Chapter 10
Skin Regeneration

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Abstract The engineering of skin substitutes and their applications on the regeneration of damaged skin have advanced dramatically in the past decades. However, scientists are still struggling with the generation of full-thickness skin with native structure and completed functions. In this chapter, classified by sources, recent developments of biomaterials for skin regeneration have been summarized. Then the most common formats of the engineering skin substitutes are introduced. The strategies of the biological functionalization in the design of skin substitutes are further summarized. Some important challenges in the field of skin substitutes such as angiogenesis, scarring, and appendages loss, and the advanced strategies including endogenous stem cells-based therapy and on-demand therapy are particularly focused on. Finally, a brief conclusion and some perspectives are given in terms of the future trend of biomaterials for skin regeneration.

Keywords Skin · Scaffold · Biofunctionalization · In situ regeneration · Regenerative medicine

10.1 Introduction

The skin, the largest organ of the human body, provides a protective barrier against physical, chemical, and biological pathogens to support and maintain human health. In addition, the skin also has the function of temperature regulation, external insult protection, and detoxing. Typically, the skin has hierarchical structures including the upper epidermal layer, interlayer dermis, and subcutaneous tissue. The epidermis

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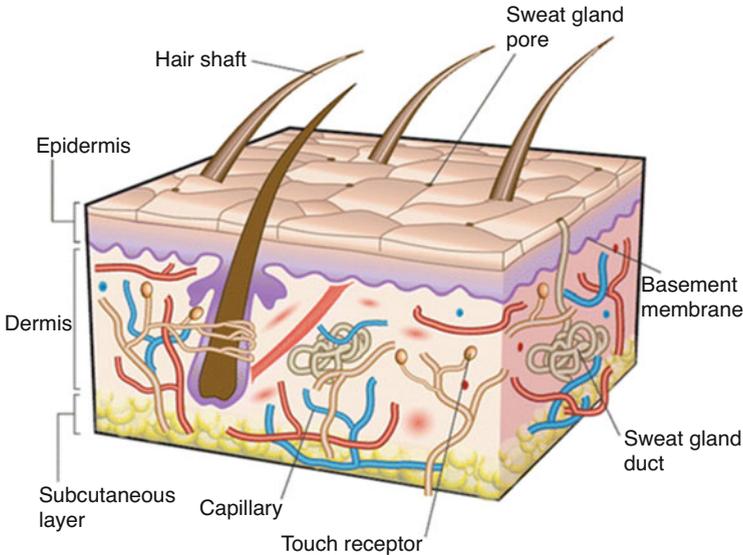e-mail: liema@zju.edu.cn; cygao@zju.edu.cn

Fig. 10.1 The structure of the human skin. (Reprinted from Ref. [1] with permission. Copyright 2007, Rights Managed by Nature Publishing Group)

whose thickness is 0.1–0.2 mm consists mainly of keratinocytes derived from the capillary network. The dermis layer composes of fibroblasts and extracellular matrix (ECM) including collagen, glycosaminoglycans (GAGs), and elastin. Skin appendages such as hair follicles, sweat glands, and sebaceous glands are from the subcutaneous tissue and play a great role in the sensation, temperature regulation, and detoxing (Fig. 10.1) [1].

Burn, trauma, or chronic diseases frequently cause the loss of the skin, leading to descent of nonspecific immunity and bacterial infection, which is one of the most severe problems affecting human life quality. Thus, skin regeneration has become a major aim in the field of wound healing. In the past several decades, surgical therapies including skin transplantation have been applied to treat the loss of the skin and have achieved great success in skin regeneration. Autologous skin graft is the "gold standard" for clinical treatment of skin defect, and allograft plays a big role in the early period of skin repair as a temporary cover until a permanent skin graft is available. However, skin autograft and allograft are limited by the timely availability and donor sites. In addition, current skin grafts often suffer from a range of problems including incomplete biological functions, scar formation, and bacterial or virus infection during surgical therapies [2]. Thus, it has been becoming more and more urgent to find effective therapy strategies for the treatment of skin loss facing with the increasing clinical need and a vast patient resource.

Recently, skin substitute based on tissue engineering is being rapidly developed to bypass the limitations of conventional tissue transplantation and provide new therapeutic strategy to restore skin function [3]. Tissue engineering combines

scaffold, cells, and biofactors to remodel the target tissue or organ in vitro, followed by in vivo transplantation according to the principles of materials, medicine, and biology. Skin tissue substitutes based on tissue engineering have been fabricating over the past several decades to provide more suitable therapeutic schemes for skin loss, and some commercial products are available in clinical application. For example, Dermagrafts®, a dermal skin substitute consists of poly(glycolic acid) (PGA), poly(lactic acid) (PLA), and fibroblasts, has been used to treat diabetic ulcers [4]. However, the fully functional skin regeneration is still a big challenge for skin tissue engineering. Better tissue engineering strategy in the aspect of exploitation of new biomaterials and novel design of biomaterial scaffolds should be developed to fulfill the increasing demand of skin regeneration.

This chapter focuses on the application of tissue engineering and regenerative medicine approach for the fabrication of bioengineered constructs for skin regeneration. First of all, the materials and the design of material scaffold for skin regeneration are summarized. Then the biofunctionalization of biomaterial scaffold is reviewed by using proteins, genes, and cytokines. Finally, some important challenges for skin regeneration including angiogenesis, scarring, appendages regeneration, and in situ tissue regeneration are discussed.

10.2   Materials for Skin Regeneration

10.2.1   Natural Materials

Collagen, one of the most important components of ECM and composed of a triple helix, is widely used in skin regeneration due to its good biocompatibility, biodegradability, flexibility, and structural and functional similarity to ECM [5]. Besides the collagen derived from animal, plant-derived human collagen has been shown to be a promising biomaterial for skin tissue engineering because of its low risk of an allergic response or disease transmission [6]. However, the poor mechanical properties of collagen limit its application in skin substitute. A variety of methods including cross-linking and blending with other substances have been established to improve the mechanical properties of collagen-based scaffold [7]. For example, synthetic human elastin/collagen composite scaffolds were fabricated by electrospinning for tissue engineering dermis [8]. The scaffold supported fibroblast infiltration, de novo collagen deposition, and new capillary formation. Recently, a full-thickness skin equivalent consists of collagen and silk was prepared to study skin biology [9]. In our lab, a collagen/chitosan scaffold cross-linked with glutaraldehyde has been fabricated to promote the growth of fibroblasts and dermis regeneration. The cross-linking of glutaraldehyde and introduction of chitosan can enhance the biostability of the scaffold [10, 11]. Wang et al. used PLGA-knitted mesh to integrate with collagen/chitosan scaffold to improve the mechanical strength of the scaffolds [12]. Some commercial products based on collagen have been applied in clinical practice. For example, Integra® fabricated by cross-linked bovine
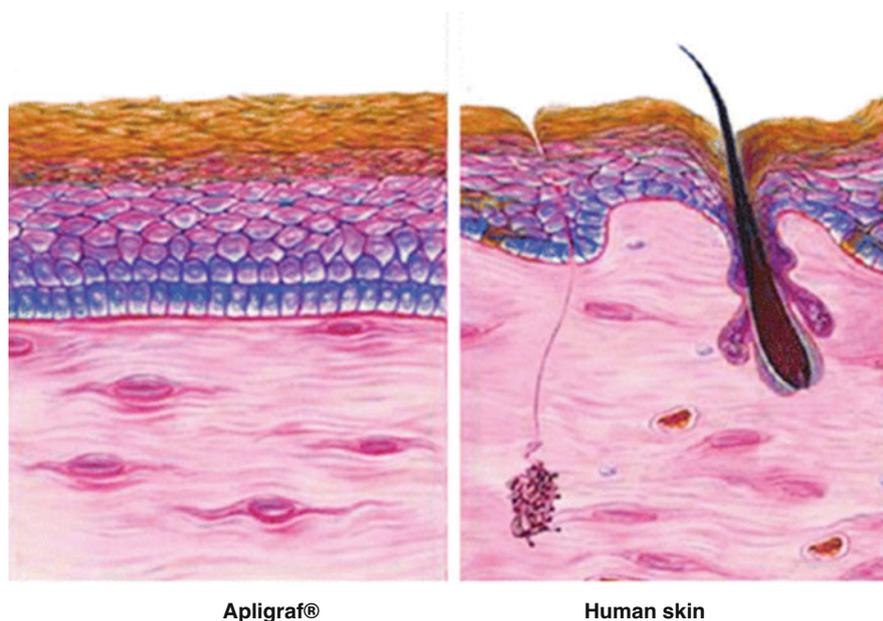
Apligraf®   **Human skin**

Fig. 10.2 Histology of Apligraf compared with normal human skin. (Reprinted from Ref. [13] with permission. Copyright 2010 John Wiley & Sons, Inc.)

collagen and chondroitin-6-sulfate was employed for dermal regeneration. Apligraf®, a collagen-based hydrogel seeded with dermal fibroblasts and epidermal cells, has been widely applied to treat burns and several kinds of ulcers in the clinic (Fig. 10.2) [13, 14]. But it remains a challenge to regenerate the skin with complete appendages.

Chitosan, the deacetylated derivative of chitin, is a linear polysaccharide consisting of glucosamine and N-acetyl glucosamine [15]. Chitosan can be tailored with various molecular weights (50–2000 kDa) as well as degrees of deacetylation (30–95%), allowing wide adjustment of mechanical and biological properties [16]. Cross-linking is usually made to control the degradation rate and enhance the mechanical properties of chitosan matrix for skin tissue engineering as well [17]. Chitosan can be applied to deliver bioactive molecules. For example, the human epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) were encapsulated in chitosan scaffold to promote wound healing [18, 19]. In addition, membranes based on chitosan are widely used as wound dressings because of the antibacterial capacity of chitosan [20]. However, the most extensive application of chitosan for skin regeneration is serving as a three-dimensional matrix. Tchemtchoua et al. prepared chitosan nanofibrillar scaffold for skin repair. Compared to chitosan sponge, the chitosan nanofibrillar scaffold induced a faster regeneration of both the epidermis and dermis (Fig. 10.3) [21]. Kiyozumi et al. fabricated a photo-cross-linkable chitosan hydrogel containing DMEM/F12 medium
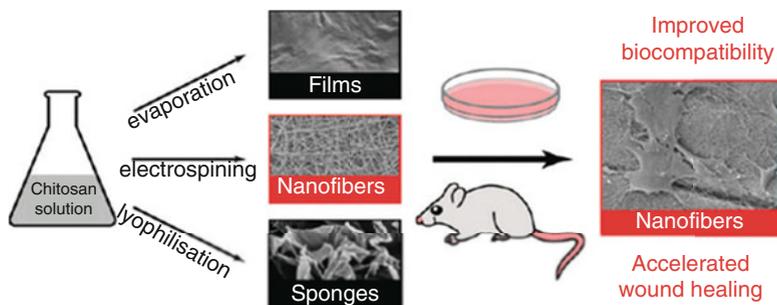
Fig. 10.3 Compared with chitosan films and sponges, the nanofibrillar structure strongly improved cell adhesion and proliferation in vitro. When used as a dressing covering full-thickness skin wounds in mice, chitosan nanofibrils induced a faster regeneration of both the epidermis and dermis compartments. (Reprinted from [21] with permission. Copyright 2011 American Chemical Society)

(medium-Az-CH-LA) for skin regeneration [22]. The hydrogel promoted re-epithelialization, vascularization, and wound repair. Moreover, compared to collagen sponge, thicker granulation tissue and earlier neovascularization were found in medium-Az-CH-LA [23].

Other natural biomaterials for skin regeneration including gelatin, hyaluronan, and fibrin are widely used as well. Shevchenko et al. designed a gelatin scaffold with attached silicone pseudo-epidermal layer for wound repair using a cryogelation technique [24]. The mechanical properties of the scaffold were comparable to the clinical product Integra®. In vivo test showed that the gelatin scaffold supports wound healing by allowing host cellular infiltration, biointegration, and remodeling. Monteiro et al. utilized a spray-assisted layer-by-layer assembly technique to fabricate a multilayer film composed of poly-L-lysine (the epidermal component) and porous hyaluronic acid scaffold (the dermal component) in a rapid and controlled manner for skin tissue engineering [25]. The multilayer film enhances cell adhesion and regeneration of the epidermal barrier functions. Losi et al. prepared a fibrin-based scaffold with incorporated VEGF- and bFGF-loaded nanoparticles to stimulate wound healing [26]. The scaffold induces re-epithelialization and enhances granulation tissue formation/maturity and collagen deposition in genetically diabetic mice. Acellular dermal matrix (ADM) is used to obtain scaffolds with similar components and structure of ECM of the natural skin. For instance, AlloDerm® made of ADM by LifeCell® Corporation is extensively employed for full-thickness skin regeneration.

10.2.2 Synthetic Polymers

The mechanical property is the biggest drawback of natural materials for skin regeneration: thus, natural materials usually need to be cross-linked or combined with other materials. On the contrary, synthetic polymers with predictable and

flexible physical and chemical properties including mechanical properties, functional groups, and degradation rate can be obtained under controlled conditions with mature techniques. Besides, synthetic polymers are biodegradable, are less expensive, and have lower immunological response than natural materials [27]. Furthermore, synthetic polymers such as PGA, PLA, poly(lactide-*co*-glycolide) (PLGA), and polycaprolactone (PCL) have been approved by the Food and Drug Administration (FDA) of the USA.

Synthetic polymers are important materials for skin regeneration. TransCyte® developed by the Advanced Tissue Science Company consisting of PLA scaffold and fibroblasts has been approved by the FDA for the healing of degree III burns [28]. PLGA matrices with fiber diameters varying from 150 to 6000 nm were fabricated via electrospinning [29]. Human skin fibroblasts acquire a well-spreading morphology and show significant growth on fiber matrices in the 350–1100 nm diameter range. Fibrous scaffolds composed of PLA and poly(ethylene glycol) (PEG) were prepared by electrospinning for skin tissue engineering [30]. The scaffold containing 30% PEG exhibited most beneficial properties including wettability, and adaptable bulk biodegradation, and promoted the penetration and growth of human dermal fibroblasts. However, synthetic polymers are usually hydrophobic, and their lack of functional groups leads to limited capacity to combine with biomolecules. To enforce the bioactivity of synthetic polymers, natural materials are widely applied with synthetic polymers to design hybrid scaffolds. Chen et al. fabricate a hybrid scaffold composed of knitted PLGA and weblike collagen microsponges to facilitate cell seeding and distribution and rapid formation of the dermal tissue [31]. A porous polycaprolactone (PCL)/collagen membrane was designed by Venugopal et al. via electrospinning. The well-defined nanostructure can well promote the growth and adhesion of cells [32]. The postmodification of synthetic polymers is another important method for the enhancement of bioactivity. Yang et al. used anhydrous ammonia plasma treatment to modify surface properties to improve the cell affinity of a PLA/PLGA scaffold [33]. The modified scaffold facilitated the growth of fibroblasts. Nanofibrous PCL/gelatin scaffolds were modified by collagen type I grafting [34]. The diameter of the fiber and porosity decreased with the increase of grafted collagen, and the collagen-modified nanofibrous PCL/gelatin scaffolds can maintain characteristic shape and promote proliferation of fibroblasts.

10.3   Scaffold Design for Skin Regeneration

The scaffold plays a vital role for skin regeneration by serving as a three-dimensional matrix for maintaining cell activities and promoting extracellular matrix formation, delivering biofactors, preserving tissue volume, and providing temporary mechanical function. As the skin is complicated with a multilayer structure, how to design a scaffold mimicking the hierarchical structure and ultrastructure of ECM of the skin is an issue of great importance. So far different formats of scaffolds have been designed

to treat different kinds of damaged skins. For example, chitosan/poly(vinyl alcohol) (PVA) nanofibrous membrane has been prepared by electrospinning as a wound dressing [35]. However, for dermal or even full-thickness skin regeneration, the most common scaffold formats are porous scaffolds and hydrogels.

10.3.1   Porous Scaffolds

The porous scaffold is the most common format for skin regeneration. Typically a porous scaffold possesses unique microstructures similar to native ECM, showing high surface area which facilitates cell attachment and growth. Both natural and synthetic polymers can be fabricated into the porous scaffolds with controlled three-dimensional structures by methods such as gas foaming, freeze-drying, and electrospinning.

A novel porous scaffold composed of collagen, hyaluronic acid, and gelatin was fabricated by freeze-drying for skin repair [36]. The average pore diameter of the scaffold was $132.5 \pm 8.4\ \mu m$, which is beneficial for cell attachment and infiltration. The in vivo histological results revealed that the scaffold promoted wound healing compared to the group without treatments. Lu et al. fabricated a funnel-like porous PLLA–collagen and PLLA–gelatin hybrid scaffolds by forming collagen or gelatin sponge on a woven PLLA mesh for skin tissue engineering [37]. PLLA–collagen and PLLA–gelatin porous scaffolds promoted the regeneration of the dermal tissue and reduced contraction during the formation of new tissues. In our lab, Ma et al. have developed a collagen/chitosan hybrid porous scaffold which was cross-linked by glutaraldehyde (GA) to improve their biostability for skin regeneration [11]. Collagen and chitosan are evenly distributed in the scaffolds with high porosity and good interconnectivity. In vitro culture suggested that the porous scaffold could maintain the original good cytocompatibility of collagen and effectively accelerate infiltration and proliferation of human dermal fibroblasts. In vivo test revealed that the scaffold could induce the fibroblast infiltration from the surrounding tissues. Besides, collagen/chitosan-silicone membrane bilayer dermal equivalent (BDE) was designed, in which the silicone membrane covers the hybrid scaffold to prevent water evaporation and infection (Fig. 10.4) [38]. The porous BDE can be functionalized by plasmid DNA to form a gene-activated scaffold for more complicated reconstruction of the damaged skin. For example, the porous BED combined with N,N,N-trimethyl chitosan chloride (TMC)/pDNA-VEGF complexes can significantly enhance the expression of VEGF, which in turn facilitates the regeneration of full-thickness incisional wounds [39]. To inhibit the scar formation during wound healing, TMC/siRNA-TGF-β1 complexes were incorporated into the BDE to interfere in transforming growth factor-β1 (TGF-β1) signal pathway and suppress the expression of TGF-β1 [40]. The functionalized porous BDE can inhibit scar formation compared to the normal BDE. These results indicate that the porous BDE holds great promise for skin regeneration in clinical application.
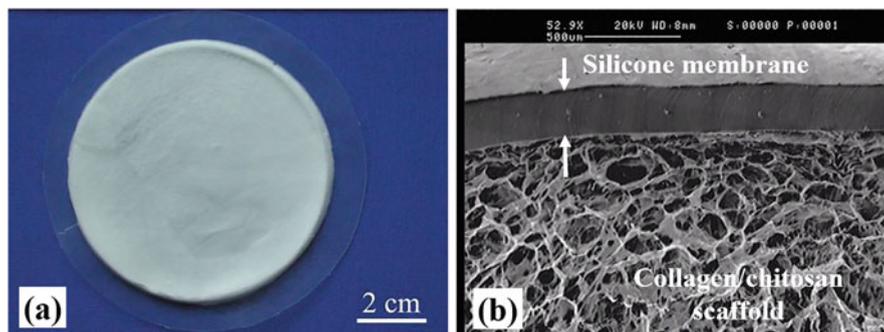
Fig. 10.4 (**a**) A view of the scaffold after being combined with a silicone membrane with a thickness of about 0.14 mm. (**b**) The microstructures of the scaffold observed under a scanning electron microscope. (Reprinted from Ref. [38] with permission. Copyright 2006 John Wiley & Sons, Ltd)

10.3.2 Hydrogel

Hydrogels are three-dimensional cross-linked polymer networks that are capable of absorbing large amount of water, which is important for the absorption of the excess of wound exudates [41]. In addition, hydrogels can protect the wound site from infection and promote the healing process by providing a moisturized environment. Moreover, hydrogels can preserve the bioactivity of growth factors, antibiotics, cytokines, and cells, making them ideal carriers for the delivery of biomolecules to realize complete skin regeneration. Many studies have been focused on the applications of hydrogel for skin regeneration [42–45].

Chitosan hydrogel is well known as a wound dressing, showing good biocompatibility, anti-infective activity, and the ability to accelerate wound healing. Thermo-responsive hydrogel was developed by using chitosan and agarose for skin regeneration [46]. The hydrogel prevented water loss and wound dehydration and was in favor of cell internalization and proliferation. A bilayer physical hydrogel of chitosan without any external cross-linking agent was used to induce inflammatory cell migration and angiogenesis [47]. A hydrogel sheet composed of alginate, chitin/chitosan, and fucoidan (ACF-HS) has been developed for wound dressing (Fig. 10.5) [48]. The hydrogel can provide a moist environment for rapid wound healing. Significantly advanced granulation tissue was observed in the healing-impaired wounds being treated with the hydrogel. Wong et al. fabricated a pullulan–collagen composite hydrogel by using a salt-induced phase inversion technique, which can recapitulate the reticular structure of human dermal ECM [49]. The hydrogel promoted wound closure due to the increased recruitment of stromal cells as well as the formation of the granulation tissue. A versatile, nontoxic, in situ cross-linkable biodegradable dextran hydrogel loaded with chitosan microparticles containing VEGF and EGF was designed for skin regeneration. In vivo results showed that the hydrogel improved the physical, chemical, and biological protection
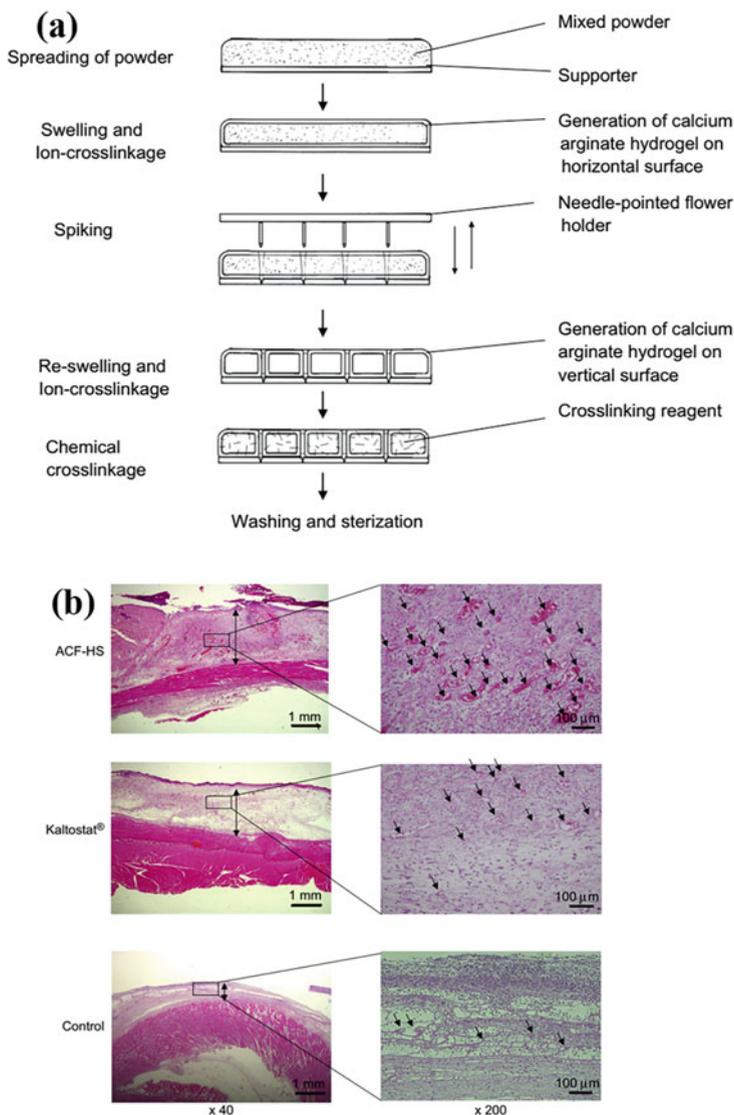
Fig. 10.5 (**a**) Preparative procedures for ACF-HS. (**b**) Histological examination of wounds covered with ACF-HS or Kaltostat® and controls (uncovered). In the *left panel*, *black arrows* show formed granulation tissues. In the *right panel*, *squares* show the sites for microphotographs, and *black arrows* show blood vessels containing erythrocytes. (Reprinted from Ref. [48] with permission. Copyright 2009 Published by Elsevier Ltd)

of the damaged skin [50]. Besides, the spatiotemporally controlled release of VEGF- and EGF-enhanced angiogenesis and re-epithelialization are crucial for the reconstruction of the native skin. More excitingly, a recent study shows that dextran-based

hydrogels supported the infiltration of inflammatory cells, resulting in its rapid degradation and promoted infiltration of angiogenic cells and endothelial cells into the healing wounds [51]. In addition, the remarkable neovascularization and regeneration with hair follicles and sebaceous glands were observed after 21 days, and new hair was observed 5 weeks later. These results indicate that dextran-based hydrogel alone without bioactive factors can promote complete skin regeneration with appendages.

10.4   Biofunctionalization of Skin Regeneration Scaffolds

Skin repair is the result of synergistic effect of different kinds of cells whose proliferation, migration, differentiation, and ECM secretion are well regulated by bioactive factors such as growth factors, genes, and cytokines. Combining bioactive factors with scaffolds is a promising way to promote the efficiency and quality of the regenerated skin.

10.4.1   Growth Factors

Growth factors are capable of stimulating cellular growth, cellular proliferation, and cellular differentiation. Usually they are proteins or steroid hormones regulating a variety of cellular processes. Growth factors typically act as signaling molecules between cells to promote cell differentiation or maturation and have been widely used in tissue engineering skin constructs. Some growth factors are promising mediators of wound healing, such as the epidermal growth factor (EGF), fibroblast growth factor (FGF), vascular endothelial growth factors (VEGF), platelet-derived growth factor (PDGF), insulin-like growth factor-1 (IGF-1), transforming growth factors α and β (TGF-α and TGF-β), and hepatocyte growth factor (HGF). Site-specific delivery of growth factors in microdevices could provide an efficient means of stimulating localized recruitment to the cell transplants and would ensure cell survival and functions. By combining growth factors with microvehicles, bioactive skin scaffolds can be constructed. Richardson et al. incorporated VEGF and PDGF into a porous PLGA scaffold to realize a controlled dose and rate of delivery, pioneering the research of a vehicle delivering multiple angiogenic factors with distinct kinetics [52]. Perets et al. incorporated bFGF-loaded microspheres with three-dimensional porous alginate scaffolds, achieving enhanced vascularization in vivo [53]. Tabata et al. combined FGF, HGF, and VEGF with collagen gels to promote the regeneration of hair follicles after implantation [54]. Mao et al. combined FGF on a substrate via a layer-by-layer technique to fabricate bioactive films, on which fibroblasts proliferated better and secreted more ECM [55]. Regarding to hair follicle regeneration, HGF, a mitogen, motogen, and morphogen for lots of different organs, is expressed by the cells in human hair follicles and involved in the

cycle of hair growth. Uijtdewilligen et al. incorporated insulin-like growth factor-2 (IGF-2) and sonic hedgehog (SHH) into a collagen type I heparin scaffold to form an embryonic-like scaffold which could help to repair the skin without contraction or scar formation [56].

10.4.2   Genes

As summarized above, with the ability to modulate and direct cells efficiently, the growth factors have been extensively used as bioactive factors to combine with tissue engineering scaffolds. However, the most challenging limitation is their short half-lives. The emerging gene technique provides an optional method to make cells produce growth factors constantly. Hence, functional genes can be incorporated into scaffolds as bioactive factors and are locally expressed to encode specific growth factors at wound site. Specific examples of application of gene therapy in skin tissue engineering will be introduced below.

Shea et al. incorporated plasmid DNA encoding platelet-derived growth factor (PDGFB) into a three-dimensional PLGA sponge, implantation of which in a rat dermis showed enhancement of granulation tissue and vascularization [57]. Other types of matrices such as collagen and PVA sponges loaded with genes are also developed and used to treat cutaneous wounds, resulting in improvement of flap survival, granulation tissue formation, angiogenesis, and re-epithelialization [39, 58].

Alternatively, the nucleic acids (e.g., plasmid, siRNA) are complexed with cationic polymers or lipids, with the design of these transfection reagents depending upon the nucleic acid properties, such as size [59]. Complexation with polymers or lipids protects against degradation, creates a less negative particle relative to naked plasmid, and facilitates internalization and intracellular trafficking [60].

10.4.3   Cytokines

Cytokines, a family of small molecules of approximately 8–10 kDa in size, are key regulators of cell migration, immune responses, and wound healing [61]. Proinflammatory cytokines, particularly IL-1 and IL-6 and TNF-α, are upregulated during the inflammatory phase of wound healing.

IL-1 is produced by monocytes, neutrophils, macrophages, and keratinocytes and is immediately released by keratinocytes during wound healing. IL-1 activates fibroblasts and promotes the secretion of FGF [62]. IL-6 is secreted by neutrophils and monocytes and has been shown to be involved in healing response. Evidence shows that IL-6 is closely related with wound healing by regulating leukocyte infiltration, angiogenesis, and collagen accumulation [63]. TNF-α can promote the

expression of FGF-7, indicating that it can favor the process of re-epithelialization [64].

Stromal cell-derived factor-1α (SDF-1α, CXCL12) chemokine is a member of the CXC family and works via the CXCR4 receptor. It plays a role in the inflammatory response by recruiting lymphocytes to the wound and promoting angiogenesis. Endothelial cells, myofibroblasts, and keratinocytes express SDF-1. A number of researches have proved that SDF-1α plays a pivotal role in the recruitment of stem cells. For example, PLGA scaffolds incorporated with SDF-1α can recruit more stem cells, which favors angiogenesis and decreases fibrotic and inflammatory responses. More interestingly, mechanical stretch can upregulate SDF-1α in the skin tissue and promote migration of circulating bone marrow-derived mesenchymal stem cells (BMSCs) [65]. The application of SDF-1α provides an avenue for the recruitment of stem cells, which is crucial for the in situ skin regeneration. For example, Nakamura et al. used mesenchymal stem cells (MSCs) genetically engineered with (SDF-1α) to heal skin wounds [66]. SDF-1α-engineered MSCs (SDF-MSCs) expressed more SDF-1α and enhanced the migration of MSCs and dermal fibroblasts and promoted skin wound closure.

10.5 Important Challenges and Strategies

Although many significant milestones of bioengineered skin have been reached for clinical therapies of full-thickness defects, challenges still remain to fulfill the criteria of "regenerated skin" with complete structural, esthetic, and functional properties as the nature of the skin. How to achieve rapid angiogenesis, inhibited scarring, and regeneration of appendages is a key issue related to the quality of the regenerated skin. In addition, in situ regeneration, which should be particularly concerned, would be less costly and complex than those approaches that require ex vivo cell manipulation.

10.5.1 Angiogenesis

One of the most critical issues for most bioengineered tissues is the rapid and appropriate angiogenesis of the constructs, since a tissue beyond a certain size generally cannot survive without the supply of nutrients and oxygen, and removal of waste products of cells [67]. For materials with a thickness larger than 0.4 mm, new blood vessels are not able to penetrate rapidly [68]. The delayed or poor angiogenesis of the reconstructed skin will hinder the nourishment of the overlaying epidermal layer and result in the failure of the graft. Moreover, if the transplantation of split-thickness skin grafts is delayed, the timely healing of the damaged skin will be hampered and the risk of death will increase [69]. It is clear that the blood supply is essential to realize the long-term integration of the reconstructed skin with the host

tissue. Therefore, acceleration of the angiogenesis rate to achieve rapid formation of new blood capillaries is urgently required, whereas still remains a research focus for improvement of existing skin substitutes.

Suitable pore size of tissue-engineered scaffold plays an important role in improving permeability, facilitating cell migration, and enhancing angiogenesis. Yannas et al. found that a pore size ranging from 90 to 150 μm and porosity larger than 90% promote vessel formation [70]. Pruitt et al. reported that only when pore size of scaffold is larger than 80 μm it can be conducive to the ingrowth of connective tissue and neovascularization [71]. Another possible approach to enhance the angiogenesis of the tissue-engineered skin is to combine the prefabricated vessels with some special kinds of cells to achieve better initial onset of revascularization for early anastomoses between graft and bed vessels. As one example, human dermal microvascular endothelial cells were incorporated into collagen or fibrin hydrogels. Three-dimensional capillaries were formed after transplantation of the prevascularized substitutes [72]. The engineered capillaries were further stabilized by pericytes and smooth muscle cells and ultimately connected to the microvessels of the wound ground. It is also found some glycosaminoglycans have angiogenetic effect. Pieper et al. reported that the incorporation of glycosaminoglycans could increase angiogenesis degree in vivo [73]. However, a sufficient vasculature still takes more than 4 weeks to develop.

The most powerful approach to induce angiogenesis in the engineered tissues is to use angiogenetic growth factors such as bFGF, VEGF, and PDGF. So far many efforts have been made to enhance angiogenesis by incorporation of angiogenic growth factors into tissue engineering scaffolds. With the loading of bFGF, the fibrin and collagen scaffolds show enhanced angiogenesis when applied to the rabbit ear ulcers, therefore greatly improving the healing of full-thickness skin defects [74]. Wissink et al. used heparin to realize a controlled release of bFGF through specific binding, which is effective in promoting the growth of endothelial cells within the collagen scaffold in vitro [75]. Perets et al. encapsulated bFGF into PLGA microspheres and then loaded the microspheres into a porous sodium alginate scaffold [53]. The formation of large mature vessels was greatly promoted by the bFGF-loaded scaffold in a rat peritoneal model.

However, delivery of the growth factors faces some challenges due to their sensitivity and instability, and their half-lives are only on the order of minutes in serum. In addition, the high cost of growth factors also limits their trial in practice. The gene technique has been considered as an alternative way in order to overcome the drawbacks of growth factors. By loading of more stable and functional genes into the scaffolds, the gene-activated matrix (GAMs) can be generated to locally transfect cells and constantly produce targeted growth factors at wound site. Endowed with the advantages of localized treatment, maintenance of effective amount of bioactive DNAs, and protection of DNAs against immune responses and nuclease degradation, the GAMs have shown great promise for the enhanced angiogenesis of the engineered skin. Mao et al. used TMC as a cationic gene delivery vector to carry plasmid DNA encoding VEGF (pDNA-VEGF) and constructed a gene-activated collagen scaffold for skin repair [76]. The in vivo application to Sprague-Dawley
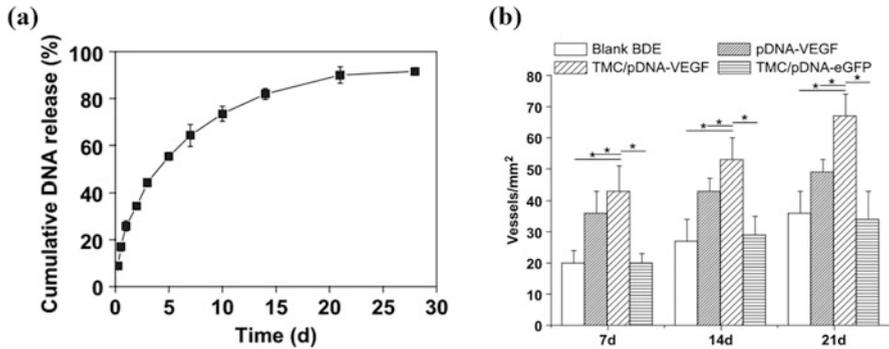
Fig. 10.6 (**a**) Cumulative release of DNA from the scaffolds as a function of time. (**b**) TMC/pDNA-VEGF group had a significantly higher number of newly formed and mature blood vessels. (Reprinted from Ref. [77] with permission. Copyright 2010 Elsevier Ltd)

mice demonstrated that the TMC/pDNA-VEGF complexes remarkably promoted the in vivo expression of VEGF and thus enhanced the angiogenesis of the scaffolds. Recently, a gene-activated collagen-chitosan/silicone membrane bilayer dermal equivalent (BDE) has been prepared and evaluated for treatment of the full-thickness incisional wounds in terms of histology, immunohistochemistry, immunofluorescence, real-time quantitative PCR, and Western blotting analysis in a porcine model [39]. The TMC/pDNA-VEGF group shows highest level of VEGF expression at both mRNA and protein levels, resulting in the highest densities of newly formed and mature vessels. After 112 days of ultrathin skin graft transplantation, the healing skin has a similar structure and ~80% tensile strength of the normal skin. Exploitation of the gene-activated BDE for the healing of full-thickness burns was also performed, showing very positive angiogenesis and repair results similar to those for incisional wounds (Fig. 10.6) [77].

10.5.2 Scarring

Although skin substitutes based on principle of regeneration have achieved important progresses, scarring still remains a problem which results in issues such as disfiguration, itching, and local ulceration [78]. Prevention of scarring is therefore a major challenge to be addressed for the repair and regeneration of skin defects, and antiscarring technologies should be incorporated for the new generation of the skin constructs.

Scars are the outcome of postnatal healing process of normal acute mammalian tissue repair including integration of bioengineered skins [79]. Scarring is a rapid tissue repairing process driven by an evolutionarily devised mechanism, allowing rapid recover of tissue integrity to fill tissue voids. Scarring involves a series of

cellular events related to tissue repair including inflammation, migration/proliferation and ECM deposition, and also the inputs of numerous cell types, matrix components, and signaling molecules. Essentially, the excessive and disordered accumulation of ECM such as collagen, as well as the imbalance of new deposition and destruction of collagen, directly leads to the scar formation. By contrast, the scar-free regeneration should have features including complete restoration of skin structure, normal collagen deposition, and regular distribution of hair follicles, capillaries, and glands, which reflects a focus of interest for the emerging fields of regenerative medicine. Central to a material-based approach for skin regeneration is to build a suitable environment where cells are exposed to a complex pattern of bioactive molecules, which direct desired cell behaviors and right tissue regeneration.

Fetal wound repair is essentially a scar-free regenerative process. It has been extensively studied and confirmed that embryonic scarless wound repair exhibits reduced fibrin clots and platelet degranulation, and suppressed inflammatory response, which has provided therapeutic strategies for scar-free repair. Most importantly, the growth factor profile is also quite different for embryonic wound and adult wound. The large family of TGF-β protein, which can be secreted by multiple cell types such as platelets, macrophages, and fibroblasts, is one of the most important biosignal molecules during the wound healing process. It acts as a chemokine for fibroblasts, induces differentiation of myofibroblasts, regulates the collagen synthesis, and modulates the matrix turnover. Recent investigations have confirmed that the level of TGF-β1 and TGF-β2 in fetal skin injuries is lower than that in adult skin, while the level of TGF-β3 is elevated [80]. Inspired by these findings, successful reduction of scarring of adult skin wounds has been reported by interrupting TGF-β1 and TGF-β2 signaling pathway through neutralization with antibodies, inactivation by proteoglycan-like decorin, and blockage of function by exogenous receptors, as well as exogenous addition of the TGF-β3 isoforms. Samuels et al. [81] found that hypertrophic scar was induced in a rabbit embryos subcutaneous model by injecting the TGF-β1 and TGF-β2, while the addition of their polyclonal neutralizing antibody could inhibit scarring and generate a normal tensile strength and more physical dermal architecture. A recent research proves that after infected by adenovirus encoding a truncated TGF-β receptor II, normal dermal fibroblasts could result in wounds with an average of 49% reduction of the scar area and less inflammatory reaction in the full-thickness incisional wounds in rats [82].

The recently emerging biomolecular cues of RNA interference (RNAi) offer a fascinating and prospective alternative to specifically silence targeted genes and downregulate targeted protein levels [83]. Delivery of exogenous small interfering RNA (siRNA) mediated by three-dimensional scaffolding materials is right now at the frontier of current research. Compared with other strategies, the RNAi not only shows higher efficiency and specificity during a long duration time but also avoids risks of immunogenicity and inactivation in the antibody method. In a recent research in our lab, TMC/siRNA complexes targeting TGF-β1 were incorporated into the collagen-chitosan/silicone membrane bilayer dermal equivalent (BDE) to fabricate an RNAi functionalized bioengineered skin (RNAi-BDE), aiming to
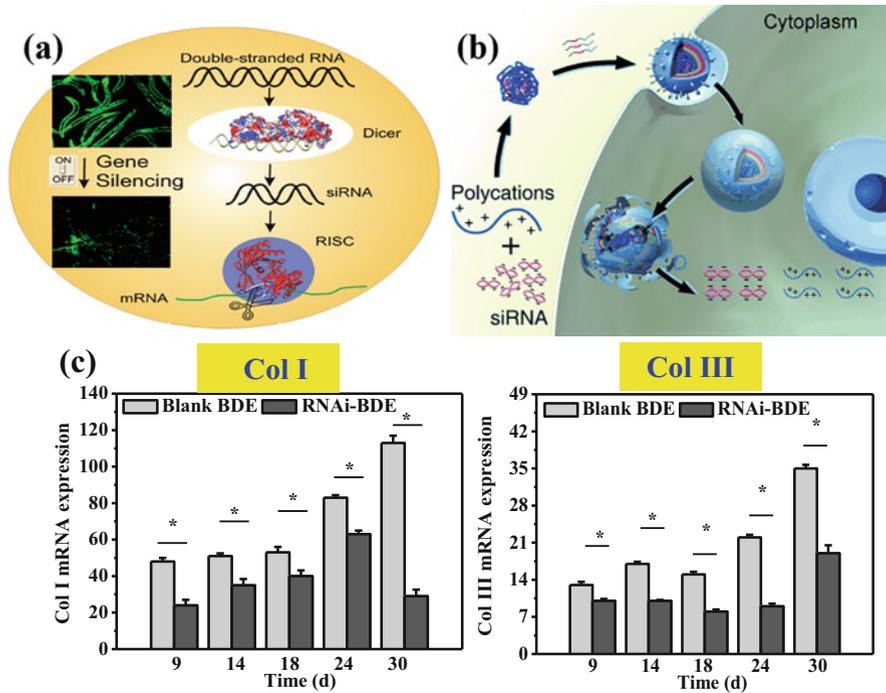
Fig. 10.7 (**a**) The siRNA could silence special gene expression. (**b**) Combination of polycations TMC with siRNA to form complexes which could be transfected into cells. Wound formation is mainly caused by massive and disordered deposition of Col I and Col III. (**c**) TMC/siRNA can suppress the expression of both Col I and Col III significantly. (Reprinted from Ref. [40] with permission. Copyright 2012 Elsevier Ltd)

interfere TGF-β1 signal pathway, directing cell behaviors, and ultimately inhibiting scarring (Fig. 10.7). The RNAi-BDE functioned as a reservoir for the incorporated TMC/siRNA complexes, enabling a prolonged siRNA release. Application of the RNAi-BDE on the full-thickness skin defects of pig backs confirmed the in vivo inhibition of TGF-β1 expression by immunohistochemistry, real-time quantitative PCR, and Western blotting during 30 days postsurgery. The levels of other scar-related factors such as collagen type I, collagen type III, and α-smooth muscle actin (α-SMA) were also downregulated. In combination with the ultrathin skin graft transplantation for 73 days, the regenerated skin by RNAi-BDE had an extremely similar structure to that of the normal one with significant scar inhibition.

10.5.3   Appendages

Skin appendages such as hair follicle, sweat gland, sebaceous gland make skin functions well in touch, temperature sensation, excretion, perspiration, and thermoregulation. Regeneration of skin appendages is an important symbol of skin recovery and functionalization. Although some commercial artificial skin substitutions can achieve a structural repair in the epidermis and the dermis, it remains a challenge to regenerate the skin with complete appendages [13]. Hence, regenerated skin cannot fully replace normal skin in function.

Hair follicle is a mini-organ which produces hair. It is composed of hair papilla, matrix, root sheath, hair fiber, bulge, and so on. There are three stages in hair growth: the growth phase (anagen), the regressing phase (catagen), and the quiescent phase (telogen). Growth cycles are controlled by chemical signals like epidermal growth factor. Efforts of reconstructing hair follicles have been made decades ago. Lin et al. combined epidermal stem cells in collagen/gelatin scaffold with fibroblasts; hair follicle-like structure was formed after implantation. It is also reported that the interaction between epidermal cells and mesenchymal cells contributes to the formation of hair follicles. More recently, MSCs are induced into hair papilla-like cells [84]. Moreover, polysaccharide, as one main component of dermal ECM, is reported to induce the regeneration of hair follicles on a mice model [51]. Recently, it was reported that hair growth was promoted by adipose-derived stem cell (ASC) transplantation in animal experiments, and a conditioned medium of ASCs (ASC-CM) induced the proliferation of hair-compositing cells in vitro. Jin et al. introduced some ASC stimulators in preconditioning to enhance hair regeneration [85]. They also highlighted the functional role of ASCs in hair cycle progression and concluded the advantages and disadvantages of their application in hair regeneration.

Sweat glands are small tubular structures of the skin that can produce sweat. There are two kinds of sweat glands. Eccrine sweat glands are distributed all over the body (except for the lips, tip of the penis, and the clitoris), although their density varies a lot from region to region, while apocrine sweat glands are larger, having different mechanisms of secretion, and are limited to axilla (armpits) and perianal areas. Sweat glands play a key role in thermoregulation and inner balance. Therefore, it is vital to reconstruct sweat glands especially for large-area burns. Fu et al. cultured sweat gland cells (SGCs) on gelatin microspheres containing EGF and delivered the SGCs-microspheres complex into an engineered skin construct mainly composed of a fibroblast-embedded collagen-based matrix [86]. This engineered skin construct was then transplanted onto full-thickness cutaneous wound in an athymic murine model. Remarkably, sweat gland-like structure can be achieved in vitro within the hybrid matrix. Huang Sha et al. designed a functional in vitro cell-laden three-dimensional extracellular matrix mimics (3D-ECM) with composite hydrogels based on gelatin and sodium alginate. It provides the spatial inductive cues for enhancing specific differentiation of epidermal lineages to regenerate sweat glands [87].

Sebaceous glands are kind of microscopic glands in the skin that secrete an oily/waxy matter, called sebum, to lubricate and waterproof the skin and hair of mammals. In human beings, they are found in greatest abundance on the face and scalp, though distributed throughout all skin sites except the palms and soles. Compared with hair follicles and sweat glands, sebaceous glands are later to be studied. But exciting progress has been made related to reconstruction of sebaceous glands. Hair bulge cells have been reported to possess the potential of differentiating into sebaceous glands. Horsley et al. firstly found a kind of progenitor cells that can secret factor Blimp1, which can stimulate the regeneration of sebaceous glands when they get hurt [88].

Appendages do have a firm interaction rather than separate growth, although most researches are still confined to one certain appendage. With further study of biology of appendages, and clarifying the interaction of materials and related cells (specifically stem cells) and appendages themselves, it is quite possible to functionalize skin constructs by reconstructing different appendages together in the future.

10.5.4 In Situ Skin Regeneration

Along with the advancement of science and technology in biology, medicine, and material science, the insight mechanism of wound healing is better understood. Diverse methods for skin repair and regeneration have also been developed. Stem cell-based therapy, which is a promising cure for a multitude of diseases and disorders, has been one of the best documented approaches in regenerative medicine. However, the ex vivo expansion of stem cells and their in vivo delivery are restricted by the low survival rate and the limited availability of stem cell sources.

It has been demonstrated that endogenous stem cells can be actively attracted to sites of injury [3]. Thus, recruiting sufficient endogenous stem cells to the wound area and inducing them to repair the structure and functions of skin become a key challenge. This technique, known as in situ regeneration, has the potential to provide new therapeutic options for all kinds of tissues and organs. The in situ tissue regeneration method relies on endogenous stem cell homing, proliferation, differentiation, and rebuilding functional skins. Such options would be less costly and complex than the traditional approaches which require substantial ex vivo cell manipulation.

Microenvironment, which could be changed by cytokines, surface topology, and so on, could influence stem cell recruiting. Some cytokines can enhance tissue regeneration by facilitating cell homing. Chen et al. fabricated a radially oriented scaffold which could effectively promote BMSCs migration, whose effect was further enhanced by addition of stromal cell-derived factor-1 (SDF-1) (Fig. 10.8) [89]. It is reported that the migration abilities of PDMSCs exposed to hypoxic conditions are significantly increased. Interestingly, decreased integrin alpha4 in PDMSCs under hypoxia increases PDMSC migration ability [90]. In addition, bone morphogenetic protein-7 (BMP-7) is another cell homing factor [3]. Shao et al.
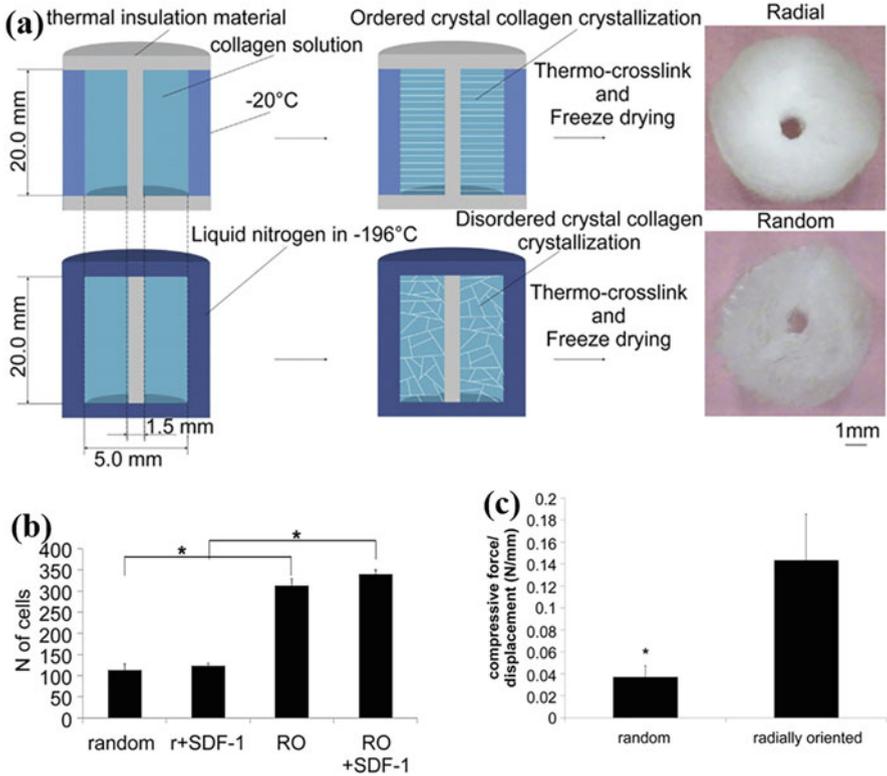
Fig. 10.8 (**a**) Fabrication of the radially oriented (RO) and random collagen scaffolds. (**b**) The radially oriented scaffolds had significantly better mechanical property compared with the random scaffolds. (**c**) The cell number was quantified at low magnification and the radially oriented scaffolds accelerated cell infiltration. (Reprinted from Ref. [89] with permission. Copyright 2014 Elsevier Ltd)

found that a peptide sequence (E7, EPLQLKM) with seven amino acids has a high specific affinity to bone marrow-derived MSCs [91]. In the subsequent work, E7 peptide was immobilized to a collagen scaffold via a collagen-binding domain (CBD) to construct a functional collagen scaffold, which could enhance the speed of healing process [92]. It is reported that E7-modified scaffolds incorporated with rhTGF-β1 could maintain a sustained release and bioactivity. A series of analyses indicate that the E7 peptide promotes BMSC initial adhesion and that the scaffolds containing both E7 and rhTGF-β1 are the most favorable for BMSC survival (Fig. 10.9) [93].
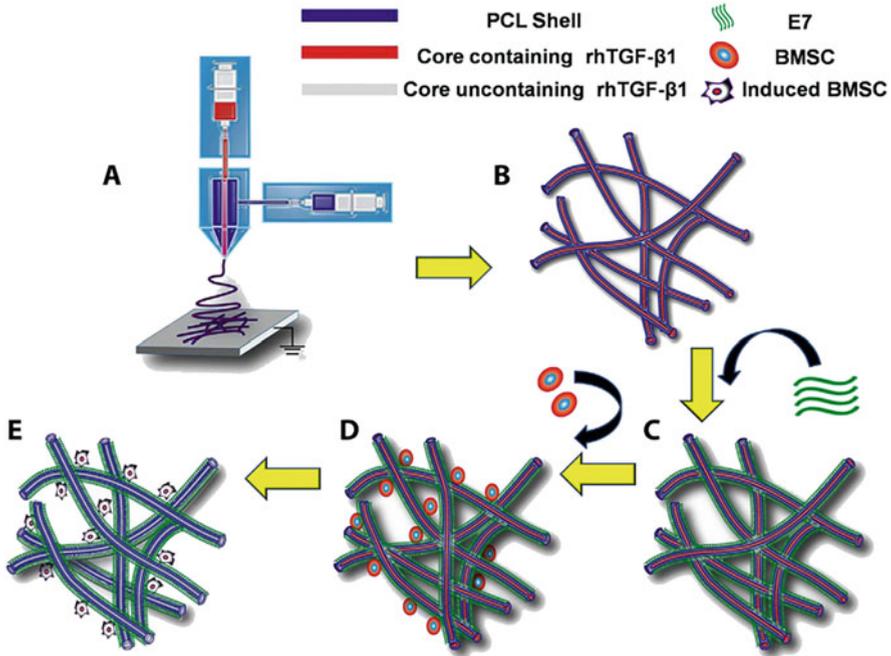
Fig. 10.9 The preparation process for coaxial electrospun fiber scaffolds. (**a**) The process of coaxial electrospinning: the spinneret is composed of two concentric needles; the outer needle is used to deliver the shell solution (*blue*, PCL), while the inner needle is used to eject the core solution (*red*, rhTGF-β 1). (**b**) Scaffold composed of electrospun coaxial fibers, with core (*red*) and shell (*blue*) structure. (**c**) Scaffold conjugated with the BMSC-specific affinity peptide (E7) (*green*). (**d**) E7-modified scaffold facilitates adhesion of BMSCs onto the surface. (**e**) The E7-modified surface and sustained release of rhTGF-β 1 in the core of the coaxial fibers promote adhesion and differentiation of BMSCs [93]. (Reprinted from Ref. [93] with permission. Copyright 2014 Elsevier Ltd)

10.5.5 On-Demand Therapy of Skin Defect

The "Black Box" status of skin regeneration caused by the passive management of traditional materials makes it difficult to obtain the real status of skin defect and provide on-demand treatment in time. Therefore, the development of next-generation skin regeneration system with the abilities of real-time monitoring, diagnosis at an early stage, and on-demand therapy is of great significance. By the integration of flexible electronics with wound dressing, a pioneer wearable skin regeneration system, which can collect physiological signals from wound sites and diagnose healing status real time, was proposed [94, 95]. On the other hand, providing therapy in time to match wound pathological need, i.e., on-demand therapy, is another designing requisite for next-generation skin regeneration system [96]. Therefore, some studies developed advanced wound dressings to provide
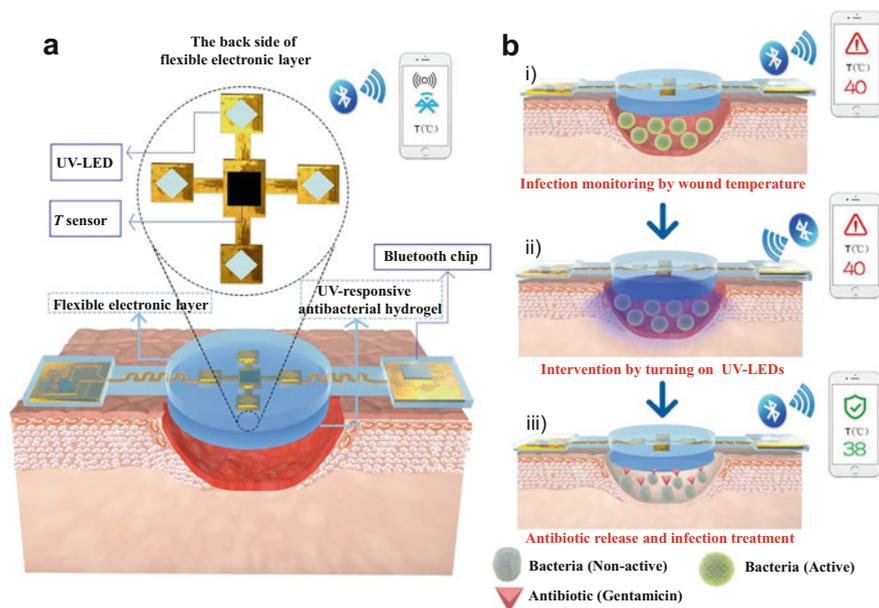
Fig. 10.10 Schematics of the structures and working principles of the smart flexible electronic-integrated wound dressing. (**a**) The integrated system consists of a polydimethylsiloxane-encapsulated flexible electronic layer and an UV-responsive antibacterial hydrogel. The flexible electronic device is integrated with a sensor for monitoring temperature and four UV-LEDs for emitting UV light (365 nm) to trigger the release of antibiotic from the UV-responsive antibacterial hydrogel when needed; a Bluetooth chip is also integrated for wireless data transmission in real time. (**b**) Conceptual view of the integrated system for infected-wound monitoring and on-demand treatment: (**i**) real-time monitoring of wound temperature and providing an alert of hyperthermia caused by infection; (**ii**) turning on UV-LEDs to trigger the release of antibiotics; (**iii**) infection inhibition by the released antibiotics, resulting in decreased wound temperature [99]. (Reprinted from Ref. [99] with permission. Copyright 2020 John Wiley & Sons, Ltd)

on-demand therapy by combining responsive drug-delivery system [97, 98]. Pang et al. reported a novel flexible electronic-integrated skin regeneration system (Fig. 10.10). It was capable of monitoring wound temperature in real-time to provide early diagnosis of pathological infection. By integrating a responsive antibacterial hydrogel into the healing system, antibiotics were released to wound sites controllably to realize on-demand therapy. Wound temperature was collected by the integrated sensor and transmitted via Bluetooth. Once wound temperature was higher than a preset value, infection would be diagnosed, and antibiotics were released responsively to kill bacteria as required [99].

10.6 Conclusions and Future Perspectives

Skin regeneration is one of the most serious problems in clinical medicine. The use of skin grafts is still an important therapy for damaged skin. So far decades of efforts have focused on the development of the tissue-engineered skin based on material technologies, chemistry, biology, and medicine. Bioengineered skins for epidermal, dermal, and full-thickness defects have been fabricated, and some of them are currently commercially available. However, most of them are not sufficient to regenerate new skin similar with native skin.

To realize the regeneration of the skin with a complicated structure and complete functions, it is becoming increasingly popular to design "smart biomaterial system" to provide instructive signals to stimulate target cell responses in the processes of skin regeneration. "Smart biomaterials" can be obtained by adjusting the properties of biomaterials including physical properties, chemical compositions, and biofunctions. Meanwhile, the application of flexible electronic devices into skin regeneration system is able to monitor the wound status in real time, and provide on demand therapy on the basis of need during the skin regeneration process. Moreover, by the combination of stem cells or in situ recruitment of endogenous stem cells, the stem cell hybrid material has attracted more and more interest for the in situ skin regeneration with appendages. We believe that with the development of biomaterial science and regenerative medicine, the skin with a complicated structure and multifunction similar to those of the native skin can be regenerated sooner or later.

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