense and uniform physical network through constraining the growth of β-sheet structures of RSF to provide high strength and good resilience of RSF hydrogels. Therefore, due to both excellent mechanical properties and high biocompatibility, this double-network hydrogel owns great potential in generating 3D bioprinted scaffolds for tissue engineering applications [209]. As gelatin-based hydrogels with tuned mechanical properties have shown outstanding cytocompatibility profile appropriate enough for tissue engineering applications, a tailorable hydrogel of gelatin with silk fibroin has been recently designed with different loading concentrations of silk fibroin. In order to explore

the effect of silk fibroin loading, biological, chemical, and physico-mechanical properties of the tailored matrix was tested. β-sheet formation of silk was enhanced applying ethanol treatment and led to deployed carbodiimide coupling, which covalently cross-linked the matrix. A considerable increase in cohesive energy was also observed with increasing the concentration of silk fibroin in the gel matrix as well as tuned surface properties to reach the maximum cell adhesion and proliferation confirmed by Rhodamine-DAPI staining. Additionally, MTT assay results verified a certain increase in mitochondrial activity of L929 fibroblast cells for silk fibroin-containing matrix as compared to the bare model making it a reasonable alternative in regenerative medicine [210].

6.6. Decellularized extracellular matrix (dECM)

dECM-based inks are another class of naturally-driven materials used as suitable substrates for 3D bioprinting applications. These substances are usually produced by decellularization of specific types of tissues (e.g., the amnion) and show amazing promises in tissue engineering due to their inherent properties for providing a tissue-specific microenvironment for mammalian regrowth cells. Indeed, including glycoprotein, proteoglycans, and collagenous protein, which can uphold native structures through supporting cell migration [211]. Different dECM-based bioinks have been validated to perform specific functions with different printability properties for target-specific skin [212], vessel [153], kidney [213], liver [214], and bone [215] tissue applications where all represent distinctive features of temperature-responsive gelation in physiological environments [216]. dECM-based bioinks can be fabricated through an applicable protocol and reprocessed as a scaffold in tissue regeneration [217]. According to the results of a

comparative study by Kim et al., ECM-derived porcine skin equivalent bioinks could successfully enhance epidermal organization through promoting dermal compartment stabilization, compared to collagen bioinks in vitro. In addition, using this dECM-based 3D skin, a promotion in re-epithelialization and neovascularization was observed as well as a satisfying wound closure in *in vivo* studies [218].

7. Synthetic 3D printable gel-inks for skin regeneration

Although natural hydrogels or polymers provide a desirable microenvironment mimicking the tissue interfaces for cell activities, their tunable properties are quite low [219]. It is also of interest to mention that synthetic polymers are favorable candidates to adjust the characteristics to improve the printability, cross-linking, and mechanical properties [220]. Among synthetic polymers, Pluronics and poly(ethylene glycol) (PEG) are the most commonly used polymers to produce 3D printable bioinks, but they are not specifically applied to the field of skin regeneration [221, 222]. From a general viewpoint, synthetic polymers can support the 3D printed tissue structures and fully degrade after being implanted without any side effects. Here, we describe a set of biocompatible synthetic polymers, including thermoplastic polycaprolactone (PCL), poly(lactic acid) (PLA), and polyurethane, which have the Food and Drug Administration (FDA) approval for use in the human body with special reference to skin regeneration and are broadly applied in 3D bioprinting field of tissue engineering [223-226].

7.1. Poly(ε-caprolactone) (PCL)

Poly(ε-caprolactone) (PCL) is a linear aliphatic polyester, which has been extensively used in biomedical applications. This polymer is hydrophobic and semicrystalline (50%), showing good biocompatibility and relatively slow degradability in the human body. The usefulness of PCL, either alone or in combination with other polymers (e.g., collagen and gelatin), in managing different skin wounds has been previously reported [227-229]. Indeed, poor hydrophilicity and slow degradation lead to designing and using PCL composites in tissue engineering applications.

PCL, as a thermoplastic polymer, shows several desirable features, including good stability under ambient conditions and ease of processability (thermal and solution), makes it an appropriate candidate for 3D bioprinting [230, 231]. PCL superior printability stems from its low melting temperature and glass transition temperature. Furthermore, it is an applicable biomaterial clinically approved by the FDA as a biodegradable and biocompatible polymer [232]. One of the challenges in employing 3D biomaterials is their degradation rate that should be carefully taken into consideration before the construction of the tissue-engineered target-specific structures. Quick degradation of 3D scaffolds leads to a possible mechanical deficiency and subsequently a rapid degradation of implants in the body. In this regard, PCL can act beneficial by controlling the degradation rate of bioinks through merging different ratios of the polymer and copolymers [233, 234]. The degradation mechanism of the PCL runs through a bulk erosion hydrolysis process in which no toxic components are released [235, 236]. Therefore, due to these useful benefits, PCL is actively utilized as an efficient bioprinting material. In a recently published study, copolymers PCL-block-poly(1,3-propylene succinate) (PCL-PPSu) containing silver particles were prepared to provide a lower processing temperature ink as compared to neat PCL [237]. This approach could enhance the degradation behavior and render antibacterial features without any adverse effects on human dermal fibroblast (HDF) viability. Applying this approach,

the inclusion of temperature-sensitive bioactive reagents into 3D printable inks might be realized. The possibility of hot-melt extrusion of PCL containing Ag, Cu, and Zn elements was also shown for 3D printing of antibacterial personalized wound dressings [238].

7.2. Poly(lactic acid) (PLA)

Poly(lactic acid) (PLA), known as polylactide, is an aliphatic polyester with a broad range of applications in biomedical engineering [239]. This polymer shows good biocompatibility and degradation without remaining toxic byproducts in the body. In addition, PLA has tailorable features and well-established processing technologies, including injection molding and extrusion [240]. However, some drawbacks are mentioned for PLA, such as its poor toughness, slow degradation rate, and hydrophobicity. Regarding tissue repair and regeneration approaches, PLA could be successfully applied as a wound-healing material in different shapes and forms [241-243]. Electrospun composites of PLA (e.g., cellulose–PLA, PCL-PLA, and PGA-PLA) have been the most widely prepared and used constructs for skin regeneration applications.

Due to its accessible thermoplastic properties, PLA has been used as a potent biomaterial in frequency 3D bioprinting applications [244]. Despite some molecular weight-dependent differences, PLA owns quite high mechanical properties with an approximate tensile strength of 50–70 MPa and tensile modulus of 3 GPa [245]. Although the molecular weight plays a major role in biodegradability, PLA with high molecular weight may cause infection and inflammation *in vivo* [246]. Thus, molecular weight properties should be carefully considered before 3D bioprinting due to the effects on the mechanical properties of the target tissues.

7.3. Polyurethane (PU)

Polyurethane (PU) is a thermoset polymer made of urethane links and is considered a versatile material for biomedical setting due to its appropriate biocompatibility, biodegradation, good oxygen and carbon dioxide permeability, mechanical integrity, toughness, durability, and moldable properties. PU either alone or blended with other polymers has been employed for skin repair and regeneration; there several commercially available PU-based dressings for managing wounds, including OpSite®, 3M® Tegaderm®, Medifoam® N, and Bioclusive® [247, 248]. Most of these constructs are commonly used as thin films; however, the use of PU-based composites has also shown promise in treating various skin injuries (e.g., full-thickness wounds) [249].

Using advanced 3D bioprinting techniques, PU can be processed closely into the native ECM of human tissues and organs to achieve improved tissue healing [250]. This polymer has been assessed in different bioprinting systems to create 3D scaffolds with uniform pore patterns and precise control over pore size, shape, and dimensions [251].

8. Conclusion

Over the last two decades, a range of 3D printing technologies have been successfully applied to prepare wound dressings based on both natural and synthetic polymers in the attempt to promote skin regeneration even in very dramatic cases, like diabetic ulcers or severe and wide burns. Man-made polymers allow fine-tuning of the physico-chemical and mechanical properties of the final product as compared to natural substances. Furthermore, their use in tissue-engineered constructs permits overcoming the limitations associated to autologous and allogenic skin grafts. Polymers can be used alone either as thin films or as micropatterned porous structures (scaffolds); some of these products (e.g. beased on polyurethane) have received FDA approval for clinical used and are currently marteketed all around the world and available to clinicians. A special set of advanced manufacturing strategies, collectively called 3D bioplotting, allows the simultaneous printing of both polymeric gels and cells, thus yielding ready-to-use cell-laden constructs; sterilization, commercialization and storage of such products still remain partially open issues.

Polymeric gels, being soft and sometimes exhibiting thermoreversible properties, are ideal biomaterials to produce printable inks. However, not all biocompatible polymers are inherently functional for promoting skin regeneration from a "biological viewpoint". In other words, while some natural polymers exhibit alone key properties for skin repair, such as pro-angiogenic (e.g. hyaluronic acid) or antibacterial functions (e.g. chitosan), synthetic polymers often do not elicit any beneficial "active" action, apart from wound protection and passive support to host tissue. Regenerative functions can be provided to polymeric gel inks by incorporating cells, therapeutic ions having, for example, a pro-angiogenic (Cu^{2+}) or antimicrobial effect (Ag^{+}), or bioactive inclusions such as bioactive glasses. The last option is highly versatile and opens new horizons in the field of wound healing, while carrying new technological challenges related to the design and actual printability of polymer-based inks that contain rigid micro- or nano-particles inside.

1. BURN, M. and W. SHEEP, *Design principles for composition and performance of cultured skin substitutes.* Burns, 2001. **27**: p. 523-33.
2. Tobin, D.J., *Biochemistry of human skin—our brain on the outside.* Chemical Society Reviews, 2006. **35**(1): p. 52-67.
3. Parenteau, N.L., et al., *The organotypic culture of human skin keratinocytes and fibroblasts to achieve form and function.* Cytotechnology, 1992. **9**(1-3): p. 163-171.
4. Supp, D.M. and S.T. Boyce, *Engineered skin substitutes: practices and potentials.* Clinics in dermatology, 2005. **23**(4): p. 403-412.
5. Groeber, F., et al., *Skin tissue engineering—in vivo and in vitro applications.* Advanced drug delivery reviews, 2011. **63**(4-5): p. 352-366.
6. Shevchenko, R.V., S.L. James, and S.E. James, *A review of tissue-engineered skin bioconstructs available for skin reconstruction.* Journal of the Royal Society Interface, 2010. **7**(43): p. 229-258.
7. Volk, S.W., S.A. Iqbal, and A. Bayat, *Interactions of the extracellular matrix and progenitor cells in cutaneous wound healing.* Advances in wound care, 2013. **2**(6): p. 261-272.
8. Pereira, R.F., et al., *Advances in bioprinted cell-laden hydrogels for skin tissue engineering.* Biomanufacturing Reviews, 2017. **2**(1): p. 1.
9. Ennis, W.J. and D. Hill, *Wound healing: a comprehensive wound assessment and treatment approach.* Skin Tissue Eng Regen Med, 2016. **239**: p. 75-81.
10. Gaur, M., M. Dobke, and V.V. Lunyak, *Mesenchymal Stem Cells from Adipose Tissue in Clinical Applications for Dermatological Indications and Skin Aging.* International Journal of Molecular Sciences, 2017. **18**(1): p. 208.
11. McGrath, J., R. Eady, and F. Pope, *Anatomy and organization of human skin.* Rook's textbook of dermatology, 2004. **1**: p. 3.2-3.80.
12. Weinstein, G.D., J.L. McCullough, and P. Ross, *Cell proliferation in normal epidermis.* Journal of investigative dermatology, 1984. **82**(6).
13. Steinhoff, M., T. Brzoska, and T.A. Luger, *Keratinocytes in epidermal immune responses.* Current opinion in allergy and clinical immunology, 2001. **1**(5): p. 469-476.
14. Visscher, M. and V. Narendran, *Neonatal Infant Skin: Development, Structure and Function.* Newborn and Infant Nursing Reviews, 2014. **14**(4): p. 135-141.

15. Cichorek, M., et al., *Skin melanocytes: biology and development.* Advances in Dermatology and Allergology/Postępy Dermatologii I Alergologii, 2013. **30**(1): p. 30.
16. Johnson, J., et al., *P53 family activities in development and cancer: relationship to melanocyte and keratinocyte carcinogenesis.* Journal of investigative dermatology, 2005. **125**(5): p. 857-864.
17. Nguyen, D., D. Orgill, and G. Murphy, *The pathophysiologic basis for wound healing and cutaneous regeneration*, in *Biomaterials for treating skin loss*. 2009, Elsevier. p. 25-57.
18. Fenner, J. and R. Clark, *Anatomy, physiology, histology, and immunohistochemistry of human skin.* Skin tissue engineering and regenerative medicine, 2016. **1**.
19. Reed, C.C. and R.V. Iozzo, *The role of decorin in collagen fibrillogenesis and skin homeostasis.* Glycoconjugate journal, 2002. **19**(4-5): p. 249-255.
20. Oxlund, H., J. Manschot, and A. Viidik, *The role of elastin in the mechanical properties of skin.* Journal of biomechanics, 1988. **21**(3): p. 213-218.
21. Nyman, E., et al., *Hyaluronic acid, an important factor in the wound healing properties of amniotic fluid: in vitro studies of re-epithelialisation in human skin wounds.* Journal of plastic surgery and hand surgery, 2013. **47**(2): p. 89-92.
22. Gallo, R.L., et al., *The Potential Role of Topically Applied Heparan Sulfate in the Treatment of Photodamage.* J Drugs Dermatol, 2015. **14**(7): p. 669-74.
23. Olivieri, J., S. Smaldone, and F. Ramirez, *Fibrillin assemblies: extracellular determinants of tissue formation and fibrosis.* Fibrogenesis & tissue repair, 2010. **3**(1): p. 24.
24. Fyrand, O., *Studies on fibronectin in the skin.* Archives of Dermatological Research, 1979. **266**(1): p. 33-41.
25. Johnson, M.B., et al., *Topical fibronectin improves wound healing of irradiated skin.* Scientific reports, 2017. **7**(1): p. 1-10.
26. Nishiyama, T., et al., *The importance of laminin 5 in the dermal–epidermal basement membrane.* Journal of dermatological science, 2000. **24**: p. S51-S59.
27. Iorio, V., L.D. Troughton, and K.J. Hamill, *Laminins: roles and utility in wound repair.* Advances in wound care, 2015. **4**(4): p. 250-263.
28. Duda, D.G., et al., *A protocol for phenotypic detection and enumeration of circulating endothelial cells and circulating progenitor cells in human blood.* Nature protocols, 2007. **2**(4): p. 805.
29. Erickson, J.R. and K. Echeverri, *Learning from regeneration research organisms: The circuitous road to scar free wound healing.* Developmental biology, 2018. **433**(2): p. 144-154.
30. Gurtner, G.C., et al., *Wound repair and regeneration.* Nature, 2008. **453**(7193): p. 314-321.
31. Marikovsky, M., et al., *Appearance of Heparin-Binding EGF-Like Growth Factor in Wound Fluid as a Response to Injury.* Proceedings of the National Academy of Sciences of the United States of America, 1993. **90**(9): p. 3889-3893.
32. Werner, S., et al., *Differential splicing in the extracellular region of fibroblast growth factor receptor 1 generates receptor variants with different ligand-binding specificities.* Mol Cell Biol, 1992. **12**(1): p. 82-8.
33. Eriksson, A., et al., *PDGF alpha- and beta-receptors activate unique and common signal transduction pathways.* Embo j, 1992. **11**(2): p. 543-50.

34. Rappolee, D.A., et al., *Wound macrophages express TGF-alpha and other growth factors in vivo: analysis by mRNA phenotyping.* Science, 1988. **241**(4866): p. 708-12.
35. Losi, P., et al., *Fibrin-based scaffold incorporating VEGF-and bFGF-loaded nanoparticles stimulates wound healing in diabetic mice.* Acta biomaterialia, 2013. **9**(8): p. 7814-7821.
36. Schmitt, S., et al., *Stathmin regulates keratinocyte proliferation and migration during cutaneous regeneration.* PLoS One, 2013. **8**(9): p. e75075.
37. Bevan, D., et al., *Diverse and potent activities of HGF/SF in skin wound repair.* J Pathol, 2004. **203**(3): p. 831-8.
38. Jackson, W.M., L.J. Nesti, and R.S. Tuan, *Mesenchymal stem cell therapy for attenuation of scar formation during wound healing.* Stem Cell Res Ther, 2012. **3**(3): p. 20.
39. Madlener, M., et al., *Regulation of the expression of stromelysin-2 by growth factors in keratinocytes: implications for normal and impaired wound healing.* Biochem J, 1996. **320 (Pt 2)**(Pt 2): p. 659-64.
40. Frank, S., M. Madlener, and S. Werner, *Transforming growth factors beta1, beta2, and beta3 and their receptors are differentially regulated during normal and impaired wound healing.* J Biol Chem, 1996. **271**(17): p. 10188-93.
41. Hübner, G., et al., *Differential regulation of pro-inflammatory cytokines during wound healing in normal and glucocorticoid-treated mice.* Cytokine, 1996. **8**(7): p. 548-56.
42. Salmon-Ehr, V., et al., *Implication of interleukin-4 in wound healing.* Lab Invest, 2000. **80**(8): p. 1337-43.
43. Mattey, D.L., *Interleukin-4 induces myofibroblast differentiation is synovial fibroblasts.* Biochem Soc Trans, 1997. **25**(2): p. 290s.
44. Peranteau, W.H., et al., *IL-10 overexpression decreases inflammatory mediators and promotes regenerative healing in an adult model of scar formation.* J Invest Dermatol, 2008. **128**(7): p. 1852-60.
45. Krishnamurthy, P., et al., *Interleukin-10 deficiency impairs bone marrow-derived endothelial progenitor cell survival and function in ischemic myocardium.* Circ Res, 2011. **109**(11): p. 1280-9.
46. King, A., et al., *Regenerative Wound Healing: The Role of Interleukin-10.* Adv Wound Care (New Rochelle), 2014. **3**(4): p. 315-323.
47. Matias, M.A., et al., *Accelerated wound healing phenotype in Interleukin 12/23 deficient mice.* J Inflamm (Lond), 2011. **8**: p. 39.
48. Clark, R.A., *Wound repair: basic biology to tissue engineering*, in *Principles of tissue engineering*. 2014, Elsevier. p. 1595-1617.
49. Schultz, G.S. and A. Wysocki, *Interactions between extracellular matrix and growth factors in wound healing.* Wound repair and regeneration, 2009. **17**(2): p. 153-162.
50. Wu, Y. and E. Tredget, *Pathology of tissue regeneration repair: skin regeneration.* 2014.
51. Babu, P.S., N. Danilovich, and M. Sairam, *Hormone-induced receptor gene splicing: enhanced expression of the growth factor type I follicle-stimulating hormone receptor motif in the developing mouse ovary as a new paradigm in growth regulation.* Endocrinology, 2001. **142**(1): p. 381-389.
52. Levy, V., et al., *Epidermal stem cells arise from the hair follicle after wounding.* The FASEB Journal, 2007. **21**(7): p. 1358-1366.
53. Ito, M., et al., *Stem cells in the hair follicle bulge contribute to wound repair but not to homeostasis of the epidermis.* Nature medicine, 2005. **11**(12): p. 1351-1354.

54. Brodsky, S., et al., *Plasmin-dependent and-independent effects of plasminogen activators and inhibitor-1 on ex vivo angiogenesis.* American Journal of Physiology-Heart and Circulatory Physiology, 2001. **281**(4): p. H1784-H1792.
55. Velnar, T., T. Bailey, and V. Smrkolj, *The wound healing process: an overview of the cellular and molecular mechanisms.* Journal of International Medical Research, 2009. **37**(5): p. 1528-1542.
56. Diegelman, R. and M. Evans, *Wound healing: Anoverview of acute, fibrotic and delayed.* Frontiers in Bioscience, 2004: p. 9,283-289.
57. Madden, J.W. and E.E. Peacock Jr, *Studies on the biology of collagen during wound healing. 3. Dynamic metabolism of scar collagen and remodeling of dermal wounds.* Annals of surgery, 1971. **174**(3): p. 511.
58. Hinz, B., *The role of myofibroblasts in wound healing.* Current research in translational medicine, 2016. **64**(4): p. 171-177.
59. Guo, S.a. and L.A. DiPietro, *Factors affecting wound healing.* Journal of dental research, 2010. **89**(3): p. 219-229.
60. Menke, N.B., et al., *Impaired wound healing.* Clinics in dermatology, 2007. **25**(1): p. 19-25.
61. Moseley, R., et al., *Comparison of oxidative stress biomarker profiles between acute and chronic wound environments.* Wound repair and regeneration, 2004. **12**(4): p. 419-429.
62. Herrick, S., et al., *Sequential changes in histologic pattern and extracellular matrix deposition during the healing of chronic venous ulcers.* The American journal of pathology, 1992. **141**(5): p. 1085.
63. Halim, A.S., T.L. Khoo, and S.J.M. Yussof, *Biologic and synthetic skin substitutes: an overview.* Indian journal of plastic surgery: official publication of the Association of Plastic Surgeons of India, 2010. **43**(Suppl): p. S23.
64. Shores, J.T., A. Gabriel, and S. Gupta, *Skin substitutes and alternatives: a review.* Advances in skin & wound care, 2007. **20**(9): p. 493-508.
65. Böttcher-Haberzeth, S., T. Biedermann, and E. Reichmann, *Tissue engineering of skin.* Burns, 2010. **36**(4): p. 450-460.
66. Zweifel, C., et al., *Initial experiences using non-cultured autologous keratinocyte suspension for burn wound closure.* Journal of Plastic, Reconstructive & Aesthetic Surgery, 2008. **61**(11): p. e1-e4.
67. Gravante, G., et al., *A randomized trial comparing ReCell® system of epidermal cells delivery versus classic skin grafts for the treatment of deep partial thickness burns.* Burns, 2007. **33**(8): p. 966-972.
68. Philandrianos, C., et al., *Comparison of five dermal substitutes in full-thickness skin wound healing in a porcine model.* Burns, 2012. **38**(6): p. 820-829.
69. van der Veen, V.C., et al., *New dermal substitutes.* Wound Repair and Regeneration, 2011. **19**: p. s59-s65.
70. Van der Veen, V.C., et al., *Biological background of dermal substitutes.* Burns, 2010. **36**(3): p. 305-321.
71. Hansen, S.L., et al., *Using skin replacement products to treat burns and wounds.* Advances in skin & wound care, 2001. **14**(1): p. 37-46.
72. Pham, C., et al., *Bioengineered skin substitutes for the management of burns: a systematic review.* Burns, 2007. **33**(8): p. 946-957.

73. Bártolo, P.J., et al., *Biofabrication strategies for tissue engineering*, in *Advances on Modeling in Tissue Engineering*. 2011, Springer. p. 137-176.
74. Nichol, J.W. and A. Khademhosseini, *Modular tissue engineering: engineering biological tissues from the bottom up.* Soft matter, 2009. **5**(7): p. 1312-1319.
75. Du, Y., et al., *Directed assembly of cell-laden microgels for fabrication of 3D tissue constructs.* Proceedings of the National Academy of Sciences, 2008. **105**(28): p. 9522-9527.
76. Khademhosseini, A., et al., *Microscale technologies for tissue engineering and biology.* Proceedings of the National Academy of Sciences, 2006. **103**(8): p. 2480-2487.
77. Bartolo, P., et al., *Biomedical production of implants by additive electro-chemical and physical processes.* CIRP annals, 2012. **61**(2): p. 635-655.
78. Bártolo, P., et al., *Biomanufacturing for tissue engineering: present and future trends.* Virtual and Physical Prototyping, 2009. **4**(4): p. 203-216.
79. Melchels, F.P., et al., *Additive manufacturing of tissues and organs.* Progress in Polymer Science, 2012. **37**(8): p. 1079-1104.
80. Guillotin, B. and F. Guillemot, *Cell patterning technologies for organotypic tissue fabrication.* Trends in biotechnology, 2011. **29**(4): p. 183-190.
81. Jakab, K., et al., *Engineering biological structures of prescribed shape using self-assembling multicellular systems.* Proceedings of the National Academy of Sciences, 2004. **101**(9): p. 2864-2869.
82. Censi, R., et al., *Hydrogels for protein delivery in tissue engineering.* Journal of Controlled Release, 2012. **161**(2): p. 680-692.
83. de Amorim Almeida, H. and P.J. da Silva Bártolo, *Virtual topological optimisation of scaffolds for rapid prototyping.* Medical engineering & physics, 2010. **32**(7): p. 775-782.
84. Hosseinkhani, H., et al., *Enhanced angiogenesis through controlled release of basic fibroblast growth factor from peptide amphiphile for tissue regeneration.* Biomaterials, 2006. **27**(34): p. 5836-5844.
85. Mohamed, A. and M.M. Xing, *Nanomaterials and nanotechnology for skin tissue engineering.* International journal of burns and trauma, 2012. **2**(1): p. 29.
86. Smith, L. and P. Ma, *Nano-fibrous scaffolds for tissue engineering.* Colloids and surfaces B: biointerfaces, 2004. **39**(3): p. 125-131.
87. Zhong, S., Y. Zhang, and C. Lim, *Tissue scaffolds for skin wound healing and dermal reconstruction.* Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology, 2010. **2**(5): p. 510-525.
88. Chandrasekaran, A.R., et al., *Fabrication of a nanofibrous scaffold with improved bioactivity for culture of human dermal fibroblasts for skin regeneration.* Biomedical materials, 2011. **6**(1): p. 015001.
89. Heunis, T. and L. Dicks, *Nanofibers offer alternative ways to the treatment of skin infections.* Journal of Biomedicine and Biotechnology, 2010. **2010**.
90. Zahedi, P., et al., *A review on wound dressings with an emphasis on electrospun nanofibrous polymeric bandages.* Polymers for Advanced Technologies, 2010. **21**(2): p. 77-95.
91. Mitchella, G.R., K.-h. Ahnb, and F.J. Davisb, *The potential of electrospinning in rapid manufacturing processes.*

92. Cunha, C., S. Panseri, and S. Antonini, *Emerging nanotechnology approaches in tissue engineering for peripheral nerve regeneration.* Nanomedicine: Nanotechnology, Biology and Medicine, 2011. **7**(1): p. 50-59.
93. Bhardwaj, N. and S.C. Kundu, *Electrospinning: a fascinating fiber fabrication technique.* Biotechnology advances, 2010. **28**(3): p. 325-347.
94. Lee, J.K.Y., et al., *Polymer-based composites by electrospinning: Preparation & functionalization with nanocarbons.* Progress in Polymer Science, 2018. **86**: p. 40-84.
95. Zhang, Y., et al., *Biomimetic and bioactive nanofibrous scaffolds from electrospun composite nanofibers.* International journal of nanomedicine, 2007. **2**(4): p. 623.
96. Dhandayuthapani, B., U.M. Krishnan, and S. Sethuraman, *Fabrication and characterization of chitosan□gelatin blend nanofibers for skin tissue engineering.* Journal of Biomedical Materials Research Part B: Applied Biomaterials, 2010. **94**(1): p. 264-272.
97. Powell, H. and S. Boyce, *Fiber density of electrospun gelatin scaffolds regulates morphogenesis of dermal–epidermal skin substitutes.* Journal of Biomedical Materials Research Part A: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials, 2008. **84**(4): p. 1078-1086.
98. Cui, W., et al., *Evaluation of electrospun fibrous scaffolds of poly (dl-lactide) and poly (ethylene glycol) for skin tissue engineering.* Materials Science and Engineering: C, 2009. **29**(6): p. 1869-1876.
99. Kumbar, S.G., et al., *Electrospun poly (lactic acid-co-glycolic acid) scaffolds for skin tissue engineering.* Biomaterials, 2008. **29**(30): p. 4100-4107.
100. Chen, H., et al., *Electrospun chitosan-graft-poly (ε-caprolactone)/poly (ε-caprolactone) cationic nanofibrous mats as potential scaffolds for skin tissue engineering.* International journal of biological macromolecules, 2011. **48**(1): p. 13-19.
101. Zhou, Y., et al., *Electrospun water-soluble carboxyethyl chitosan/poly (vinyl alcohol) nanofibrous membrane as potential wound dressing for skin regeneration.* Biomacromolecules, 2008. **9**(1): p. 349-354.
102. Nazarnezhad, S., et al., *Electrospun Nanofibers for Improved Angiogenesis: Promises for Tissue Engineering Applications.* Nanomaterials, 2020. **10**(8): p. 1609.
103. Yildirimer, L., N.T. Thanh, and A.M. Seifalian, *Skin regeneration scaffolds: a multimodal bottom-up approach.* Trends in biotechnology, 2012. **30**(12): p. 638-648.
104. Choi, J.S., K.W. Leong, and H.S. Yoo, *In vivo wound healing of diabetic ulcers using electrospun nanofibers immobilized with human epidermal growth factor (EGF).* Biomaterials, 2008. **29**(5): p. 587-596.
105. Yang, Y., et al., *Promotion of skin regeneration in diabetic rats by electrospun core-sheath fibers loaded with basic fibroblast growth factor.* Biomaterials, 2011. **32**(18): p. 4243-4254.
106. Shalumon, K., et al., *Sodium alginate/poly (vinyl alcohol)/nano ZnO composite nanofibers for antibacterial wound dressings.* International journal of biological macromolecules, 2011. **49**(3): p. 247-254.
107. Suganya, S., et al., *Herbal drug incorporated antibacterial nanofibrous mat fabricated by electrospinning: an excellent matrix for wound dressings.* Journal of Applied Polymer Science, 2011. **121**(5): p. 2893-2899.

108. Tavakoli, S. and A.S. Klar, *Advanced Hydrogels as Wound Dressings.* Biomolecules, 2020. **10**(8): p. 1169.
109. Gupta, P., K. Vermani, and S. Garg, *Hydrogels: from controlled release to pH-responsive drug delivery.* Drug discovery today, 2002. **7**(10): p. 569-579.
110. Ahmed, E.M., *Hydrogel: Preparation, characterization, and applications: A review.* Journal of advanced research, 2015. **6**(2): p. 105-121.
111. Pereira, R.F. and P.J. Bártolo, *Photopolymerizable hydrogels in regenerative medicine and drug delivery*. 2014, Future Medicine.
112. Yang, J.-A., et al., *In situ-forming injectable hydrogels for regenerative medicine.* Progress in Polymer Science, 2014. **39**(12): p. 1973-1986.
113. Hunt, N.C., R.M. Shelton, and L. Grover, *An alginate hydrogel matrix for the localised delivery of a fibroblast/keratinocyte co□culture.* Biotechnology journal, 2009. **4**(5): p. 730-737.
114. Lootens, L., et al., *Keratinocytes in the treatment of severe burn injury: an update.* International wound journal, 2013. **10**(1): p. 6-12.
115. Mironov, V., et al., *Organ printing: tissue spheroids as building blocks.* Biomaterials, 2009. **30**(12): p. 2164-2174.
116. Mironov, V., et al., *Organ printing: computer-aided jet-based 3D tissue engineering.* TRENDS in Biotechnology, 2003. **21**(4): p. 157-161.
117. Zhang, Y.S., et al., *3D bioprinting for tissue and organ fabrication.* Annals of biomedical engineering, 2017. **45**(1): p. 148-163.
118. Guillotin, B., et al., *Laser assisted bioprinting of engineered tissue with high cell density and microscale organization.* Biomaterials, 2010. **31**(28): p. 7250-7256.
119. Unkovskiy, A., et al., *Additive manufacturing: a comparative analysis of dimensional accuracy and skin texture reproduction of auricular prostheses replicas.* Journal of Prosthodontics, 2019. **28**(2): p. e460-e468.
120. Hakimi, N., et al., *Handheld skin printer: in situ formation of planar biomaterials and tissues.* Lab on a Chip, 2018. **18**(10): p. 1440-1451.
121. Sun, W., et al., *The bioprinting roadmap.* Biofabrication, 2020. **12**(2): p. 022002.
122. Michael, S., et al., *Tissue engineered skin substitutes created by laser-assisted bioprinting form skin-like structures in the dorsal skin fold chamber in mice.* PloS one, 2013. **8**(3): p. e57741.
123. Koch, L., et al., *Laser assisted cell printing.* Current pharmaceutical biotechnology, 2013. **14**(1): p. 91-97.
124. Seol, Y.-J., et al., *3D bioprinted biomask for facial skin reconstruction.* Bioprinting, 2018. **10**: p. e00028.
125. Obata, K., et al., *High-aspect 3D two-photon polymerization structuring with widened objective working range (WOW-2PP).* Light: Science & Applications, 2013. **2**(12): p. e116-e116.
126. Malda, J., et al., *25th anniversary article: engineering hydrogels for biofabrication.* Advanced materials, 2013. **25**(36): p. 5011-5028.
127. Singh, D., D. Singh, and S.S. Han, *3D printing of scaffold for cells delivery: Advances in skin tissue engineering.* Polymers, 2016. **8**(1): p. 19.
128. Peng, W., D. Unutmaz, and I.T. Ozbolat, *Bioprinting towards physiologically relevant tissue models for pharmaceutics.* Trends in biotechnology, 2016. **34**(9): p. 722-732.

129. Seol, Y.-J., et al., *Bioprinting technology and its applications.* European Journal of Cardio-Thoracic Surgery, 2014. **46**(3): p. 342-348.
130. Gudapati, H., M. Dey, and I. Ozbolat, *A comprehensive review on droplet-based bioprinting: past, present and future.* Biomaterials, 2016. **102**: p. 20-42.
131. Li, K., et al., *Controllable printing droplets on demand by piezoelectric inkjet: applications and methods.* Microsystem technologies, 2018. **24**(2): p. 879-889.
132. Matai, I., et al., *Progress in 3D bioprinting technology for tissue/organ regenerative engineering.* Biomaterials, 2020. **226**: p. 119536.
133. Mandrycky, C., et al., *3D bioprinting for engineering complex tissues.* Biotechnology advances, 2016. **34**(4): p. 422-434.
134. McCormack, A., et al., *3D Printing in Suspension Baths: Keeping the Promises of Bioprinting Afloat.* Trends in Biotechnology, 2020. **38**(6): p. 584-593.
135. Shim, J.-H., et al., *Bioprinting of a mechanically enhanced three-dimensional dual cell-laden construct for osteochondral tissue engineering using a multi-head tissue/organ building system.* Journal of Micromechanics and Microengineering, 2012. **22**(8): p. 085014.
136. Gao, G., et al., *Recent strategies in extrusion-based three-dimensional cell printing toward organ biofabrication.* ACS Biomaterials Science & Engineering, 2019. **5**(3): p. 1150-1169.
137. Khalil, S. and W. Sun, *Biopolymer deposition for freeform fabrication of hydrogel tissue constructs.* Materials Science and Engineering: C, 2007. **27**(3): p. 469-478.
138. Tigner, T.J., et al., *Comparison of photo cross linkable gelatin derivatives and initiators for three-dimensional extrusion bioprinting.* Biomacromolecules, 2019. **21**(2): p. 454-463.
139. Turner, P.R., et al., *Peptide chitosan/dextran core/shell vascularized 3D constructs for wound healing.* ACS Applied Materials & Interfaces, 2020. **12**(29): p. 32328-32339.
140. Yue, Z., et al., *Advances in printing biomaterials and living cells: implications for islet cell transplantation.* Current opinion in organ transplantation, 2016. **21**(5): p. 467-475.
141. Wang, Z., et al., *A simple and high-resolution stereolithography-based 3D bioprinting system using visible light crosslinkable bioinks.* Biofabrication, 2015. **7**(4): p. 045009.
142. Zhou, X., et al., *Three-Dimensional Printing Biologically Inspired DNA-Based Gradient Scaffolds for Cartilage Tissue Regeneration.* ACS Applied Materials & Interfaces, 2020. **12**(29): p. 33219-33228.
143. Lin, H., et al., *Application of visible light-based projection stereolithography for live cell-scaffold fabrication with designed architecture.* Biomaterials, 2013. **34**(2): p. 331-339.
144. Melchels, F.P., J. Feijen, and D.W. Grijpma, *A review on stereolithography and its applications in biomedical engineering.* Biomaterials, 2010. **31**(24): p. 6121-6130.
145. Donderwinkel, I., J.C. Van Hest, and N.R. Cameron, *Bio-inks for 3D bioprinting: recent advances and future prospects.* Polymer Chemistry, 2017. **8**(31): p. 4451-4471.
146. Liang, Y., et al., *Direct electrohydrodynamic patterning of high-performance all metal oxide thin-film electronics.* ACS nano, 2019. **13**(12): p. 13957-13964.
147. He, J., et al., *High-resolution electrohydrodynamic bioprinting: a new biofabrication strategy for biomimetic micro/nanoscale architectures and living tissue constructs.* Biofabrication, 2020. **12**(4): p. 042002.
148. Gao, D. and J.G. Zhou, *Designs and applications of electrohydrodynamic 3D printing.* International Journal of Bioprinting, 2019. **5**(1).

149. Mao, M., et al., *Multi-directional cellular alignment in 3D guided by electrohydrodynamically-printed microlattices.* Acta Biomaterialia, 2020. **101**: p. 141-151.
150. Sutterby, E., et al., *Microfluidic Skin□on□a□Chip Models: Toward Biomimetic Artificial Skin.* Small, 2020: p. 2002515.
151. Xu, J., et al., *Advances in the Research of Bioinks Based on Natural Collagen, Polysaccharide and Their Derivatives for Skin 3D Bioprinting.* Polymers, 2020. **12**(6): p. 1237.
152. Au, A.K., et al., *3D□printed microfluidics.* Angewandte Chemie International Edition, 2016. **55**(12): p. 3862-3881.
153. Gao, G., et al., *Tissue engineered bio□blood□vessels constructed using a tissue□specific bioink and 3D coaxial cell printing technique: a novel therapy for ischemic disease.* Advanced functional materials, 2017. **27**(33): p. 1700798.
154. Montero, F.E., et al., *Development of a Smart Bioink for Bioprinting Applications.* Frontiers in Mechanical Engineering, 2019. **5**: p. 56.
155. Gopinathan, J. and I. Noh, *Recent trends in bioinks for 3D printing.* Biomaterials research, 2018. **22**(1): p. 11.
156. Valot, L., et al., *Chemical insights into bioinks for 3D printing.* Chemical Society Reviews, 2019. **48**(15): p. 4049-4086.
157. Smandri, A., et al., *Natural 3D-Printed Bioinks for Skin Regeneration and Wound Healing: A Systematic Review.* Polymers, 2020. **12**(8): p. 1782.
158. Colosi, C., et al., *Microfluidic bioprinting of heterogeneous 3D tissue constructs using low□viscosity bioink.* Advanced materials, 2016. **28**(4): p. 677-684.
159. Aderibigbe, B.A. and B. Buyana, *Alginate in wound dressings.* Pharmaceutics, 2018. **10**(2): p. 42.
160. Pourchet, L.J., et al., *Human Skin 3D Bioprinting Using Scaffold-Free Approach.* Advanced Healthcare Materials, 2017. **6**(4): p. 1601101.
161. Liu, P., et al., *3D bioprinting and in vitro study of bilayered membranous construct with human cells-laden alginate/gelatin composite hydrogels.* Colloids and Surfaces B: Biointerfaces, 2019. **181**: p. 1026-1034.
162. Datta, S., et al., *Alginate-honey bioinks with improved cell responses for applications as bioprinted tissue engineered constructs.* Journal of Materials Research, 2018. **33**(14): p. 2029-2039.
163. Hunt, N.C. and L.M. Grover, *Cell encapsulation using biopolymer gels for regenerative medicine.* Biotechnology letters, 2010. **32**(6): p. 733-742.
164. Skardal, A., et al., *Bioprinted amniotic fluid□derived stem cells accelerate healing of large skin wounds.* Stem cells translational medicine, 2012. **1**(11): p. 792-802.
165. Augustine, R., *Skin bioprinting: a novel approach for creating artificial skin from synthetic and natural building blocks. Prog Biomater 7: 77–92*. 2018.
166. Xue, Z., M. Yang, and D. Xu, *Nucleation of biomimetic hydroxyapatite nanoparticles on the surface of type I collagen: molecular dynamics investigations.* The Journal of Physical Chemistry C, 2019. **123**(4): p. 2533-2543.
167. Nocera, A.D., et al., *Development of 3D printed fibrillar collagen scaffold for tissue engineering.* Biomedical microdevices, 2018. **20**(2): p. 26.
168. Heidenreich, A.C., et al., *Collagen and chitosan blends for 3D bioprinting: A rheological and printability approach.* Polymer Testing, 2020. **82**: p. 106297.

169. Albanna, M., et al., *In situ bioprinting of autologous skin cells accelerates wound healing of extensive excisional full-thickness wounds.* Scientific reports, 2019. **9**(1): p. 1-15.
170. Osidak, E.O., et al., *Viscoll collagen solution as a novel bioink for direct 3D bioprinting.* Journal of Materials Science: Materials in Medicine, 2019. **30**(3): p. 31.
171. Lee, V., et al., *Design and fabrication of human skin by three-dimensional bioprinting.* Tissue Engineering Part C: Methods, 2014. **20**(6): p. 473-484.
172. Kim, J.E., S.H. Kim, and Y. Jung, *Current status of three-dimensional printing inks for soft tissue regeneration.* Tissue engineering and regenerative medicine, 2016. **13**(6): p. 636-646.
173. Ulrich, T.A., et al., *Probing cellular mechanobiology in three-dimensional culture with collagen–agarose matrices.* Biomaterials, 2010. **31**(7): p. 1875-1884.
174. Kim, G., et al., *Coaxial structured collagen–alginate scaffolds: fabrication, physical properties, and biomedical application for skin tissue regeneration.* Journal of Materials Chemistry, 2011. **21**(17): p. 6165-6172.
175. Ma, L., et al., *Collagen/chitosan porous scaffolds with improved biostability for skin tissue engineering.* Biomaterials, 2003. **24**(26): p. 4833-4841.
176. Han, C.-m., et al., *Application of collagen-chitosan/fibrin glue asymmetric scaffolds in skin tissue engineering.* Journal of Zhejiang University Science B, 2010. **11**(7): p. 524-530.
177. Shin, J.-Y., S.-J. Jeong, and W.-K. Lee, *Fabrication of porous scaffold by ternary combination of chitosan, gelatin, and calcium phosphate for tissue engineering.* Journal of Industrial and Engineering Chemistry, 2019. **80**: p. 862-869.
178. Choi, D.J., et al., *Effect of the pore size in a 3D bioprinted gelatin scaffold on fibroblast proliferation.* Journal of Industrial and Engineering Chemistry, 2018. **67**: p. 388-395.
179. Xu, W., et al., *On low-concentration inks formulated by nanocellulose assisted with gelatin methacrylate (gelma) for 3D printing toward wound healing application.* ACS applied materials & interfaces, 2019. **11**(9): p. 8838-8848.
180. Shi, L., et al., *Three□dimensional printing alginate/gelatin scaffolds as dermal substitutes for skin tissue engineering.* Polymer Engineering & Science, 2018. **58**(10): p. 1782-1790.
181. Huang, L., et al., *Bacterial cellulose nanofibers promote stress and fidelity of 3D-printed silk based hydrogel scaffold with hierarchical pores.* Carbohydrate polymers, 2019. **221**: p. 146-156.
182. Chen, C.-S., et al., *Three-dimensionally printed silk-sericin-based hydrogel scaffold: a promising visualized dressing material for real-time monitoring of wounds.* ACS applied materials & interfaces, 2018. **10**(40): p. 33879-33890.
183. Wang, X., et al., *Generation of three-dimensional hepatocyte/gelatin structures with rapid prototyping system.* Tissue engineering, 2006. **12**(1): p. 83-90.
184. Ouyang, L., et al., *Effect of bioink properties on printability and cell viability for 3D bioplotting of embryonic stem cells.* Biofabrication, 2016. **8**(3): p. 035020.
185. Roehm, K.D. and S.V. Madihally, *Bioprinted chitosan-gelatin thermosensitive hydrogels using an inexpensive 3D printer.* Biofabrication, 2017. **10**(1): p. 015002.
186. Sharma, R., et al., *3D bioprinting pluripotent stem cell derived neural tissues using a novel fibrin bioink containing drug releasing microspheres.* Frontiers in bioengineering and biotechnology, 2020. **8**: p. 57.

187. Shin, J.H. and H.-W. Kang, *The development of gelatin-based bio-ink for use in 3D hybrid bioprinting.* International Journal of Precision Engineering and Manufacturing, 2018. **19**(5): p. 767-771.
188. Xiong, S., et al., *A gelatin-sulfonated silk composite scaffold based on 3D printing technology enhances skin regeneration by stimulating epidermal growth and dermal neovascularization.* Scientific reports, 2017. **7**(1): p. 1-12.
189. Das, S., et al., *Bioprintable, cell-laden silk fibroin–gelatin hydrogel supporting multilineage differentiation of stem cells for fabrication of three-dimensional tissue constructs.* Acta biomaterialia, 2015. **11**: p. 233-246.
190. Gauvin, R., et al., *Microfabrication of complex porous tissue engineering scaffolds using 3D projection stereolithography.* Biomaterials, 2012. **33**(15): p. 3824-3834.
191. Chen, X., et al., *Development of rhamnose-rich hydrogels based on sulfated xylorhamno-uronic acid toward wound healing applications.* Biomaterials science, 2019. **7**(8): p. 3497-3509.
192. Patrulea, V., et al., *Chitosan as a starting material for wound healing applications.* European Journal of Pharmaceutics and Biopharmaceutics, 2015. **97**: p. 417-426.
193. Pahlevanzadeh, F., et al., *Three-Dimensional Printing Constructs Based on the Chitosan for Tissue Regeneration: State of the Art, Developing Directions and Prospect Trends.* Materials (Basel, Switzerland), 2020. **13**(11): p. 2663.
194. Intini, C., et al., *3D-printed chitosan-based scaffolds: An in vitro study of human skin cell growth and an in-vivo wound healing evaluation in experimental diabetes in rats.* Carbohydrate Polymers, 2018. **199**: p. 593-602.
195. Ng, W.L., W.Y. Yeong, and M.W. Naing, *Polyelectrolyte gelatin-chitosan hydrogel optimized for 3D bioprinting in skin tissue engineering.* International Journal of Bioprinting, 2016. **2**(1).
196. Ng, W.L., W.Y. Yeong, and M.W. Naing, *Development of polyelectrolyte chitosan-gelatin hydrogels for skin bioprinting.* Procedia Cirp, 2016. **49**: p. 105-112.
197. Hafezi, F., et al., *3D printed chitosan dressing crosslinked with genipin for potential healing of chronic wounds.* International Journal of Pharmaceutics, 2019. **560**: p. 406-415.
198. Gholipourmalekabadi, M., et al., *Silk fibroin for skin injury repair: where do things stand?* Advanced drug delivery reviews, 2020. **153**: p. 28-53.
199. Jao, D., X. Mou, and X. Hu, *Tissue regeneration: a silk road.* Journal of functional biomaterials, 2016. **7**(3): p. 22.
200. Kamalathevan, P., P.S. Ooi, and Y.L. Loo, *Silk-based biomaterials in cutaneous wound healing: a systematic review.* Advances in skin & wound care, 2018. **31**(12): p. 565-573.
201. Wang, F., et al., *Tunable Biodegradable Polylactide–Silk Fibroin Scaffolds Fabricated by a Solvent-Free Pressure-Controllable Foaming Technology.* ACS Applied Bio Materials, 2020.
202. Keirouz, A., et al., *High-throughput production of silk fibroin-based electrospun fibers as biomaterial for skin tissue engineering applications.* Materials Science and Engineering: C, 2020: p. 110939.
203. Egan, P.F., *Integrated design approaches for 3D printed tissue scaffolds: Review and outlook.* Materials, 2019. **12**(15): p. 2355.
204. Chawla, S., et al., *Silk□based bioinks for 3D bioprinting.* Advanced healthcare materials, 2018. **7**(8): p. 1701204.

205. Wang, Q., et al., *3D printing of silk fibroin for biomedical applications.* Materials, 2019. **12**(3): p. 504.
206. Zheng, Z., et al., *3D bioprinting of self□standing silk□based bioink.* Advanced healthcare materials, 2018. **7**(6): p. 1701026.
207. Gupta, S., et al., *Evaluation of silk□based bioink during pre and post 3D bioprinting: A review.* Journal of Biomedical Materials Research Part B: Applied Biomaterials, 2020.
208. Kim, S.H., T.H. Lim, and C.H. Park, *Silk Fibroin Bioinks for Digital Light Processing (DLP) 3D Bioprinting*, in *Bioinspired Biomaterials*. 2020, Springer. p. 53-66.
209. Gong, D., et al., *Preparing 3D-printable silk fibroin hydrogels with robustness by a two-step crosslinking method.* RSC Advances, 2020. **10**(45): p. 27225-27234.
210. Kulkarni, G., et al., *Tailorable hydrogel of gelatin with silk fibroin and its activation/crosslinking for enhanced proliferation of fibroblast cells.* International Journal of Biological Macromolecules, 2020. **164**: p. 4073-4083.
211. Kim, B.S., et al., *Decellularized extracellular matrix: a step towards the next generation source for bioink manufacturing.* Biofabrication, 2017. **9**(3): p. 034104.
212. Kim, B.S., et al., *3D cell printing of perfusable vascularized human skin equivalent composed of epidermis, dermis, and hypodermis for better structural recapitulation of native skin.* Advanced healthcare materials, 2019. **8**(7): p. 1801019.
213. Ali, M., et al., *A photo□crosslinkable kidney ECM□derived bioink accelerates renal tissue formation.* Advanced healthcare materials, 2019. **8**(7): p. 1800992.
214. Lee, H., et al., *Development of liver decellularized extracellular matrix bioink for three-dimensional cell printing-based liver tissue engineering.* Biomacromolecules, 2017. **18**(4): p. 1229-1237.
215. La, W.-G., et al., *Systemically replicated organic and inorganic bony microenvironment for new bone formation generated by a 3D printing technology.* RSC advances, 2016. **6**(14): p. 11546-11553.
216. Jang, J., et al., *Biomaterials-based 3D cell printing for next-generation therapeutics and diagnostics.* Biomaterials, 2018. **156**: p. 88-106.
217. Dzobo, K., K.S.C.M. Motaung, and A. Adesida, *Recent trends in decellularized extracellular matrix bioinks for 3D printing: an updated review.* International journal of molecular sciences, 2019. **20**(18): p. 4628.
218. Kim, B.S., et al., *3D cell printing of in vitro stabilized skin model and in vivo pre-vascularized skin patch using tissue-specific extracellular matrix bioink: a step towards advanced skin tissue engineering.* Biomaterials, 2018. **168**: p. 38-53.
219. Burdick, J.A. and G.D. Prestwich, *Hyaluronic acid hydrogels for biomedical applications.* Advanced materials, 2011. **23**(12): p. H41-H56.
220. Guvendiren, M. and J.A. Burdick, *Engineering synthetic hydrogel microenvironments to instruct stem cells.* Current opinion in biotechnology, 2013. **24**(5): p. 841-846.
221. Kang, H.-W., et al., *A 3D bioprinting system to produce human-scale tissue constructs with structural integrity.* Nature biotechnology, 2016. **34**(3): p. 312-319.
222. Hockaday, L., et al., *Rapid 3D printing of anatomically accurate and mechanically heterogeneous aortic valve hydrogel scaffolds.* Biofabrication, 2012. **4**(3): p. 035005.
223. Jeong, H.-J., et al., *3D Bioprinting Strategies for the Regeneration of Functional Tubular Tissues and Organs.* Bioengineering, 2020. **7**(2): p. 32.
224. Sabir, M.I., X. Xu, and L. Li, *A review on biodegradable polymeric materials for bone tissue engineering applications.* Journal of materials science, 2009. **44**(21): p. 5713-5724.

225. Wu, W., A. DeConinck, and J.A. Lewis, *Omnidirectional printing of 3D microvascular networks.* Advanced materials, 2011. **23**(24): p. H178-H183.
226. Kim, B.S., et al., *Three-dimensional bioprinting of cell-laden constructs with polycaprolactone protective layers for using various thermoplastic polymers.* Biofabrication, 2016. **8**(3): p. 035013.
227. He, J., et al., *Anti-oxidant electroactive and antibacterial nanofibrous wound dressings based on poly (ε-caprolactone)/quaternized chitosan-graft-polyaniline for full-thickness skin wound healing.* Chemical Engineering Journal, 2020. **385**: p. 123464.
228. Dodero, A., et al., *Multilayer Alginate–Polycaprolactone Electrospun Membranes as Skin Wound Patches with Drug Delivery Abilities.* ACS applied materials & interfaces, 2020. **12**(28): p. 31162-31171.
229. Yang, S., et al., *Multifunctional Chitosan/Polycaprolactone Nanofiber Scaffolds with Varied Dual-Drug Release for Wound-Healing Applications.* ACS Biomaterials Science & Engineering, 2020. **6**(8): p. 4666-4676.
230. Mondal, D., M. Griffith, and S.S. Venkatraman, *Polycaprolactone-based biomaterials for tissue engineering and drug delivery: Current scenario and challenges.* International Journal of Polymeric Materials and Polymeric Biomaterials, 2016. **65**(5): p. 255-265.
231. Borkar, T., V. Goenka, and A.K. Jaiswal, *Application of poly-ε-caprolactone in extrusion-based bioprinting.* Bioprinting, 2020: p. e00111.
232. Li, Z. and B.H. Tan, *Towards the development of polycaprolactone based amphiphilic block copolymers: molecular design, self-assembly and biomedical applications.* Materials Science and Engineering: C, 2014. **45**: p. 620-634.
233. Woodruff, M.A. and D.W. Hutmacher, *The return of a forgotten polymer—Polycaprolactone in the 21st century.* Progress in polymer science, 2010. **35**(10): p. 1217-1256.
234. Guo, B. and P.X. Ma, *Synthetic biodegradable functional polymers for tissue engineering: a brief review.* Science China Chemistry, 2014. **57**(4): p. 490-500.
235. Lam, C.X., et al., *Evaluation of polycaprolactone scaffold degradation for 6 months in vitro and in vivo.* Journal of Biomedical Materials Research Part A: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials, 2009. **90**(3): p. 906-919.
236. Lam, C.X., et al., *Dynamics of in vitro polymer degradation of polycaprolactone-based scaffolds: accelerated versus simulated physiological conditions.* Biomedical materials, 2008. **3**(3): p. 034108.
237. Afghah, F., et al., *3D printing of silver-doped polycaprolactone-poly (propylene succinate) composite scaffolds for skin tissue engineering.* Biomedical Materials, 2020. **15**(3): p. 035015.
238. Muwaffak, Z., et al., *Patient-specific 3D scanned and 3D printed antimicrobial polycaprolactone wound dressings.* International Journal of Pharmaceutics, 2017. **527**(1): p. 161-170.
239. Patrício, T., et al., *Fabrication and characterisation of PCL and PCL/PLA scaffolds for tissue engineering.* Rapid Prototyping Journal, 2014.
240. Casalini, T., et al., *A perspective on polylactic acid-based polymers use for nanoparticles synthesis and applications.* Frontiers in bioengineering and biotechnology, 2019. **7**.

241. Foong, C.Y., et al., *Influence of poly (lactic acid) layer on the physical and antibacterial properties of dry bacterial cellulose sheet for potential acute wound healing materials.* Fibers and Polymers, 2018. **19**(2): p. 263-271.
242. Nguyen, T.T.T., et al., *Characteristics of curcumin-loaded poly (lactic acid) nanofibers for wound healing.* Journal of materials science, 2013. **48**(20): p. 7125-7133.
243. Ren, Y., et al., *Stereocomplexed electrospun nanofibers containing poly (lactic acid) modified quaternized chitosan for wound healing.* Carbohydrate Polymers, 2020. **247**: p. 116754.
244. Zhang, B., et al., *3D printing of high-resolution PLA-based structures by hybrid electrohydrodynamic and fused deposition modeling techniques.* Journal of Micromechanics and Microengineering, 2016. **26**(2): p. 025015.
245. Farah, S., D.G. Anderson, and R. Langer, *Physical and mechanical properties of PLA, and their functions in widespread applications—A comprehensive review.* Advanced drug delivery reviews, 2016. **107**: p. 367-392.
246. Liu, S., et al., *Current applications of poly (lactic acid) composites in tissue engineering and drug delivery.* Composites Part B: Engineering, 2020: p. 108238.
247. Nair, L.S. and C.T. Laurencin, *Biodegradable polymers as biomaterials.* Progress in Polymer Science, 2007. **32**(8): p. 762-798.
248. Lee, S.M., et al., *Physical, morphological, and wound healing properties of a polyurethane foam-film dressing.* Biomaterials research, 2016. **20**(1): p. 15.
249. Bankoti, K., et al., *Accelerated healing of full thickness dermal wounds by macroporous waterborne polyurethane-chitosan hydrogel scaffolds.* Materials Science and Engineering: C, 2017. **81**: p. 133-143.
250. Eppa, Ł., et al., *Deposition of mannose□binding lectin and ficolins and activation of the lectin pathway of complement on the surface of polyurethane tubing used for cardiopulmonary bypass.* Journal of Biomedical Materials Research Part B: Applied Biomaterials, 2018. **106**(3): p. 1202-1208.
251. Hung, K.-C., C.-S. Tseng, and S.-H. Hsu, *3D printing of polyurethane biomaterials*, in *Advances in Polyurethane Biomaterials*. 2016, Elsevier. p. 149-170.