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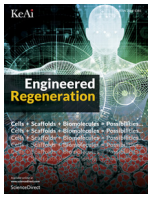

Engineered artificial skins: Current construction strategies and applications

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ABSTRACT

Skin damage resulting from burns, injuries, or diseases can lead to significant functional and esthetic deficits. However, traditional treatments, such as skin grafting, have limitations including limited donor skin availability, poor aesthetics, and functional impairment. Skin tissue engineering provides a promising alternative, with engineered artificial skins offering a highly viable avenue. Engineered artificial skin is designed to mimic or replace the functions of natural human skin and find applications in various medical treatments, particularly for severe burns, chronic wounds, and other skin injuries or defects. These artificial skins aim to promote wound healing, provide temporary coverage, permanent skin replacement, and restore the skin's barrier function. Artificial skins have diverse applications in medicine and wound care, addressing burns, chronic wounds, and traumatic injuries. They also serve as valuable tools for research in tissue engineering, offering experimental models for studying wound healing mechanisms, testing new biomaterials, and exploring innovative approaches to skin regeneration. This review provides an overview of current construction strategies for engineered artificial skin, including cell sources, biomaterials, and construction techniques. It further explores the primary application areas and future prospects of artificial skin, highlighting their potential to revolutionize skin reconstruction and advance the field of regenerative medicine.

1. Introduction

As the body's largest organ, the skin acts as a defensive shield, safeguarding against external environmental factors [1,2]. When the skin is damaged due to burns, injuries, or diseases, it can result in significant functional and esthetic deficits [3,4]. Traditional treatments for skin injuries, such as skin grafting, may have limitations, including limited availability of donor skin, poor aesthetics, and functional impairment [5,6]. The field of skin tissue engineering presents a promising alternative approach to tackle these challenges [7], with engineered artificial skin being a highly promising avenue [8–10]. Engineered artificial skin refers to a material or product that is designed to mimic or replace the functions of natural human skin [11]. It is used in various medical applications, particularly in the management of severe burns, wounds, and other skin injuries or defects [12]. These artificial skins aim to promote wound healing, provide temporary coverage or permanent replacement for damaged skin, and restore the barrier function of the skin [13].

The main types of artificial skin include synthetic and biological artificial skin. Synthetic artificial skin is composed of artificial materials, such as polymers, that are engineered to resemble the structure and properties of natural skin [14]. These artificial skins are often made from materials like silicone, polyurethane, or collagen-based scaffolds [15,16]. Biological artificial skins are derived from natural sources and aim to closely mimic the structure and function of native skin. They can be further divided into acellular and cellular artificial skin [17]. Acellular artificial skins are composed of extracellular matrix (ECM) composition, such as collagen, elastin, and glycosaminoglycans [18]. These materials are obtained from human or animal sources and processed to remove cellular components while retaining the structural and biochemical properties of the ECM [19,20]. Cellular artificial skins, often involve the use of living cells along with a scaffold to create a more complex and functional tissue construct. These cells can be autologous, allogeneic, or stem cells [21]. The cells are seeded onto a biomaterial scaffold, which provides structural support and promotes cell attachment, proliferation, and differentiation [22].

Engineered artificial skin has various applications in the field of medicine and wound care, including burns, chronic wounds, and traumatic injuries [23]. In addition, engineered artificial skin are valuable tool in the field of tissue engineering [24]. They serve as experimental models for studying wound healing mechanisms, testing new biomaterials, and exploring innovative approaches to skin regeneration [25,26]. In this review, we first introduced the current main construction strategies of the engineered artificial skin, including the cell sources, biomaterials, and construction techniques (Fig.1). We further elaborated on the current primary application areas and prospects of the engineered artificial skin.

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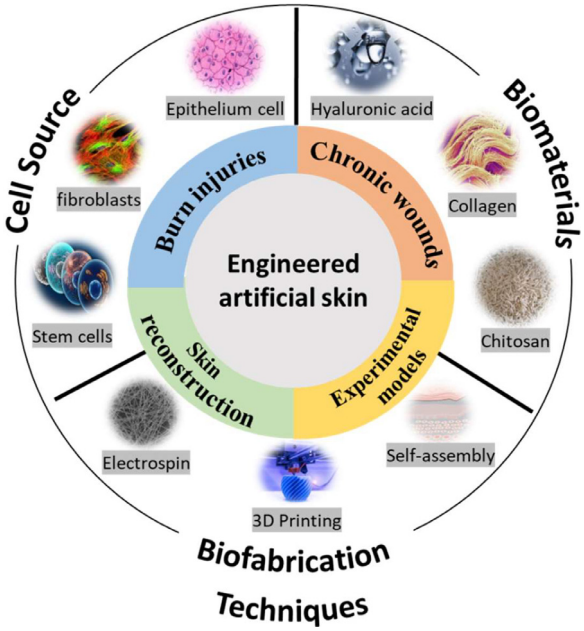

Fig. 1. The schematic image of the construction strategies and applications of the engineered artificial skin.

2. Construction strategies of engineered artificial skin

2.1. Cellular components

Engineered artificial skin can be classified into two main categories: acellular and cellular constructs. Acellular substitutes typically consist of biomaterial scaffolds that provide a framework for cell migration, angiogenesis, and tissue regeneration [27–29]. Cellular constructs, on the other hand, involve seeding or incorporating living cells into the scaffolds [30]. These cells can be dermal fibroblasts [31], keratinocytes [32], adipose-derived stem cells [33], or other cell types relevant to skin tissue. The presence of cells in the engineered artificial skin further promotes tissue regeneration, as they actively participate in the healing process, produce extracellular matrix components, and release growth factors [34]. Cellular constructs offer the advantage of more active wound healing and the potential for more complex skin structures [35]. Here, we introduce the main cell sources that are involved in the engineered artificial skin construction.

2.1.1. Epidermal cells

Epidermal cells are of utmost importance in the development of artificial skin due to their unique properties and superior capabilities [36,37]. These cells form the outer layer of the skin, which serves as a protective shield against external factors and helps maintain the body's homeostasis. In the context of artificial skin, epidermal cells are often used to create engineered skin grafts or tissue-engineered constructs that are used to manage wounds, burns, and other skin defects [38]. Due to the remarkable adhesive property, the epidermal cells can be seeded on the artificial dermal and construct the epithelium layer [39].

Epidermal cells used in engineered artificial skin can be obtained from different cell sources, including autologous (from the patient's skin), allogeneic (from another individual of the same species), or even stem cells [40]. The choice of cell source depends on various factors such as availability, scalability, immune compatibility, and the specific requirements of the intended application. Autologous epidermal cells, harvested from a patient's own skin, are commonly used in the production of artificial skins [41]. This approach eliminates the risk of immune rejection and offers a personalized treatment option. The cells can be obtained through a small biopsy, which is then processed in the laboratory to isolate and expand the epidermal cells for subsequent seeding onto a suitable scaffold [42]. Allogeneic epidermal cells, derived from healthy human donors, can also be used in artificial skins [43]. These cells are obtained from skin tissue donors, expanded in culture, and then incorporated into the artificial skin product. Allogeneic cell-based products provide an off-the-shelf solution and can be readily available for patients in need. However, the use of allogeneic cells carries a potential risk of immune rejection, requiring careful matching and immune modulation strategies.

2.1.2. Fibroblast cells

Fibroblast cells are a type of connective tissue cell found in various organs and tissues, including the skin, tendons, and organs. They are primarily responsible for producing and maintaining the extracellular matrix (ECM), which is the framework that supports and gives structure to tissues [44]. Within the skin, fibroblasts reside in the dermis, situated beneath the epidermis layer. They possess a vital function in the healing of wounds by migrating toward the injury site and releasing extracellular matrix (ECM) components to facilitate tissue restoration [45]. Fibroblasts actively participate in the production and arrangement of collagen fibers, which significantly contribute to the skin's tensile strength and elasticity [46]. Apart from their role in ECM production, fibroblasts also play a part in tissue homeostasis, immune responses, and signaling processes. They can secrete growth factors, cytokines, and other signaling molecules that regulate cell proliferation, differentiation, and migration [47].

Similar to epidermal cells, the sources of fibroblast cells can also be classified into autologous and allogeneic. For clinical use, autologous fibroblasts are the first choice. Autologous fibroblasts are derived from the patient's skin obtained from a small biopsy [41]. Autologous fibroblasts offer excellent immune compatibility since they are a perfect match to the patient's cells. These cells can be expanded in culture and incorporated into the artificial skin, enhancing its acceptance and integration within the body [48]. Neonatal fibroblasts are also an alternative cell source, which is derived from the skin of newborns [49]. These cells exhibit high mitotic activity and have enhanced regenerative capabilities, making them a valuable source for tissue engineering applications [50].

In addition, to obtain the specific characteristics or functionalities desired for the artificial skin, engineered fibroblasts can be created by modifying the genetic characteristics or properties of fibroblasts through techniques such as genetic engineering or cell reprogramming. Engineered fibroblasts can be tailored to exhibit enhanced collagen production or growth factor secretion [51,52]. The type of cells used to engineer fibroblasts can vary depending on the specific research or medical application. Both autologous fibroblasts (derived from the patient's cells) and neonatal fibroblasts (derived from newborns or neonates) have been used in various tissue engineering and regenerative medicine contexts. The choice of cell source for engineering fibroblasts, such as autologous or neonatal fibroblasts, can impact the immune compatibility of the resulting engineered cells. Autologous cells are typically preferred for their high immune compatibility, but they may not always be available or practical. Allogeneic cells may require additional measures to reduce immune rejection, such as genetic modifications or immunosuppressive therapies, depending on the specific application and context.

2.1.3. Other cell types

In addition to the key cell types like keratinocytes and fibroblasts, several other cell types have shown promising applications in engineered artificial skin construction. These include endothelial cells, adipocytes, and melanocytes. Each of these cell types contributes unique characteristics and functions to the development of functional and biomimetic artificial skins.

Endothelial cells were essential for forming blood vessels, known as angiogenesis. In engineered artificial skin, the incorporation of endothelial cells aims to promote vascularization, enabling proper blood

supply to the regenerated tissue [51,52]. Endothelial cells contribute to the formation of capillary networks within the artificial skin, enhancing oxygen and nutrient delivery to support cell survival and tissue viability [53,54]. This vascularization is vital for efficient wound healing and the long-term integration of the artificial skin with the surrounding tissue.

Adipocytes, also known as fat cells, are involved in the formation and maintenance of adipose tissue, which provides insulation, energy storage, and mechanical cushioning [55,56]. The inclusion of adipocytes in artificial skins can help recreate the natural structure and function of the subcutaneous adipose layer. Adipocytes contribute to the overall aesthetics and contouring of the regenerated artificial skin, providing a more natural appearance [57]. Furthermore, the presence of adipocytes can enhance the mechanical properties and elasticity of the artificial skin, improving its functionality and integration.

Melanocytes are accountable for the production of melanin, the pigment that imparts color to the skin, hair, and eyes [58]. Incorporating melanocytes into artificial skin can help restore the pigmentation of the regenerated tissue. This is particularly relevant for patients with conditions such as vitiligo or for those undergoing reconstructive procedures that involve areas with specific pigmentation requirements [59]. By including melanocytes, artificial skin can provide a more realistic and aesthetically pleasing appearance, improving patient satisfaction and quality of life.

2.2. Scaffold materials

In skin tissue engineering, biomaterials play a vital role by offering a supportive framework for cells to adhere, multiply, and transform into functional skin tissue. The biocompatibility, biodegradability, and capacity to emulate the natural extracellular matrix (ECM) of the skin have positioned natural biomaterials as promising candidates for skin tissue engineering. By creating a favorable microenvironment for cell adhesion, proliferation, and differentiation, these biomaterials facilitate the regeneration of damaged or diseased skin. Meanwhile, single-component biomaterials often fail to meet the requirements, leading to the development of composite materials. Composite biomaterials are a type of synthetic biomaterials that are composed of two or more different types of materials, each with its unique properties. By combining these materials, a composite material is formed, which exhibits a unique blend of properties tailored for specific applications. In this section, we will introduce commonly used natural materials and their derived composite materials in constructing artificial skins.

2.2.1. Collagen

Collagen, a prevalent fibrous protein found in the human body, is extensively utilized as a natural biomaterial in skin tissue engineering [60]. Its abundance in the skin's extracellular matrix (ECM) has spurred significant research into its potential as a scaffold material for constructing artificial skins [61,62]. Recent advancements have led to the successful development of a bi-layered dermal construct [63]. This construct incorporates a collagen hydrogel fortified with a nanofibrous poly-L-lactide (PLLA) membrane previously populated with fibroblasts (as illustrated in Fig. 2a). The inclusion of a fibrin mesh within the construct facilitates the attachment, proliferation, and migration of fibroblasts towards the upper regions of the collagen hydrogel. Notably, the migrated fibroblasts within the collagen hydrogel display reduced contractile forces, resulting in minimal shrinkage of the hydrogel. Subsequently, the surface of the collagen layer is seeded with human dermal keratinocytes, which form a basal layer comprising highly active mitotic cells and a suprabasal layer.

Moreover, studies have shown that native collagen networks enhance cell adhesion and proliferation on the surfaces of different commercially accessible dermal templates. Fig. 2b showcases tightly packed collagen patches within the dermis of native human skin, featuring

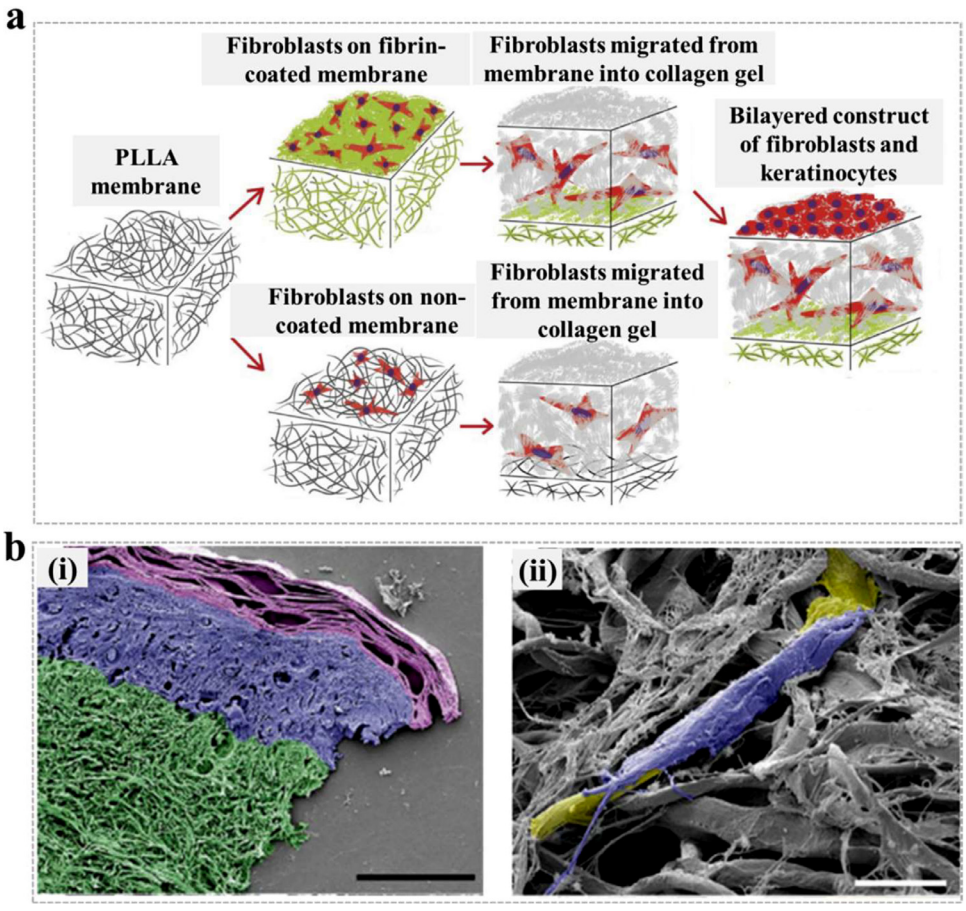

Fig. 2. (a) The process of preparing the fibrin-coated PLLA nanofibrous membrane is illustrated. PLLA stands for poly-L-lactide. (b) A field emission scanning electron microscopy (FESEM) image of human skin specimens is shown. Scale bar, 50 μm. (a) Reproduced from Ref. [63] with permission. (b) Reproduced from Ref. [64] with permission.

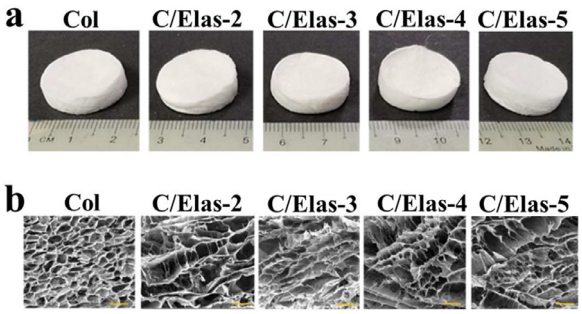

Fig. 3. (a) The gross appearance of genipin-crosslinked scaffolds after crosslinking at 25 °C. (b) Cross-sectional FESEM images of the scaffolds at 100 × magnification. Scale bar, 200 μm. (a-b) Reproduced from Ref. [65] with permission.

regions with diverse fiber orientations [64]. Researchers have examined cell adherence, guidance, and morphology, finding that biological dermal templates possessing the greatest concentration of natural collagen networks predominantly stimulate these cellular processes. This study underscores the significant advantages of native collagen networks in constructing artificial skins and promoting wound healing.

In addition to its direct application, collagen can be combined with active molecules to achieve additional functionalities. In one study, researchers explore the combination of elastin peptide with collagen to mimic the structural, physiological, and functional aspects of ECM microenvironment (Figs. 3a, 3b) [65]. The inclusion of elastin peptide enhances the strength and elasticity of the hybrid scaffolds, resulting in optimum physicochemical and mechanical properties. These hybrid scaffolds hold promise as acellular artificial skins in wound management.

2.2.2. Chitosan

Chitosan, a natural biomaterial utilized in skin tissue engineering, is derived from the deacetylation process of chitin, a polysaccharide present in the exoskeleton of crustaceans [66]. Its application has demonstrated beneficial effects on cellular adhesion, proliferation, wound healing, and tissue regeneration [67]. Advancements in chitosan-based scaffolds have led to the development of functional electrospun nanofibers incorporating active substances [68]. In a recent investigation, chitosan and gelatin electrospun nanofibers ranging from 240 to 360 nm were fabricated and integrated with a glass-ceramic (GC) material. Mouse embryonic fibroblasts (MEFs) were loaded onto these nanofibers to expedite the healing process (Fig. 4a) [69]. GC possesses properties such as angiogenesis promotion and anti-inflammatory effects due to its ionic dissolution products. To enhance antibacterial properties, silver (Ag) was incorporated into GC. The MEFs exhibited complete attachment and well-spread morphology on all the scaffolds, indicating the considerable potential of this scaffold in promoting wound healing (Fig. 4b) [69].

Recent research has highlighted the benefits of employing multilayer scaffolds over single-layer dressings for the management of full-thickness wounds. These scaffolds provide functional substitutes for both the dermal and epidermal layers of the skin. Within the domain of porcine wound reconstruction, researchers have created a bilayer scaffold, incorporating polydopamine (PDA) enhancements, to enhance mechanical strength and biological support [70]. The bilayer scaffold comprises a porous collagen/polysaccharide foam that mimics the structure of the dermis, along with a nanofibrous layer that resembles the basal membrane. The fibrous layer is composed of polymers such as gelatin, polycaprolactone (PCL), and a calcium oxide composite (CaOC). Scanning electron microscopy (SEM) images of the non-cross-linked bilayers and PDA-coated cross-linked bilayers demonstrate the substantial impact of both interventions. The nanofibrous is visibly present on the sample's surface (indicated by the yellow arrow), and a porous structure is observed beneath it (indicated by the red arrow) (Fig. 5a). Direct application of this bi-layer scaffold to the wound bed enhances wound healing and diminishes scar formation (as shown in Fig. 5b, 5c).

2.2.3. Hyaluronic acid

Hyaluronic acid (HA), a natural biomaterial, finds application in the field of skin tissue engineering [71]. As a glycosaminoglycan, HA was critical to the extracellular matrix (ECM) of the skin [18]. Researchers have developed HA-based hydrogels capable of supporting the growth and differentiation of skin cells [72]. Notably, the selection of HA with an appropriate molecular weight is critical and depends on the specific goals of the constructed artificial skin. For example, high molecular weight HA may be preferred for creating a scaffold that maintains tissue hydration and supports cell growth, while lower molecular weight HA might be used for its potential pro-inflammatory effects in promoting tissue repair. Recent investigations have demonstrated that HA-based scaffolds can be modified with growth factors and peptides to enhance the regeneration of skin tissue.

In a particular study, a novel dermal substitute was presented, consisting of polyvinyl alcohol (PVA) incorporated with collagen supplemented with hyaluronic acid and silver nanoparticles (AgNP) [73]. The porosity characteristics of col-HA and PVA-AgNP were examined using SEM and CLSM (Fig. 6a, 6b). HR-TEM revealed that the dimensions of

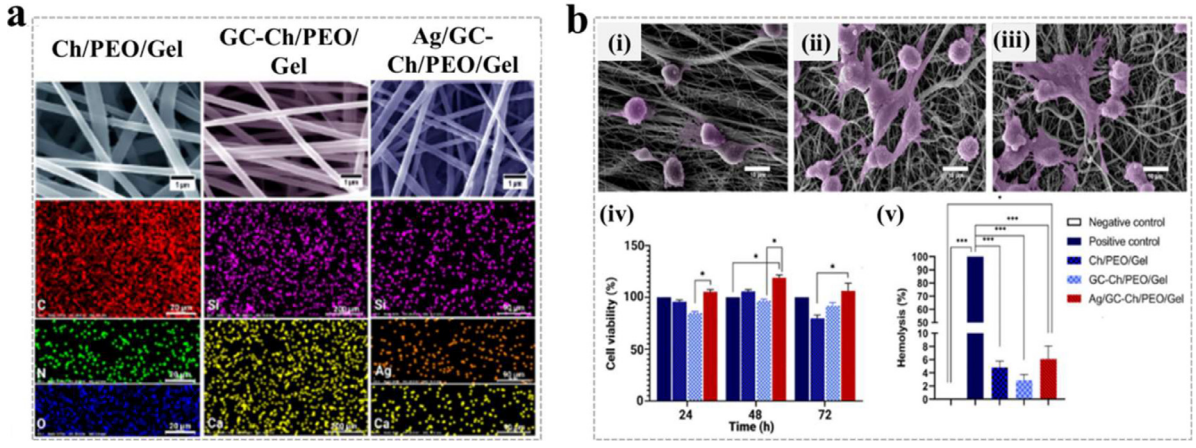

Fig. 4. (a) SEM micrographs and corresponding energy dispersive x-ray analysis of the elemental distribution with the Ch/PEO/Gel, GC–Ch/PEO/Gel, and Ag/GC–Ch/PEO/Gel scaffolds. Abbreviation: Ch, chitosan. (b) SEM of fibroblasts cultured for 24 h on (i) Ch/PEO/gel, (ii) GC–Ch/PEO/gel, and (iii) Ag/GC–Ch/gel scaffolds. In vitro cytotoxicity of the three experimental scaffolds using the MTT assay with mouse embryonic fibroblasts at 24, 48, and 96 h ($^*p < 0.05$) (iv). Percentage of hemolysis induced by different experimental scaffolds (v). Abbreviation: Ch, chitosan. (a-b) Reproduced from Ref. [69] with permission.

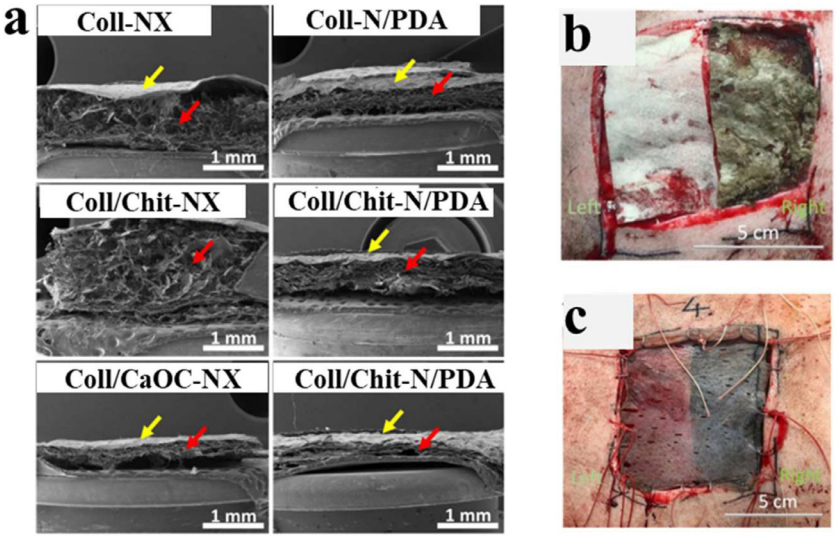

Fig. 5. (a) Scanning electron microscopy (SEM) images displaying the structure of bilayers comprised of porous foam and nanofibers are shown. (i) The bilayers consisted of three types: Coll-N, which is collagen foam with cross-linked nanofibers; Coll/Chit-N, composed of collagen/chitosan foam with cross-linked nanofibers; and Coll/CaOC–N, comprising collagen/oxidized cellulose foam with cross-linked nanofibers. The nanofibrous layer is visibly positioned on the surface of the sample (indicated by the yellow arrow), while a porous scaffold structure can be observed beneath it (indicated by the red arrow). Additionally, the SEM image depicts a bilayer scaffold constructed from collagen/oxidized cellulose foam with cross-linked nanofibers, known as Coll/CaOC–N (left), and a bilayer scaffold coated with polydopamine, denoted as Coll/CaOC–N/PDA (right), which is applied directly to the wound bed. (b-c) The application of split-thickness skin graft (STSG) onto the bilayer scaffold, effectively covering the full-thickness skin defect. (a-c) Reproduced from Ref. [70] with permission.

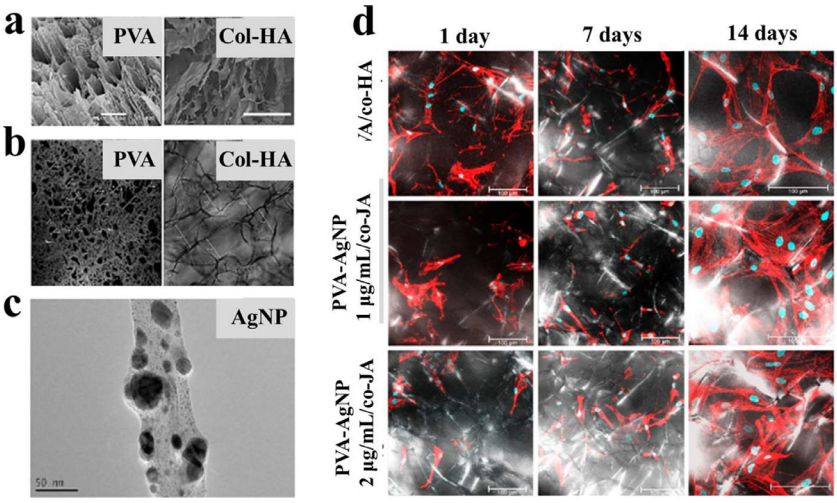

Fig. 6. (a-b) PVA (left) and col-HA (right) were observed by SEM (a) and LSCM (b). SEM bars were 5 μm (left) and 100 μm (right). (c) HR-TEM observation of AgNP dispersed in formvar-coated Cu-grids. (d) LSCM images of hMSCs adhered to PVA/col-HA containing or not AgNP at two concentrations. Scale bar was 100 μm. (a-d) Reproduced from Ref. [73] with permission.

the AgNPs ranged between 5 and 20 nm on average (Fig. 6c). The artificial skin exhibited remarkable antimicrobial effects due to the incorporation of AgNPs. The growth and morphology of human mesenchymal stem cells (hMSCs) cultivated on the scaffold, which contained AgNP at 1 and 2 μg/mL remained unaffected, indicating the absence of scaffold cytotoxicity (Fig. 6d).

Nevertheless, the widespread utilization of hyaluronic acid has been constrained due to its inadequate mechanical properties, including rapid contraction and degradation. To overcome this challenge, scientists have devised hydrogels by utilizing plasma-derived fibrin in combination with thiolated-hyaluronic acid (HA-SH) crosslinked with poly(ethylene glycol) diacrylate (PEGDA) at concentrations ranging from 0.05 % to 0.2 % w/v. at varying ratios of thiol to acrylate [74]. These innovative hydrogels showcased decreased contraction levels in vitro and displayed enhanced mechanical properties, as evidenced by improvements in elastic modulus and load-bearing capacity (Fig. 7a). Utilizing this new material, in vitro skin constructs were fabricated, a more uniform suprabasal K10 expression and a stratum corneum with improved hydration, resulting in a more homogeneous appearance.

In order to evaluate the potential of hyaluronic acid-based artificial skim as an ideal biomaterial for engineered skin, a study was conducted comparing them with autograft (the gold standard treatment) and a human tissue-engineered artificial skin manufactured using fibrin–agarose biomaterial (AG-Skin) (Fig. 7b) [75]. The findings revealed that eight weeks after treatment, the HA-Skin, Autograft and AG-Skin displayed favorable clinical integration and epithelization. Scar evaluation exhibited superior outcomes for the Autograft group and the HA-Skin group. The study indicates that human tissue-engineered artificial skins utilizing fibrin-hyaluronic acid biomaterial hold promise for clinical use in the management of diverse dermatological conditions, particularly in wound treatment.

2.3. Biofabrication techniques

2.3.1. Electrospinning

Electrospinning is a versatile and scalable technology that uses an electric field to generate fibers with diameters spanning from nanometers to micrometers [76,77]. The process entails subjecting a polymer solution or melt to an applied electric field, which leads to the formation of a charged jet that is then stretched and solidified to form fibers [78]. Electrospinning has been widely used in various fields, including drug delivery, tissue engineering, and filtration [79]. In artificial skin construction, the process of electrospinning has been employed to create scaffolds that emulate the structure and functionality of natural skin. Electrospun scaffolds can provide a 3D microenvironment that promotes cell adhesion, proliferation, and differentiation. The fibers can be aligned to mimic the orientation of collagen fibers in native skin, which is important for the mechanical properties of the artificial skin [24]. Furthermore, electrospun scaffolds can be modified with bioactive molecules like growth factors and peptides to augment cell

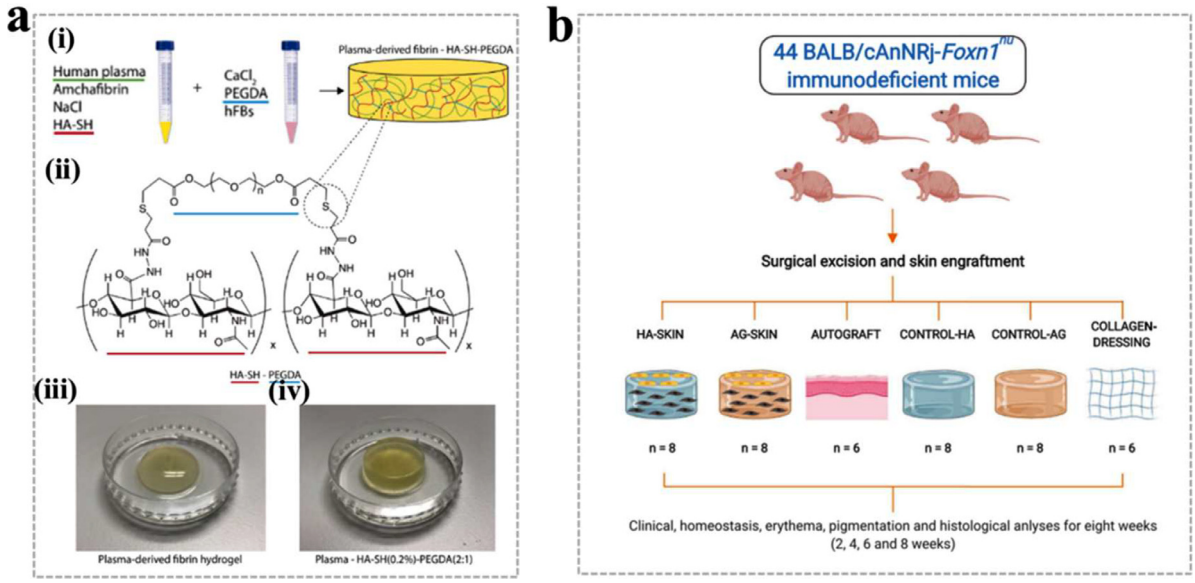

Fig. 7. (a) The preparation of plasma/HA-SH-PEGDA hydrogels is depicted, showcasing the components and mixture conditions. (i) The illustration highlights the various components involved and the specific conditions for mixing. (ii) The chemical structures of both the HA-SH and PEDGA components are shown, emphasizing how covalent bonds are formed through the thiol and diacrylate functional groups via the Michael addition reaction. (iii) A photograph of the plasma hydrogel in a petri dish is provided at time zero. (iv) Additionally, a photograph of the plasma/HA-SH-PEDGA hydrogels in a petri dish with a 0.2 % HA-SH content and a 2:1 crosslinking ratio is presented at time zero. (b) The in vivo study design is represented schematically. (a) Reproduced from Ref. [74] with permission. (b) Reproduced from Ref. [75] with permission.

attachment and promote cellular differentiation. In a recent study, researchers presented a poly(lactic-co-glycolic acid) scaffold that cooperated with collagen to enhance cell adhesion using coating and common solvent methods [80]. As shown in Fig. 8a, the fibers are beadles and their arrangement is randomly oriented. In addition, the in vitro study demonstrated that cell adhesion and spreading of the HDF cells are enhanced on the collagen-coated scaffold, while the effect was not observed on the HaCaT cells (Fig. 8b) [80].

Aside from the traditional 2D membrane-like fibers produced by traditional electrospinning (TE), 3D fibers with controllable thickness and shape have gained great attention. Scientists have suggested utilizing electrospun-based fibroin nanofibers as a foundation for constructing engineered artificial skin [81]. The fabrication of the 3D nanofibers was achieved through the utilization of a specifically designed immersion chiller, which can cool to the temperature rapidly, as schemed in Fig. 9a. The low temperature allowed the layer-by-layer deposition of the nanofiber. After the satisfying accumulation, the scaffold experienced crystallization, and the “sacrificial” PEO was removed. The nanofibers generated through CPE exhibited exceptional full-thickness characteristics. Furthermore, with the help of mold, these nanofibers can be adapted to typical structures. It was demonstrated that the nanofibers can serve as an excellent scaffold for artificial skin construction. After 5 days of culture and 16 days of air-lift culture, the artificial skin with an epidermal and dermal-like structure was successfully constructed (Fig. 9b) [81].

Overall, electrospinning technology has shown great potential for the development of engineered artificial skin that replicates the structure and functionality of natural skin. The latest advancements in electrospinning techniques have been dedicated to enhancing the mechanical and biological properties of electrospun scaffolds, as well as developing precise and scalable electrospinning systems.

2.3.2. 3D printing

3D printing, also referred to as additive manufacturing, is a revolutionary technology that allows for the creation of intricate three-dimensional structures by building them layer by layer [82,83]. In recent years, 3D printing has found diverse applications across various

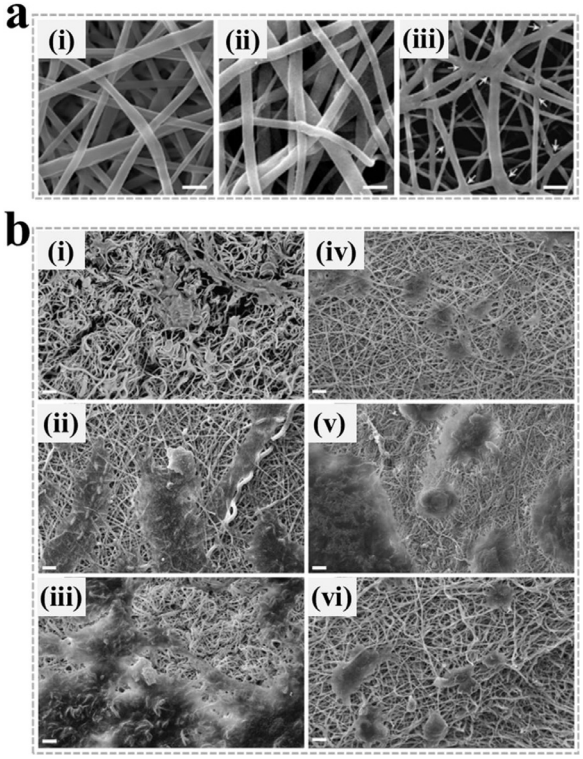

Fig. 8. (a) Scanning electron microscopy (SEM) images of different samples are presented: (i) Pure PLGA electrospun fibers, (ii) coated samples, and (iii) samples prepared using a common solvent. Scale bar, 2 μm. (b) SEM images showcasing cell attachment are displayed for HDF cell lines: (i) Pure PLGA, (ii) coated samples, and (iii) samples prepared using a common solvent. Additionally, SEM images for HaCat cell lines are shown: (iv) Pure PLGA, (v) coated samples and (vi) samples prepared using a common solvent. Scale bar, 10 μm. (a-b) Reproduced from Ref. [80] with permission.

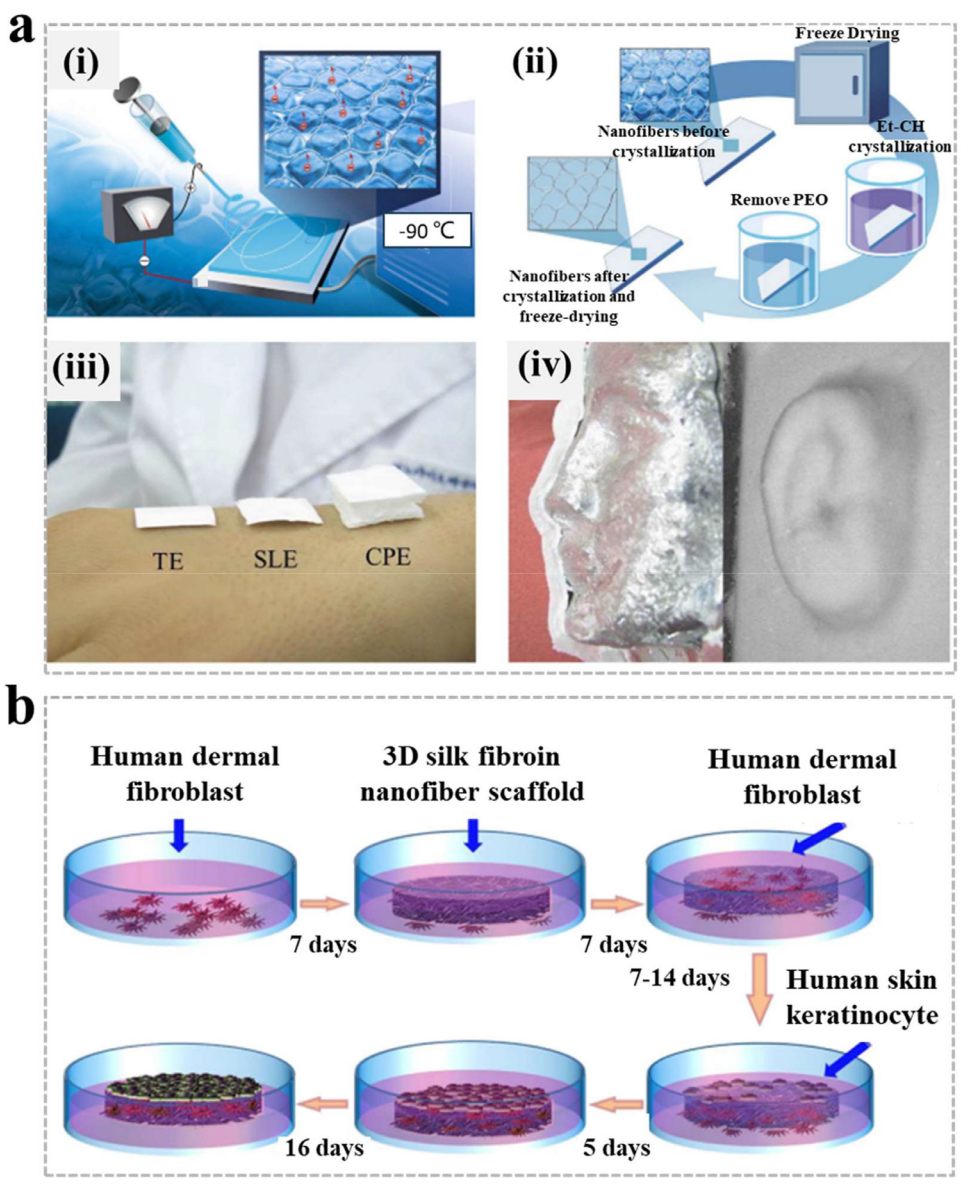

Fig. 9. (a) The cold-plate electrospinning technique, employed to create highly porous nanofibers, is illustrated schematically (i). A schematic illustration of the crystallization method for 3D electrospun silk fibroin nanofibers is also provided (ii). Gross findings of the TE (Tissue Engineering), SLE (Self-assembly Lateral Epitaxy), and CPE (Cold-Plate Electrospinning) techniques are shown (iii). Furthermore, photographs of the full-thickness 3D bionic face and ear, fabricated using the CPE technique, are included (iv). (b) A schematic illustration of the co-cultured method, involving fibroblasts and keratinocytes, utilizing an air-liquid culture system is presented. (a-b) Reproduced from Ref. [81] with permission.

fields, particularly in biomedical engineering, showcasing significant potential for tissue engineering and regenerative medicine [84,85]. In skin tissue engineering, 3D printing has been used to fabricate scaffolds with a precise and reproducible architecture mimicking the native skin [86,87]. The 3D-printed scaffolds can provide a 3D microenvironment that promotes cell adhesion, proliferation, and differentiation. They can also be functionalized with bioactive molecules such as growth factors and peptides to enhance cell behavior. Keratin, a protein extracted from hair and wool fibers, encompasses cell adhesion sequences like leucine-aspartic acid-valine (LDV) and Arg-Gly-Asp (RGD). Hence, blending the keratin into the hydrophobic material is a promising method to enhance cell adhesion and affinity. Based on this, the researchers proposed a keratin/polycaprolactone PCL scaffold for constructing a multi-layer artificial skin (Fig. 10a) [88]. NHDF seeded on the platform form a thick layer with high expression of fibronectin and HaCaT cells exhibited favorable adhesion on the NHDF layers, which leads to the successful fabrication of the artificial skin. In addition, the multi-layer artificial skin showed great efficacy in promoting wound healing, as evidenced by the accelerated healing duration and evaluated collagen deposition.

Aside from the bio-modification, tailoring the microstructure of the scaffold to better reproduce the characteristics of the human skin is another essential research direction. To meet the requirement, researchers combined the electrospinning and 3D bioprinting techniques and presented a 3D skin asymmetric construct (3D_SAC) [89]. The epidermis-like layer was produced with poly(caprolactone) and silk sericin using electrospinning, while the dermis-like layer was produced with chitosan/sodium alginate hydrogel using layer-by-layer 3D printing (Fig. 10b). The fibroblast cells show great migration and proliferation on the asymmetric construct, making it possible to construct ECM and secret essential growth factors (Fig. 10c).

To achieve the 3D printed scaffold with favorable stiffness and biodegradable behavior, researchers proposed a PLGA scaffold with epidermal growth factor (EGF) cooperation using solvent exchange deposition modeling (SEDM) technology [90]. The rapid in situ formation system was constructed with the 3D printer consisting of an N2 pressure pump, alcohol solution solidification reservoir and an ink container, as shown in Fig. 11a. Compared with the traditional fused filament fabrication (FDM) 3D product, the scaffolds fabricated with SEDM technology demonstrated enhanced porosity and flexibility. In addition, by immobilizing the EGF on the scaffold (SEDM/E), the cell viability and adhesion of the seeded NIH 3T3 cells were significantly enhanced (Fig. 11b).

In general, 3D printing technology has shown great potential in the development of engineered artificial skin and wound dressings that can

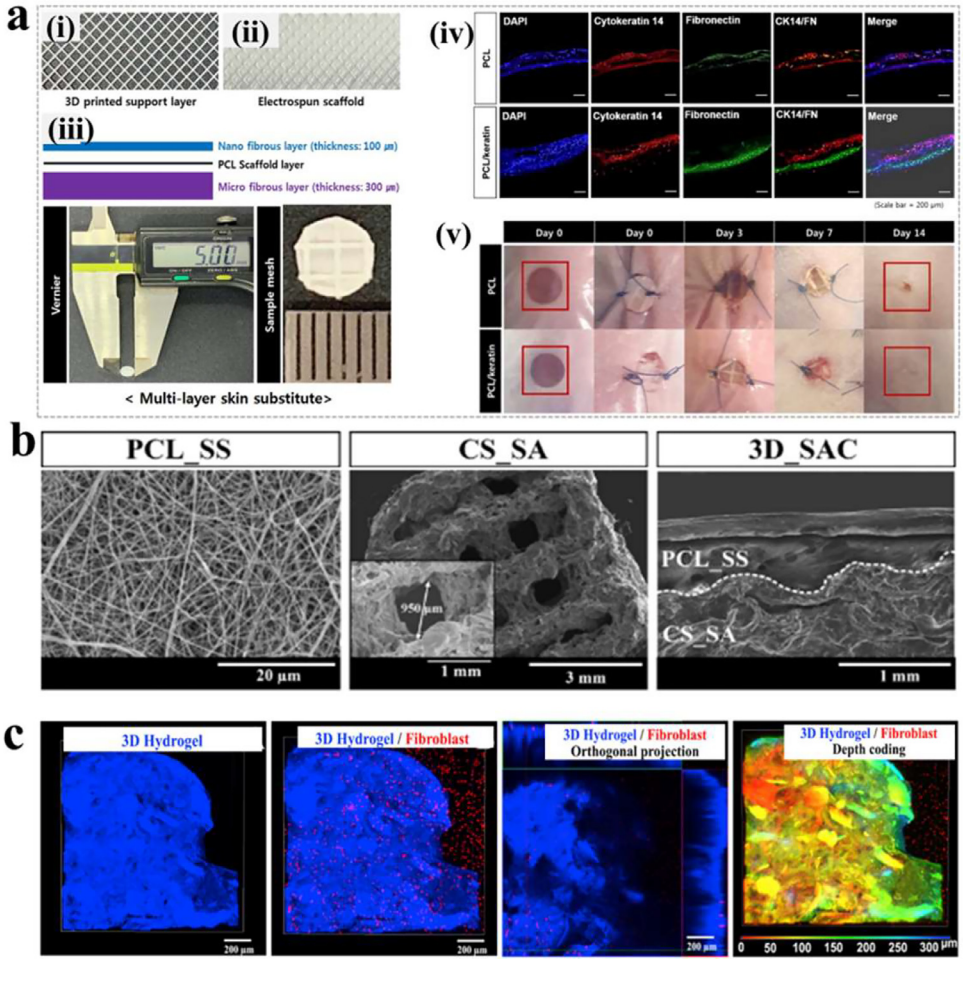

Fig. 10. (a) Fabrication and characterization of the multi-layer scaffolds. (i) Optical image of the 3D-printed support layer, (ii) optical image of the electrospun and 3D-printed scaffold, and (iii) optical image of the three-layer electrospinning 3D-printed hybrid scaffold. (iv) Immunocytochemistry results of the co-cultured scaffolds. Scale bar, 200 μm. (v) In vivo wound-healing results of PCL and PCL/keratin scaffolds. (b) Morphological analysis was conducted on the layers of the 3D_SAC. S, with a focus on their structural characteristics. (c) CLSM images were captured to visualize the cellular distribution within the CS_SA hydrogel. (a) Reproduced from Ref. [88] with permission. (b-c) Reproduced from Ref. [89] with permission.

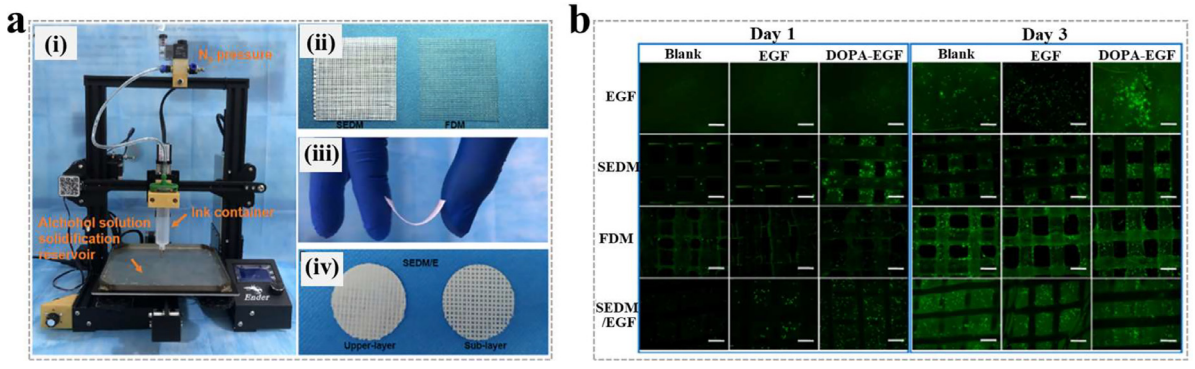

Fig. 11. (a) The photographs of (i) the 3D printer for SEDM. (ii) The SEDM sample (left) and FDM (right) scaffold. (iii) The exhibition of elastic and flexible SEDM scaffold. (iv) The upper layer (left) and sub-layer (right) of the SEDM/E scaffold. (b) Live cell staining images: The morphology and distribution of the NIH3T3 fibroblast cells on the EGF, SEDM, FDM, SEDM/EGF scaffolds loaded with EGF using physical adsorption and DOPA adhesion at day 1 and 3. Scale bar is 500 μm. (a-b) Reproduced from Ref. [90] with permission.

mimic the structure and function of native skin. The latest advances in 3D printing technology have focused on improving the physical and chemical properties of 3D-printed scaffolds, as well as developing precise and scalable 3D printing systems. Additional research is required to enhance the design and manufacturing processes of 3D-printed scaffolds tailored for skin tissue engineering purposes.

2.3.3. Self-assembly

Self-assembly methods have emerged as a promising approach for preparing artificial skin to reduce the immunogenicity of artificial skin and better simulate the structure and function of natural skin. Self-assembly methods avoid the use of exogenous proteins and biomaterials, instead utilizing living cells to synthesize ECM and facilitate assembly, resulting in an ideal dermal scaffold. Self-assembly methods typically follow the following process: First, fibroblasts are cultured to form cell sheets. Then, these cell sheets are stacked to create the dermal layer. Finally, epidermal cells are seeded onto the dermal layer to form the epidermal layer and stratum corneum.

Great effort has been devoted to reducing the preparation time of the self-assembled artificial skin, thus enhancing the timelessness of

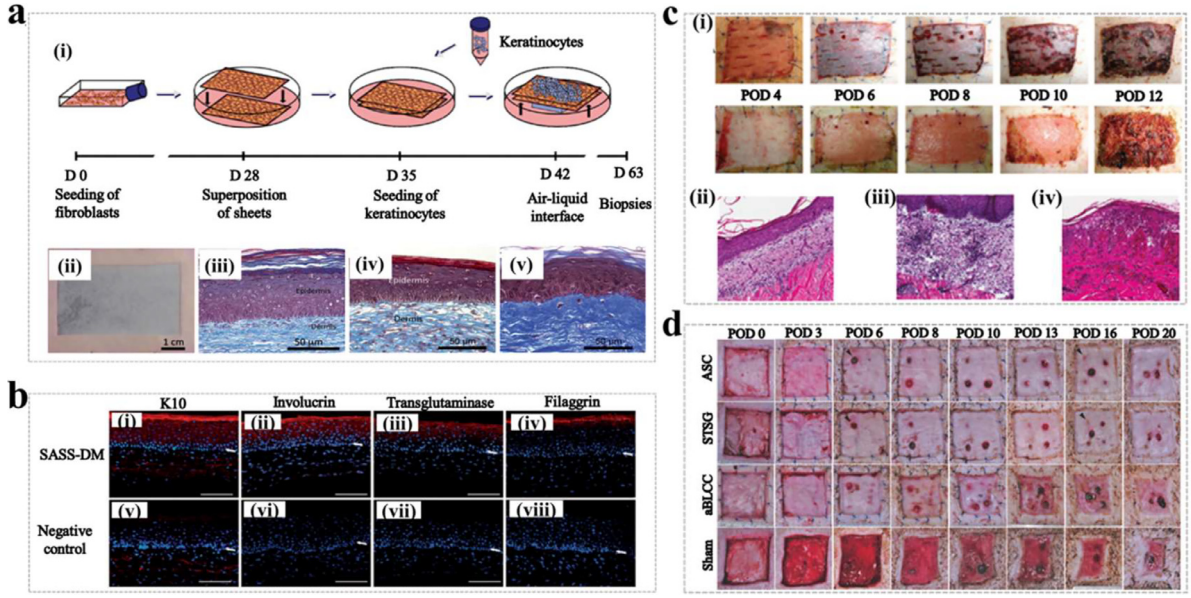

Fig. 12. (a) The fabrication process of SASS-DM (Scaffold-Assisted Self-Assembly of Dermal Matrix) is depicted in a schematic image (i). The macroscopic appearance of SASS-DM after 10 days of culture at the air-liquid interface is shown (ii). Masson staining images of SASS-DM (iii), SASS (iv), and human skin (v) are presented to examine the tissue characteristics. (b) Immunofluorescence labeling of keratin 10 (K10) (i, v), involucrin (ii, vi), transglutaminase (iii, vii), and filaggrin (iv, viii) in SASS-DM cultured for 10 days at the air-liquid interface is displayed. The scale bar represents 100 μm. (c) Clinical appearances of DD-STSG (Donor Dominant - Split-Thickness Skin Graft) rejection (upper) and DD-SC (Donor Dominant - Skin Construct) rejection (lower) are shown (i). Hematoxylin and eosin (HE) staining images of DD-SC-treated wounds on postoperative day 4 (ii), DD-SC-treated wounds on postoperative day 6 (iii), and DD-STSG-treated wounds on postoperative day 6 (iv) are provided. The images are magnified at 20X. (d) A representative time course of the full-thickness wounds (FTWs) treated with ASC (Autologous Skin Cell), STSG (Split-Thickness Skin Graft), aBlCC (acellular bilayer collagen-chitosan), or dressings alone (sham) from Yorkshire 2 is shown. (a-b) Reproduced from Ref. [91] with permission. (c) Reproduced from Ref. [92] with permission. (d) Reproduced from Ref. [93] with permission.

treatment. For example, researchers have developed a self-assembled dermal matrix (DM) using a single population of newborn cells [91]. Cells derived from newborns have characteristics of low immunogenicity and high vitality. Using the layer-by-layer construction, researchers obtain fibroblast-produced tissue templates. After decellularization, the donor's fibroblast and keratinocytes were seeded on the DM and formed the artificial skin (Fig. 12a). K10, involucrin, transglutaminase and filaggrin were positively expressed in the epidermal layer and stratum corneum, indicating the preserved structure and function of the artificial skin (Fig. 12b) [91].

Additionally, the mechanism of the skin transplant rejection was also studied to provide more clues for developing artificial skin with immune tolerance. It was demonstrated that the absence of the foreign ECM and professional passenger antigen-presenting cells (APC) can effectively reduce the rejection response in the allogeneic situation (Fig. 12c) [92]. Another study presents a comparison of the treatment efficacy between autologous skin construct (ASC), split-thickness skin grafts (STSG), and bilayer living cellular construct (BLCC). As shown in Fig. 10d, ASC exhibited enhanced skin construct vascularization and healing, as well as reduced dermal thickness and skin contraction [93].

3. Applications of engineered artificial skins

The primary purpose of constructing engineered human skin is to mimic the structure and functionality of human skin. It is primarily used as an in vitro model for research and the treatment of skin-defect-related diseases. Although the construction strategies or methods used may be similar, the focus of engineering artificial skin varies depending on the intended purpose. When constructing in vitro models, artificial skin aims to recreate certain features of the disease process, such as using seed cells derived from patient skin. In the context of therapeutic purposes, artificial skin focuses on reducing its immunogenicity and enhancing repair effects, for example, by incorporating growth factors and other drugs. In the following sections, we will introduce the specific engineered artificial skins based on their application.

3.1. Wound healing

Engineered artificial skin has emerged as a promising approach for enhancing wound healing outcomes. These artificial skins are designed to replace or assist the damaged or lost skin tissue, providing a supportive environment for the natural healing process. They offer several advantages over traditional wound dressings by mimicking the structure and function of native skin, promoting cellular interactions, and delivering bioactive molecules. The application of engineered artificial skins in wound healing has shown significant potential and encompasses various approaches.

To assess the safety and effectiveness of artificial skin transplantation, a phase I clinical trial was conducted [40]. A 4 cm^2 skin biopsy specimen was obtained from the patients with partial-or full-thickness skin defects. The autologous keratinocytes and fibroblasts were extracted from the specimen and cuture on the collagen-based scaffold to generate the artificial skin graft with 49 cm^2. It was demonstrated that the patients undergoing the transplant exhibited successful wound healing, as evidenced by the near-natural epidermis and dermal reconstruction.

In addition, artificial skin finds extensive application in the treatment of burns, providing effective solutions for wound coverage and promoting healing. It can be used as temporary coverage for partial-thickness burns, protecting the wound and facilitating the natural healing process. For more severe burns, permanent artificial skin grafts offer a long-term solution by promoting tissue regeneration and restoring the skin's protective functions. Artificial skin also supports split-thickness skin grafts and aids in scar revision, enhancing cosmetic outcomes. A case report demonstrated that the self-assembled artificial skin (SASS) transplantation successfully survived an 8-year-old boy with burns covering 86 % of

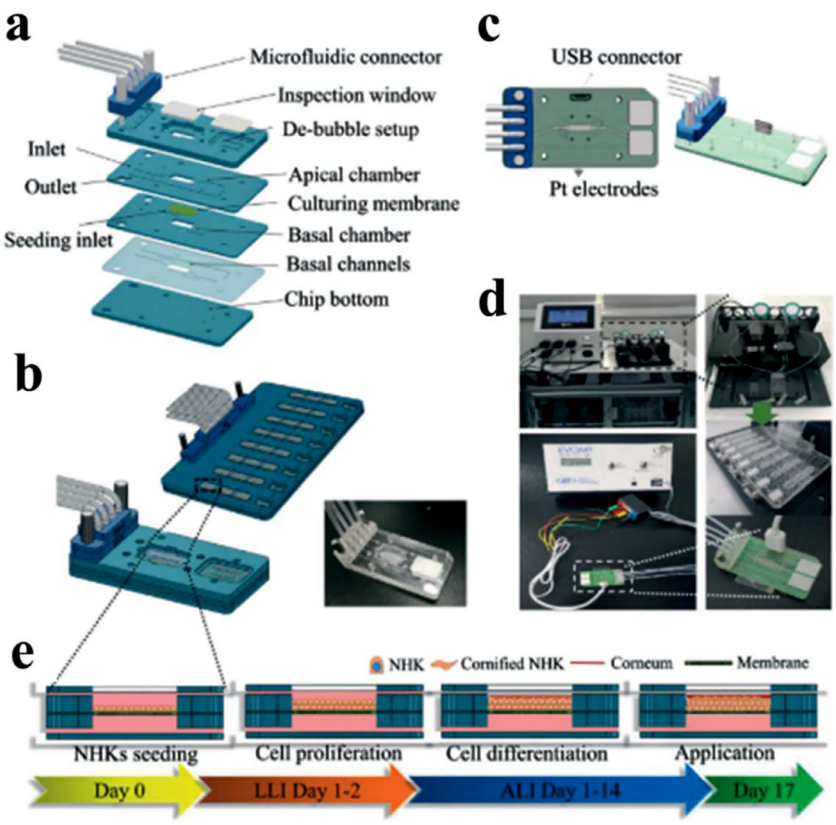

Fig. 13. (a) A detailed exploded view of the iEOC (in vitro Epidermal Organ-on-a-Chip) device is presented, consisting of four main layers made of PMMA (Polymethyl methacrylate), along with a microfluidic connector. The virtual representation showcases the basal microfluidic channels. (b) An illustration of the multi-chamber chip and the overall appearance of the iEOC device is provided, highlighting its design and structure. (c) The iEOC device is depicted with TEER (Trans Epithelial Electrical Resistance) attachments, demonstrating the integration of TEER measurement capabilities. (d) The dynamic culturing process controlled by the microfluidic system and the in situ TEER detection on the chip are shown, emphasizing the real-time monitoring capabilities of the device. (e) A schematic diagram is presented to illustrate the process of epidermis formation, describing the key steps involved in the development of the epidermal layer. (a-e) Reproduced from Ref. [95] with permission.

his body surface area (BSA) [42]. Considering far inadequate unburned skin for donation, a temporary dermal replacement (BTM) was used for coverage of the wound and wait for donation. During the period, a skin biopsy was obtained and the SASS graft was constructed based on the living cells extracted from the biopsy. The SASS exhibited a closed structure and function of the human skin. The wound area covered with the SASS revealed favorable scarring characteristics.

3.2. Reconstructive applications

Engineered artificial skin has also shown great potential in the reconstruction of complex tissue defects and congenital anomalies. In cases where large areas of skin are missing or damaged, engineered artificial skin can be utilized to replace or augment the lost tissue. These artificial skins can be tailored to match the specific needs of the defect, providing mechanical stability, promoting tissue integration, and supporting the regeneration of functional skin. By combining different types of biomaterials, cells, and growth factors, researchers aim to create constructs that closely mimic the native skin's structure, function, and esthetic appearance. For instance, researchers used the allogenic acellular dermal matrix (ADM) to reconstruct the extensive skin defect [94]. In the cases of the cranial defect, deep traumatic defect in the pre-patellar region, and abdominal wound, the ADM served as an ideal artificial skin and effectively improved the quality of the scar. The early revascularization and the reduced bacterial infection were also observed in the cases.

3.3. Experimental models

Engineered artificial skin can also serve as a valuable tool in research and development, allowing for the study of wound healing mechanisms, the evaluation of novel therapeutic strategies, and the testing of drug candidates. They provide a controlled and reproducible platform for investigating cellular behavior, tissue regeneration processes, and the efficacy of various interventions. Through these applications, artificial skins contribute to the advancement of our understanding of skin biology and wound healing, leading to the development of more effective treatment modalities in the future.

As the field of organ-on-chip (OOC) technology continues to advance, researchers can recreate the mechanical and biomedical cues present in human organs. Integrated with OOC, the artificial skin can generate the skin-on-chip, and provide a more accurate and reliable model for studying various aspects of human skin, such as drug testing, toxicity screening, disease modeling, and personalized medicine. In a recent study, researchers developed an epidermis-on-a-chip (iEOC) system by seeding the human keratinocytes in the microfluidic chip [95]. The microfluidic chip unit (shown in Figs. 13a and b) consists of four PMMA thermally bonded layers to prevent drug from PDMS. In addition, we have incorporated four electrodes within the chip to enable in situ detection of trans-epithelial electrical resistance. The electrodes were linked to a TEER measuring device using a USB adapter, as illustrated in Fig. 13c. A microfluidic template incorporating multiple micropumps was utilized to control fluid loading and perfusion (Fig. 13d). NHK cells are implanted in the chamber and after 1–2 days of proliferation phase and 14 days of growth phase, they can form artificial epidermis with a multilayered structure (Fig. 13e). The iEOC demonstrates the ability to accurately differentiate between toxins and non-toxins in irritation measurements, aligning with OECD standards. Additionally, it shows promising results in the preliminary identification of irritation responses, which can predict diverse irritation reactions.

4. Conclusion and perspective

In conclusion, the current construction strategies and applications of engineered artificial skins have significantly advanced the field of regenerative medicine and provided valuable alternatives for skin reconstruction. These strategies encompass a multidisciplinary approach, combining biomaterials, cells, growth factors, and advanced fabrication techniques to create functional and biocompatible constructs that mimic the

properties of native skin. Engineered artificial skins have demonstrated promising results in various reconstructive applications, including burn treatment, chronic wound healing, and tissue defect reconstruction.

The development of biomaterials with tailored properties has enabled the creation of scaffolds that provide mechanical support, promote cell attachment and proliferation, and facilitate tissue regeneration. Natural and synthetic polymers offer versatility in scaffold design, while the incorporation of bioactive molecules and growth factors enhances the regenerative capacity of artificial skins. Advanced fabrication techniques, such as 3D bioprinting and electrospinning, have allowed for precise control over scaffold architecture, cellular distribution, and growth factor delivery, enabling the creation of complex and patient-specific constructs.

Looking ahead, further advancements in the field of engineered artificial skin hold great potential. Ongoing research focuses on improving the long-term stability and functionality of artificial skin, as well as enhancing its integration with the host tissue. Strategies to enhance vascularization within the constructs are being explored to improve nutrient and oxygen supply, promoting faster and more efficient tissue regeneration. The development of more sophisticated and physiologically relevant in vitro models for preclinical testing will contribute to the translation of engineered artificial skin into clinical practice.

Personalized medicine and tissue engineering approaches are also gaining traction, aiming to create patient-specific artificial skins that closely match the individual's anatomical, functional, and esthetic requirements. This involves utilizing patient-derived cells and incorporating personalized genetic and epigenetic information to optimize the regenerative potential of the constructs. Anticipated progress in stem cell research and regenerative medicine holds the potential to significantly improve the effectiveness and versatility of engineered artificial skin in the future.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

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