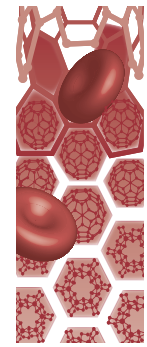
Review

Nanomedicine



Recent advances in electrospun nanofibers for wound healing

Electrospun nanofibers represent a novel class of materials that show great potential in many biomedical applications including biosensing, regenerative medicine, tissue engineering, drug delivery and wound healing. In this work, we review recent advances in electrospun nanofibers for wound healing. This article begins with a brief introduction on the wound, and then discusses the unique features of electrospun nanofibers critical for wound healing. It further highlights recent studies that have used electrospun nanofibers for wound healing applications and devices, including sutures, multifunctional dressings, dermal substitutes, engineered epidermis and full-thickness skin regeneration. Finally, we finish with conclusions and future perspective in this field.

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Keywords: electrospun nanofibers • local drug delivery • scaffold • skin regeneration • wound healing

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Skin is the largest organ of the human body [1]. It interfaces with the external environment and protects the human body against pathogens and excessive water loss [2,3]. Skin wounds may be divided into two categories: acute (e.g., surgical and traumatic wounds, abrasions and burns) and chronic (e.g., diabetic foot ulcers and pressure ulcers) [4]. According to the National Center for Health Statistics, there were 40 million inpatient surgical procedures performed in the USA in 2000, along with 31.5 million outpatient procedures [5]. In addition, there are about 41 million trauma cases each year in the USA with an economic burden of >$670 billion annually [6]. Fire- and heat-induced burns result in 35 million injuries each year worldwide [7], with 2.9 million hospitalizations and 238,000 deaths [8]. Chronic wounds affect 6.5 million patients in the USA, with >$25 billion spent annually on their treatment [5]. This situation is even more serious in developing countries [9,10]. Additionally, 2.5 million pressure ulcers are treated in the USA in acute care facilities alone each year. Management of a single full-thickness pressure ulcer costs approximately $70,000; total annual expenditures for treating pressure ulcers in the USA have been estimated at $11 billion [5]. Therefore, human skin wounds are a major, rapidly growing threat for public health and the economy [5].

Treatment of skin wounds is dictated in part by the size, depth and the extent of undermining of the wound. Skin wounds may be classified as superficial, partial-thickness or full-thickness wounds (Figure 1) [11,12]. Alternatively, skin wounds can be classified into burn, traumatic or chronic wounds. Burn wounds and traumatic wounds could be superficial, partial thickness or full thickness. Chronic wounds are mostly full-thickness wounds. Superficial wounds, defined as a defect of the epidermis and the papillary dermis, heal by re-epithelialization from surviving hair follicles and other dermal append-

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ages. Wound dressings help prevent infection and maintain an appropriate wound environment for healing of superficial wounds [13]. Partial dermal wounds are associated with the epidermis and the deeper dermis. These injuries (e.g., partial-thickness burns and deep ulcers) may not heal adequately secondary to an inadequate mass of dermal appendages available that can form regenerative buds [14]. Treatment of partial-thickness wounds may require dermal substitutes (including acellular and cell-seeded substitutes) to promote migration of nearby cells into the defect site [15,16]. Full-thickness wounds involve subcutaneous fat or deeper tissues in addition to the epidermal and dermal layers, and are more difficult to heal than superficial or partial-thickness wounds. Healing of these injuries usually necessitates the use of skin autografts [17] or artificial skin substitutes [18].

Critical features of electrospun nanofibers for wound healing

Compositional mimicry

Materials utilized for studies of skin wound healing have included hydrogels, decellularized porcine dermal matrix and freeze-dried or gas-foaming formed scaffold [1,19,20]. These materials, however, lack capability to recapitulate the architecture of extracellular matrix (ECM) of skin [21]. Recently, electrospinning, an enabling nanotechnology, has attracted a lot of attention in wound healing, because this technology can produce biomimetic nanofibrous materials from a wide variety of natural and synthetic polymers with biologically relevant features [22].

The ECM of skin consists of fibrous structural proteins including collagens, elastins, laminins, and a variety of polysaccharides and proteoglycans (e.g., dermatan sulfate and hyaluronan) [23]. The versatility of electrospinning allows the production of nanofibers made of type I and III collagens which occupy 80–85 and 8–11% of the dermal matrix, respectively [24]. In fact, researchers have produced electrospun laminin nanofibers [25]. Through blending with other polymers, Feijen *et al.* demonstrated that the addition of polyethylene oxide (PEO; Mw = 8×10^6 kDa) and NaCl was essential for generating continuous and homogeneous collagen/PEO, elastin/PEO and collagen/elastin/PEO blended nanofibers [26]. Schenke-Layland *et al.* also fabricated decorin, a structural and functional proteoglycan that resides in the complex network of ECM proteins in many tissues, containing polycaprolactone (PCL)/gelatin nanofibers [27].

ECM molecules have also been immobilized to the surface of electrospun nanofibers through electrostatic interactions, hydrogen bonding or covalent bonding [28,29]. For example, polyelectrolyte polysaccharides, notably chitosan and hyaluronic acid, were used to coat PCL nanofibers through a layer-by-layer deposition via electrostatic interactions [30]. In another study, perlecan domain I–biotin proteins were attached to electrospun collagen and gelatin nanofibers using a coupling reaction with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride and *N*-hydroxysulfosuccinimide [31]. Table 1 summarizes electrospun nanofibers that mimic the ECM compositions of skin tissue. Through direct electrospinning, blending or surface immobilization, it is possible to generate electrospun nanofibers that can recapitulate the composition of the ECM of native skin to a greater degree.

Structural mimicry

Early studies examined the microarchitecture of human

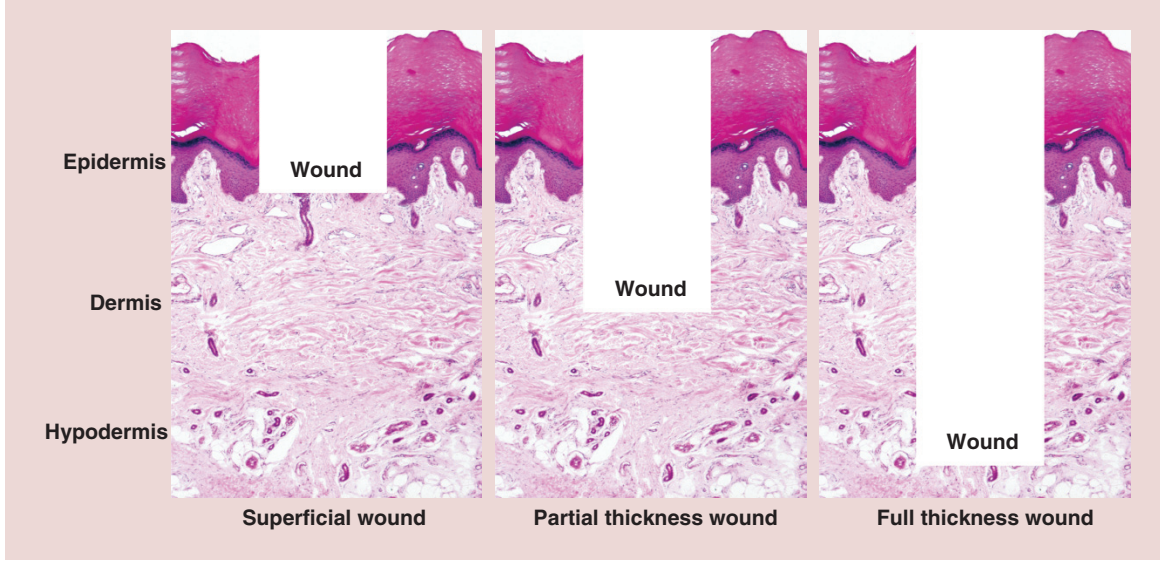

Figure 1. Schematic of a superficial versus partial-thickness versus full-thickness wound.

Table 1. Nanofibers for mimicking skin extracellular matrix composition.

Composition	Solvents	Average diameter	Ref.
Collagen	HFIP	460 nm	[24,32]
Collagen/chitosan	Acetic acid solution	134 ± 42 nm	[33]
Collagen/PCL	HFP	170 ± 0.075 nm	[34]
Collagen/Zein	Acetic acid solution	423–910 nm	[35]
Collagen/elastin/PEO	Hydrochloric acid solution	220–600 nm	[26]
Laminin I	HFP	90–300 nm	[25]
PCL/gelatin	TFE	470 ± 120 nm; 409 ± 88 nm	[14,36]
Gelatin	TFE	570 ± 10 nm	[37]
Polyurethane/gelatin	TFE	0.4–2.1 μm	[38,39]
HA/PEO	DMEM solution	70–110 nm	[40]
Silk fibroin/chitosan	HFIP/TFA	185.5–249.7 nm	
Silk fibroin/PEO	H_2O	414 ± 73 nm; 1 μm	[41,42]
Chitin	HFIP	163 nm	[43]
Carboxyethyl chitosan/PVA	H2O	131–456 nm	[44]
Chitosan/gelatin	TFA	120–220 nm	[45]
PLGA	THF/DMF	150–225 nm	[46]
PLGA/collagen	HFIP	170–650 nm	[47]
Chitosan/PEO	Acetic acid solution	130–150 nm	[48]
Hyperbranched polyglycerol	Methanol/DMF	58–80 nm	[49]

DMF: Dimethylformamide; HA: Hyaluronic acid; HFIP: Hexafluoroisopropanol; HFP: Hexafluoropropylene; PCL: Polycaprolactone; PEO: Polyethylene oxide; PLGA: Polylactic glycolic acid; PVA: Polyvinyl alcohol; TFA: Trifluoroacetic acid; TFE: Trifluoroethanol; THF: Tetrahydrofuran.

skin using scanning electron microscopy [50,51]. Three structural zones were observed in the dermis: papillary, mid and deep zones, composed of a thin layer of fine fibers (0.3–3 μm width) adjacent to the epidermis, a thick layer of densely packed fiber bundles (10–40 μm width) and a loosely arranged layer of fiber bundles, respectively. The fiber bundles consisted of fine fibrils, aligned in parallel arrays. Later studies showed that collagen in the dermis of normal skin formed a 'basket-weave' structure, with perpendicular collagen fibers intersecting at approximately 90° angles [52–54]. Interestingly, several groups assembled electrospun nanofibers into an ordered structure like bundles or yarns [55–57]. Other groups report using weaving techniques from the textile industry to generate nanofiber yarns into basket-weave structures [58,59]. Recently, we also prepared 3D nanofiber scaffolds in a basket-weave structure, composed of uniaxially aligned electrospun nanofiber strips using a 'noobing' process [60].

Ease of incorporation of bioactive materials

Site-direct, local treatment with therapeutic agents for wound healing is independent of systemic circulation (e.g., poor vascular perfusion) and can reduce pain, stimulate new tissue healing, increase vascular perfusion and decrease bioburden [61]. Electrospun nanofiber matrices provide an excellent platform for local delivery of therapeutic agents [62]. Table 2 lists different therapeutic agents (e.g., antimicrobial agents, antioxidants, anti-inflammatory drugs, anesthetics, enzymes and growth factors) that have been incorporated into electrospun nanofibers for wound healing purposes. Compared with traditional nanofiber drug-delivery systems, environment-responsive electrospun nanofibers could allow shorter response time and more precise control over the release rate of therapeutic agents for wound healing [63,64]. Therapeutic agents are hydrophilic, hydrophobic or both. Emulsion electrospinning or coaxial electrospinning is often used to encapsulate hydrophilic molecules [65,66]. Hydrophobic molecules along with polymers can be readily dissolved in organic solvents, and physically mixed with polymers after formation of nanofibers [67]. In addition, therapeutic agents can also be incorporated into electrospun nanofibers through postprocessing (e.g., CO_2 impregnation/infusion and surface immobilization) [68,69].

In principle, researchers can incorporate virtually any molecule into electrospun nanofibers. Furthermore, multiple therapeutic agents can be simultaneously incorporated into electrospun nanofibers, increasing functionality of the nanofiber matrices.

Mechanical mimicry

Physical parameters of scaffolds can affect cellular behavior and tissue regeneration [90]. Mechanical properties have attracted attention because of their capability to modulate biological processes and determine cell fate, similar to the capability of biochemical signals [91]. Electrospun nanofibers typically display tensile strengths below 300 MPa and Young's moduli below 3 GPa due to the low degree of orientation and chain extension of the polymer chains along the fiber axis [92].

Selecting an appropriate raw material can achieve desired mechanical properties. Other methods to tailor the mechanical properties of nanofiber matrix include the incorporation of metals, inorganic materials or carbon materials into electrospun nanofibers to form composites/hybrid materials/blends [93]. In addition, postprocessing (e.g., thermal treatment, mechanical treatment or surface coating) is also used to alter the mechanical properties of electrospun nanofibers [94–96]. For reference, the tensile modulus, ultimate tensile stress and ultimate tensile strain of human skin range from 15 to 150 MPa, 1 to 32 MPa and 35 to 115%, respectively [97]. Sun and coworkers fabricated electrospun poly(ε-caprolactone-*co*-lactide)/poloxamer (PLCL/poloxamer) nanofibers with different ratios, the tensile strength and modulus of which were within the range of human skin [98]. The data in Table 3 suggest that electrospun nanofibers can closely match the mechanical properties of human skin by careful selection of nanofiber composition.

Regulation of skin cell response

Skin cell proliferation, migration and ECM deposition are critical for wound healing [102]. Re-epithelialization requires epidermal cell migration and proliferation. Granulation tissue formation requires dermal fibroblast proliferation and collagen deposition. Electrospun nanofibers can regulate skin cell behavior via transmembrane receptors or intracellular signaling

Table 2. Therapeutic agents incorporated into nanofibers to improve wound healing.

Therapeutic agents	Nanofibers	Purpose	Ref.
Lysozyme	Chitosan/PVA	Antibacterial	[70]
Silver	Gelatin/polyurethane; gelatin; polyurethane; poly(ethylene-*co*-vinyl alcohol)	Antibacterial	[39,71–73]
ZnO	PCL; alginate/PVA	Antibacterial	[74–76]
Cefoxitin sodium	PLGA	Antibacterial	[77]
Gentamicin	Chitosan	Antibacterial	[78]
Ciprofloxacin HCl	Polyurethane/dextran; PVA/ poly(vinyl acetate)	Antibacterial	[79,80]
Polyhexamethylene biguanide	Cellulose acetate/polyester urethane	Antibacterial	[81]
Lidocaine, mupirocin	PLLA	Pain management and antibacterial	[82]
Fibrinogen	PLLA	Hemostasis	[39]
Curcumin	PCL	Antioxidant	[83]
VEGF	Chitosan/PEO; HA/collagen	Angiogenesis	[48,84]
PDGF-BB	Polyurethane; HA/collagen	Angiogenesis, granulation tissue formation	[84,85]
EGF	PCL–PEG/PCL; poly(l-lactic acid)-*co*-poly-(ε-caprolactone); HA/collagen; PCL/PEG	Keratinocytes migration and maturation, angiogenesis	[84,86,87]
Basic-FGF	PELA; HA/collagen	Cell adhesion, proliferation, ECM secretion, re-epithelialization and skin appendages regeneration, angiogenesis	[84,88,89]

ECM: Extracellular matrix; HA: Hyaluronic acid; PCL: Polycaprolactone; PELA: Poly(ethylene glycol-*co*-lactide); PEO: Polyethylene oxide; PLLA: Poly-L-lactic-acid; PVA: Polyvinyl alcohol.

pathways. Min *et al.* observed that electrospun nanofibers coated with type I collagen, laminin and integrin ligands promoted normal human epidermal keratinocyte adhesion, and around 50% of proliferating cells displayed a spreading morphology [32]. Yoo and coworkers reported that keratinocytes cultured on PCL nanofibers chemically conjugated with recombinant human EGF expressed higher mRNA levels for keratin 1 and loricrin specific markers than normal keratinocytes [86]. Klee *et al.* observed that keratinocytes selectively adhered to nanofibers modified with either collagen IV segment GEFYFDLRLKGDK or a 1:1 mixture of the two peptide sequences GEFYFDLRLKGDK and GRGDS [103]. Kristl *et al.* showed that nanotopographic cues rendered by aligned PVA nanofibers could direct the migration and proliferation of keratinocytes, which might be used for scaffolds that promote re-epithelialization [104].

Ramakrishna and coworkers showed that a PCL-blended collagen nanofibrous membrane is suitable for the attachment and proliferation of human dermal fibroblasts [34]. Bayat and coworkers found that silk fibroin fibers with diameters of 250–300 nm enhanced primary human dermal fibroblast proliferation compared with 1-μm fibers [105]. In addition, the expression of ECM genes, type I and III collagen, and proliferation markers (e.g., proliferating cell nuclear antigen) increased with decreasing fiber diameter. In contrast, Laurencin *et al.* showed the highest cellular proliferation on polylactic glycolic acid (PLGA) nanofiber scaffolds that had a diameter in the range of 600–1200 nm [106]. These conflicting findings necessitate more work to determine the size effect on human dermal fibroblast proliferation.

Wang *et al.* showed that human dermal fibroblasts cultured on aligned (anisotropically organized) collagen-containing nanofibrous matrices displayed elongated cell morphology, clustering of focal adhesions, accelerated cell migration and increased fibroblast-to-myoblast differentiation [107]. The integrin β1 signaling pathway induced by spatial arrangements of nanofibers was mainly responsible for these cellular responses. The same group also demonstrated that human dermal fibroblasts on fibrinogen-containing nanofiber matrices exhibited a differentiated phenotype in the presence of exogenous TGF-β1, characterized by lower proliferation, faster migration and higher expression of α-smooth muscle actin, in contrast to the proliferative phenotype on collagen-containing nanofiber matrices. This appeared to be secondary to higher expression of TGF-β1/Smad2/3 phosphorylation in fibroblasts cultured on fibrinogen-containing nanofibers [108]. Similarly, Bacakova *et al.* found that fibrin coating of electrospun poly(lactic acid) (PLA) nanofibers significantly enhanced human dermal fibroblast cell spreading, mitochondrial activity and cell population density. Fibrin coating also stimulated the expression and synthesis of type I collagen in human dermal fibroblasts [109]. Leong *et al.* demonstrated potential mitigation of hypertrophic scar contraction using electrospun poly(lactide-caprolactone) (PLCL) and polyurethane random nanofiber scaffolds [110,111]. Due to their elastomeric properties, human dermal fibroblasts that were seeded on nanofiber scaffolds had less contraction and fewer α-smooth muscle actin-positive myofibroblasts compared with fibroblasts seeded on collagen lattices. Collagen-coated polyurethane or PLCL nanofiber scaffolds implanted beneath skin grafts significantly reduced hypertrophic scar contraction and scar stiffness compared with standard management in a murine hypertrophic model. Cheng *et al.* showed that electrospun PCL/collagen nanofibers could sustain delivery of active TGF-β1, which enhanced myofibroblast differentiation, as confirmed by cell metabolic activity, gene expression and protein expression [112].

In addition to effects on keratinocytes and dermal fibroblasts, Savkovic *et al.* showed that PCL fiber meshes were capable of providing a niche for normal human epidermal melanocytes as well as hair-follicle-derived melanocytes from the outer root sheath that supported the cells' melanotic properties. Low proliferation rates, along with enhanced expression of terminal melanotic differentiation genes PAX3, MITF, TYR and PMEL in 3D cultures, suggested that PCL nanofi-

Table 3. Comparison of mechanical properties between human skin tissue and electrospun nanofibers.

Materials	Tensile modulus (MPa)	Ultimate tensile stress (MPa)	Ultimate tensile strain (%)	Ref.
Human skin	15–150	1–32	35–115	[99]
PCL/collagen	21.42 ± 0.04	8.63 ± 1.44	24.0 ± 7.16	[100]
HA/PLGA	28.0	1.52	60.07	[101]
PLGA/collagen	40.43 ± 3.53	1.22 ± 0.12	96 ± 13	[99]

HA: Hyaluronic acid; PCL: Polycaprolactone; PLGA: Polylactic glycolic acid.

ber scaffolds support melanocyte differentiation rather than proliferation [113]. Finally, Nobakht *et al.* seeded rat hair-follicle stem cells (nestin and CD34 positive, K15 negative) on electrospun random PCL nanofiber scaffolds, and demonstrated cellular attachment and spreading [114].

Applications in wound healing

Sutures

Sutures are normally used to close the wound, facilitate wound healing, reduce inflammation, prevent surgical site infection and relieve pain. Electrospinning successfully creates nanofiber yarns and bundles which can be used as surgical sutures [55,115,116]. Menon *et al.* fabricated mechanically robust electrospun core–sheath yarns as sutures, with a central PLA core and an aceclofenac- or insulin-eluting PLGA sheath. These showed reduced epidermal hyperplasia and enhanced cellular migration in a skin-inflammation animal model and wound healing assay for aceclofenac and insulin-eluting sutures [117]. Hu and Huang braided uniaxially aligned poly-L-lactic-acid (PLLA) nanofibers and subsequently coated them with chitosan coating for use as sutures [118]. These resulting sutures had tensile and knot strength comparable with commercial sutures, promoted cell growth and had minimal toxicity. In addition, the chitosan-coated PLLA sutures showed better histological compatibility than silk sutures when implanted into muscle tissues of rats. Liu and coworkers made braided PLLA nanofiber sutures loaded with an antibiotic (cefotaxime sodium) which performed better *in vivo* than silk sutures [119]. Similarly, He *et al.* incorporated tetracycline into aligned PLLA fibrous threads for suture applications [120].

We recently described a twisting method for fabrication of nanofiber-based sutures, which could deliver silver and gentamicin simultaneously [121]. The release profiles of silver and gentamicin from these sutures exhibited an initial burst followed by a sustained release over 5 weeks. These gentamicin/silver co-encapsulated nanofiber sutures killed bacteria much more effectively than those containing either antibiotic gentamicin or silver alone, but without an obvious impact on proliferation and migration of dermal fibroblasts and keratinocytes. Kohane *et al.* fabricated PLGA nanofiber sutures loaded with the local anesthetic bupivacaine, exhibiting a sustained release of entire drug payload over the period of 12 days [122]. Local analgesia was achieved 1 day after surgery, and lasted about 1 week for 90% of subjects in a rat skin wound model. While most studies describe production of nanofiber sutures with antimicrobial, anti-inflammatory and analgesic functions, it is feasible to consider growth factors and other therapeutic agents beneficial to wound healing that could be co-incorporated into nanofiber sutures to confer multiple activities.

Wound dressings

The major roles of wound dressings include maintenance of wound hydration, absorption of excess wound exudate, minimization of wound trauma and as a barrier to external microorganisms [15,123]. Recently, scientists have focused on developing nanofiber-based dressings that can mimic the native dermal ECM, have high surface area-to-volume ratio and high porosity. In addition to traditional functions, these nanofiber dressings were intended to reduce infection, inflammation and promote wound healing by creating a positive environment [124]. To prevent and treat wound infections, antimicrobial agents have been incorporated into electrospun nanofibers [125]. Antibacterial delivery from electrospun nanofiber dressings has existed for some time and responsive systems now aim to trigger release of antimicrobial agents only if infection occurs [126]. Besides, the porosity and pore spacing are important factors of electrospun nanofiber membranes that act as microbial barrier property to prevent microbial invasion [127]. To provide anti-inflammatory activity and promote the rapid healing of mild skin burns, Athanassiou *et al.* developed an electrospun poly(octyl cyanoacrylate) nanofiber dressing loaded with polypropylene fumarate which provides conformal coverage of the injured tissue and reduces the level of pro-inflammatory cytokines by 80% in the first 48 h and enhances re-epithelialization [128].

To enhance wound healing of diabetic ulcers in mice, Yoo and coworkers immobilized human EGF to electrospun PCL–PCL/PEG nanofibers via amine-terminated PEG linkers [86]. The EGF-conjugated nanofibers showed superior *in vivo* wound healing activities relative to control groups or EGF solutions. Similarly, Li *et al.* reported the incorporation of basic FGF (bFGF) into core–sheath fibers for treatment of diabetic wounds in rats [88]. bFGF-loaded scaffolds had a higher wound recovery rate with complete re-epithelialization and regeneration of skin appendages [88]. Furthermore, Wang *et al.* designed electrospun composite nanofibers with staged release of multiple growth factors (including bFGF, EGF, VEGF and PDGF-BB) for healing diabetic wounds in rats [129]. These composite nanofibers rapidly delivered bFGF and EGF mimicking the early stage of the wound healing process and slowly delivered VEGF and PDGF-BB imitating the late stage of skin reconstruction. Similar to sutures described above, nanofiber dressings can possess multiple activities through incorporation of antimicrobial agents, anti-inflammatory drugs, growth hormones and other molecules (Figure 2) [130,131]. Furthermore,

electronics can be incorporated into nanofiber dressings for detection of infection, monitoring of healing and release of therapeutics, performed remotely and in real time [132].

Dermal regeneration/substitute

For extensive skin defects, collagen-based sponges (e.g., Integra® and Pelnec®) have been used as temporary coverage to promote granulation tissue formation prior to autografting. Traditional electrospinning techniques produce 2D densely packed nanofiber membranes that restrict the cellular penetration and thus limit their use for *in situ* dermal tissue engineering. On the other hand, 3D scaffolds composed of electrospun nanofibers promote cellular infiltration, and could sever as an improved synthetic matrix for dermal repair/regeneration. Many approaches have been recently developed to fabricate 3D electrospun nanofiber scaffolds. Tan *et al.* used a needle collector to fabricate 3D electrospun PCL nanofiber scaffolds with a larger pore size compared with traditional 2D nanofiber membranes [133]. After modification with gelatin, the scaffolds improved human dermal fibroblast infiltration and proliferation throughout the scaffolds and the secretion of ECM proteins from the cells, showing potential for dermal tissue engineering.

Our group reported a simple approach for preparing 3D scaffolds in a basket-weave structure composed of uniaxially aligned electrospun nanofiber strips [60]. Human adipose-derived stem cells distributed uniformly throughout the scaffolds after seeding and could proliferate, and were organized by the nanotopographic cues rendered by aligned nanofiber arrays. Though the scaffolds have not yet been used in dermal tissue engineering, the basket-weave structure could be used to recapitulate the native ECM architecture in human dermis. Our most recent studies developed a modified gas-foaming approach to expand 2D electrospun nanofiber membranes into the third dimension (Figure 3) [134,135]. The resulting nanofiber scaffolds showed layered structures with controllable gap widths and layer thicknesses on the order of micrometers (Figure 3A & B). Expanded scaffolds possessed higher porosity than traditional 2D nanofiber membranes, while simultaneously retaining nanotopographic cues. The expanded scaffolds promoted cellular infiltration/tissue integration, a regenerative response and neovascularization after subcutaneous implantation in rats;

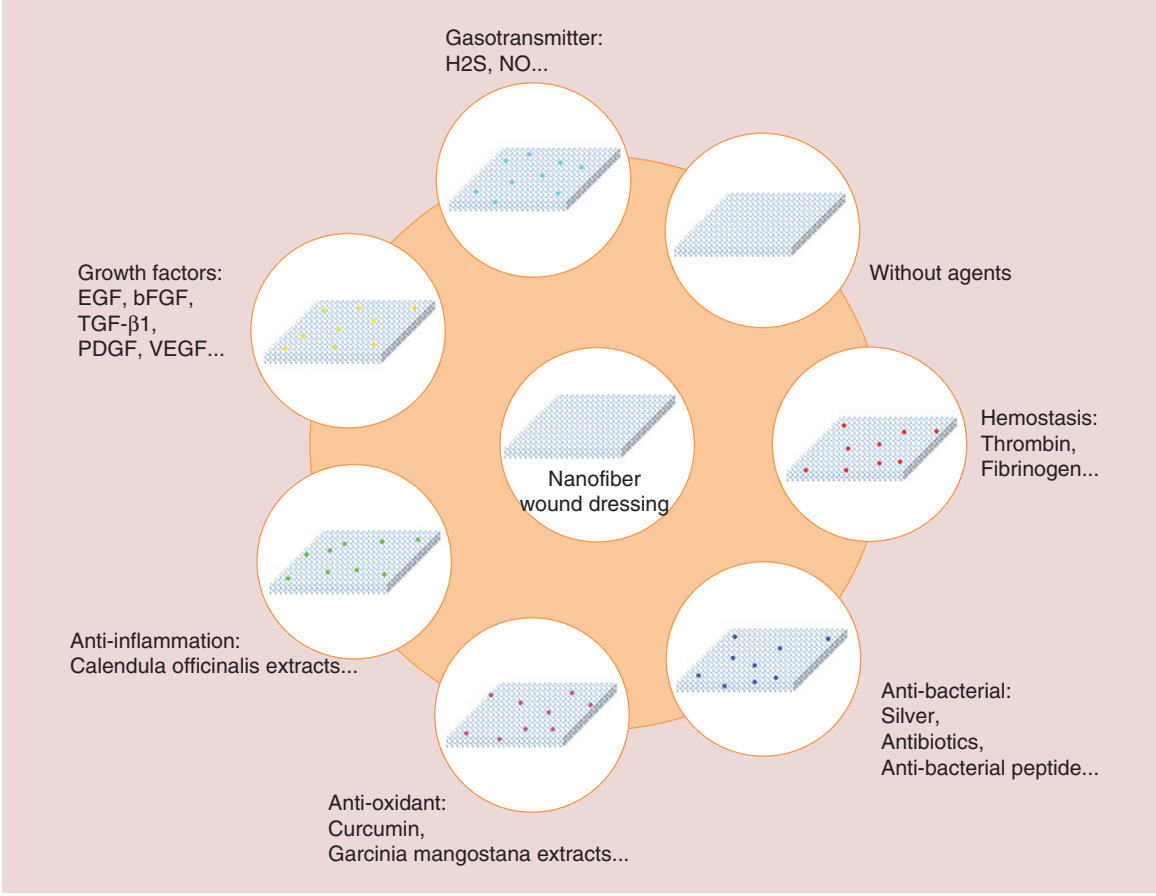

Figure 2. Schematic illustrating multifunctional wound dressings made of electrospun nanofibers.

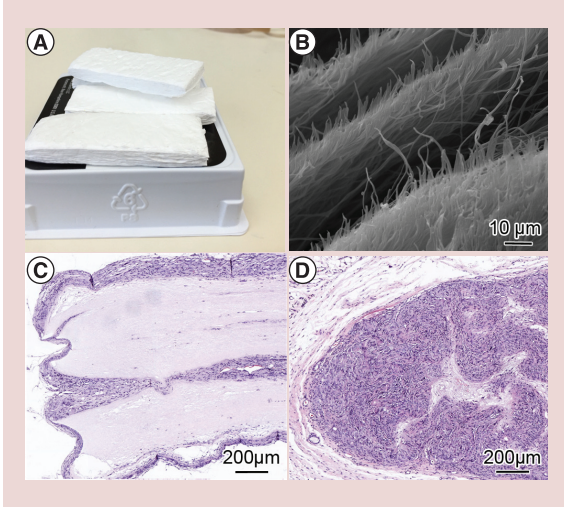

Figure 3. Expanded nanofiber scaffolds.
(A) Photograph of expanded nanofiber scaffolds (50 mm × 70 mm × 5 mm). **(B)** SEM images showing cross-section of expanded nanofiber scaffolds. **(C)** H&E staining of unexpanded nanofiber scaffolds and surrounding tissues after subcutaneous implantation into rats for 8 weeks. **(D)** H&E staining of expanded nanofiber scaffolds and surrounding tissues after subcutaneous implantation into rats for 8 weeks.
H&E: Hematoxylin and eosin; SEM: Scanning electron microscopy.
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no cell penetration was observed for unexpanded scaffolds (Figure 3C & D). Such expanded nanofiber scaffolds could be useful for *in situ* dermal tissue regeneration.

Epidermal regeneration

Epidermal engineering of electrospun PCL nanofibers modified with gelatin may improve mechanical and handling properties such that these materials could replace epidermal autografts – keratinocyte sheets (e.g., Epicel®) [36]. HaCaT cells and human keratinocytes isolated from foreskin specimens were cultured on gelatin/PCL nanofiber membranes for 7 days, and then implanted on the backs of nude mice with a 1-cm diameter full-thickness circular wound. Direct growth of human keratinocytes on a nanofiber substrate eliminates the need for a seeder layer of mouse cells. This is important as human keratinocytes are grown on a layer of irradiated mouse cells for current Epicel epidermal grafts, making it a xenotransplantation product as defined by the US Public Health Services and US FDA.

Full-thickness skin regeneration

Nanofiber scaffolds combined with skin cells

Electrospun nanofiber scaffolds seeded with skin cells (e.g., dermal fibroblasts and keratinocytes) have been tested for full-thickness wound repair. Park *et al.* reported a cold-plate electrospinning technique for fabrication of 3D nanofiber structures that were co-cultured with human dermal fibroblasts and keratinocytes to make a skin substitute [136]. In a separate study, Bellis *et al.* created a microporous dermal-mimetic electrospun nanofiber scaffold, seeded with F344 fibroblasts, which promoted tissue regeneration in full-thickness skin wounds in rats characterized by a normal-appearing dermal matrix and hair-follicle regeneration [137]. In addition, Wang and coworkers made a skin tissue construct with electrospun PCL/collagen nanofibers and foreskin cells (fibroblasts and keratinocytes), using a layer-by-layer technique (Figure 4) [138,139]. After *in vitro* culture for 2 weeks, this construct was implanted onto full-thickness wounds in nude mice, which resulted in an effective healing of these wounds with complete wound closure and epidermal regeneration.

Nanofiber scaffolds combined with stem cells

Mesenchymal stem cells (MSCs) have shown great potential in tissue regeneration, including myocardium [140], blood vessels [141], bone [142], cartilage [143] and skin [144,145]. MSCs are involved in almost all the phases of wound healing, stimulating new blood vessel formation in the granulation tissue, modulating the inflammatory environment, encouraging the migration of keratinocytes and enhancing ECM production [146]. MSCs also display antimicrobial activity that is attributed to the secretion of antimicrobial proteins or immune-modulating factors [147]. Owing to the above advantages, MSCs have been studied extensively in wound healing. Direct injection of MSCs to the defect site, however, often induces rapid cell death/clearance. Using electrospun nanofiber scaffolds as ideal substrates could facilitate cell attachment, proliferation, differentiation, migration and ECM production during wound healing.

Ma *et al.* reported bone mesenchymal stem cell (BMSC)-seeded nanofiber scaffolds for acute full-thickness skin wound healing [148]. The implanted BMSCs seemed to promote epithelial edge ingrowth and collagen production. Prabhakaran and coworkers investigated the potential of human BMSCs for epidermal cell differentiation on electrospun collagen/poly(l-lactic acid)-*co*-poly(3-caprolactone) nanofibrous scaffolds. BMSCs had a round keratinocyte morphology and expressed keratin 10, filaggrin and partial involucrin protein (epidermal differentiation markers) in epidermal induction medium [149]. In a different study, Bayati *et al.* reported that PCL nanofiber matrices seeded with adipose-derived stem cells served as an efficient skin equivalent for acute wound healing [150].

These results suggest that MSCs could provide good alternatives as cell sources for full-thickness skin repair/regeneration. However, Steffens *et al.* tested poly DL lactic acid and poly DL lactic acid/*Sp* nanofiber scaffolds seeded with MSCs for the treatment of third-degree burns in mice C57/B17N for 7 days without showing re-epithelialization, keratinization or presence of hair follicles on the lesion site [151]. The presence of ulceration, inflammation and fibrosis was noted among all the treatment groups 7 days after injury. It appears that the role of seeded MSCs in wound healing will require further investigation, particularly in regard to the type of injury.

Nanofiber scaffolds combined with skin tissues

Development of skin substitutes is time-consuming and expensive. Skin grafting (e.g., split-thickness skin grafts in the form of meshed graft or skin pieces) remains the most popular treatment for skin replacement [152]. Our group developed a novel approach that combined tissue engineering strategies (e.g., making use of scaffolds) with currently viable clinical approaches (e.g., autologous skin grafting) to fabricate a 'sandwich-type' multifunctional nanofiber skin graft (Figure 5) [153]. This construct was fabricated by placing nanofiber membranes with square-arrayed microwells and nanostructured topographic cues at the bottom, followed by seeding minced microskin tissue islands into the microwells and then placing the radially aligned nanofiber membrane on the top. This composite had the following unique features: guidance and acceleration of cell migration due to both nanotopographic cues and eluted biological cues; confinement of microskin tissue islands in a uniform distribution with square-arrayed microwells; a larger expansion ratio compared with current techniques (e.g., meshed split-thickness skin grafting); prevention of infection due to local sustained release of antimicrobial agents; permanent; immediate availability and ease of operation; and safe toxicity profile. The performance of sandwich-type nanofiber scaffolds was tested in a rat-skin excision model. Our findings indicate all transplanted microskin grafts in sandwich-type nanofiber scaffolds which contained an epithelial layer and a dermal layer were 'take' satisfactorily by the wound with a uniform distribution at day 7 postsurgery (Figure 6A). We observed re-epithelialization along the wound bed derived from microskin grafts at day 14 after surgery (Figure 6B). Wounds were completely closed by re-epithelialization (derived from microskin grafts) at day 21 after surgery (Figure 6C). This nanofiber skin graft could prove useful in repairing extensive burns or to treat nonhealing chronic wounds. Similarly, MacNeil developed a one-step approach for the reconstruction of full-thickness skin defects using minced split-thickness skin grafts and PLLA nanofiber scaffolds as a skin substitute (Figure 7) [154]. It was found that skin cells migrated along the fibers of the scaffold and formed new collagen. Further studies are required to determine the extent of vascularization of scaffolds seeded with minced skin tissues, which is crucial for their survival on the wound bed.

New electrospinning technologies

Portable handheld electrospinning apparatus

Although many studies show a great potential of electrospun nanofibers for biomedical applications, electrospinning has certain limitations in flexible practicability because of its conventional setup that is usually quite bulky and excessively dependent on a plug. Recently, Long *et al.* and Ye and coworkers developed a battery-operated portable handheld electrospinning apparatus, which was composed of two AAA batteries and one high-voltage converter instead of the typical high-voltage generation (Figure 8A). The entire apparatus was lightweight (about 120 g) with a small volume (10.5 × 5 × 3 cm^3) and low cost. The apparatus was able to work with a battery and was no longer dependent on the high-voltage supply. In addition, different polymers such as polyvinylpyrrolidone, PCL, polystyrene, PLA and poly(vinylidene fluoride) were electrospun into fibers successfully. The handheld electrospinning apparatus was able to greatly overcome the restricted conditions of traditional electrospinning and hopefully contribute to promote the electrospinning technique for practical day-to-day applications such as

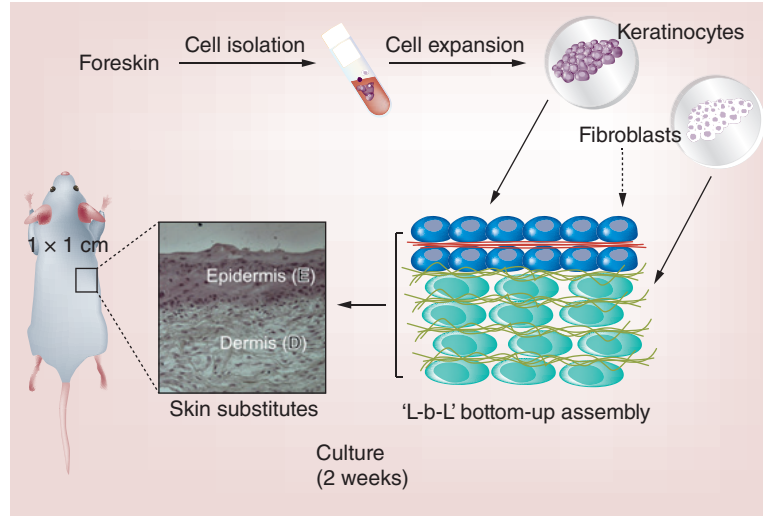

Figure 4. Full-thickness wound repair with skin substitutes assembled from electrospun nanofibers and isolated skin cells using a layer-by-layer technique.

L-b-L: Layer by layer.

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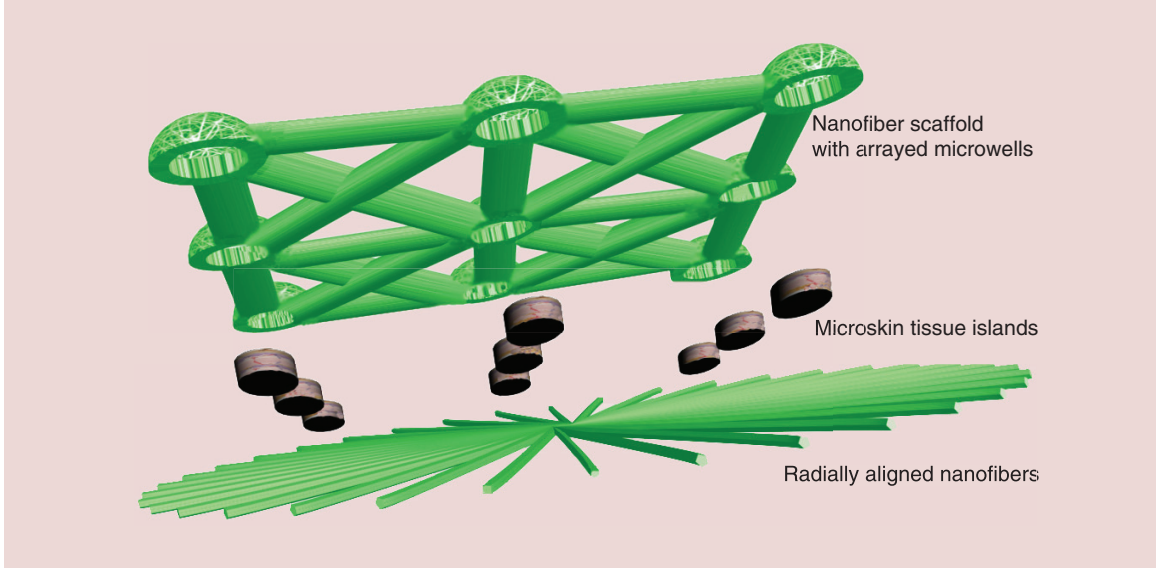

Figure 5. Schematic illustrating the 'sandwich-type' nanofiber skin grafts composed of nanofiber scaffolds with arrayed microwells on the top and radially aligned nanofibers on the bottom and microskin tissue islands in the middle.

personal healthcare devices, especially in biomedical fields such as skin damage, wound healing and rapid hemostasis [155–157].

Melt electrospinning writing

Melt electrospinning is a processing technique to produce fibrous materials from polymer melts. Melt electrospinning writing (melt electrospinning combined with moving collectors) is a relatively new processing technology, and it is also considered a type of 3D printing technology (Figure 8B) [158,159]. 3D structures can be designed and built up to millimeter thicknesses based on the accurate deposition of fibers upon each other via melt electrospinning writing (Figure 8C) [158,160]. This technology has a number of advantages in terms of fabricating scaffolds. For example, it is solvent free, and thus the solvent toxicity and accumulation are avoided. The small fiber diameters (down to 800 nm) lead to flexible constructs that enable even relatively rigid polymers to be fabricated as soft, compliant structures. The 3D structure exhibited a full cellular penetration for dermal fibroblasts

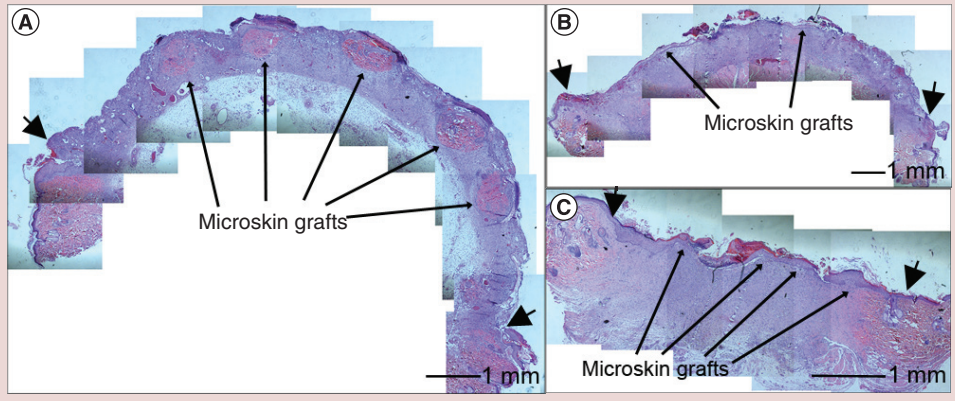

Figure 6. Hematoxylin and eosin staining of skin tissue sections illustrating the healing process of a wound after surgical treatment with sandwich-type nanofiber skin grafts at days 7, 14 and 21. Black arrowheads in the images indicate the boundaries between wound and surrounding normal skin. **(A)** Transplanted microskins indicated by small black arrows in sandwich-type nanofiber scaffolds were grafted onto wounds with a uniform distribution at day 7 postsurgery. **(B)** Re-epithelialization from microskin grafts occurred along the wound bed at day 14 after surgery. **(C)** The wound was completely closed by microskin re-epithelialization as indicated by black arrows at day 21 after surgery.
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after 14 days *in vitro* culture [161]. So far, melt electrospinning has been used to process the following polymers: poly(ε-caprolactone), poly(lactide-*co*-glycolide), PLA, PCL-*block*-poly(ethylene glycol), poly(lactide-*co*-caprolactone-*co*-acryloyl carbonate), polypropylene, poly(methyl methacrylate) [162].

Combination of melt electrospinning writing & traditional electrospinning

Melt electrospinning writing has been used to produce 3D scaffolds due to its ability to construct various 3D structures. However, the large pore size of obtained 3D scaffolds limits the cell seeding efficiency and tissue formation. Therefore, sufficient surface areas for cell adhesion are needed for these 3D scaffolds. To

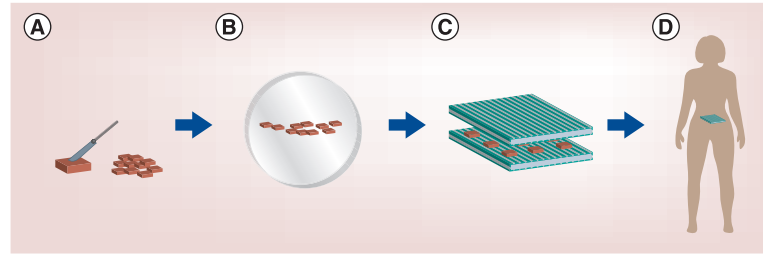

Figure 7. Schematic illustrates an approach for the reconstruction of full-thickness skin defects using minced split-thickness skin grafts and electrospun fiber scaffolds. **(A)** Mincing biopsy of patient skin. **(B)** Placing minced skin tissues in 1% methylcellulose. **(C)** Placing minced skin tissues in between two layers of electrospun nanofiber scaffolds. **(D)** Applying to patients.
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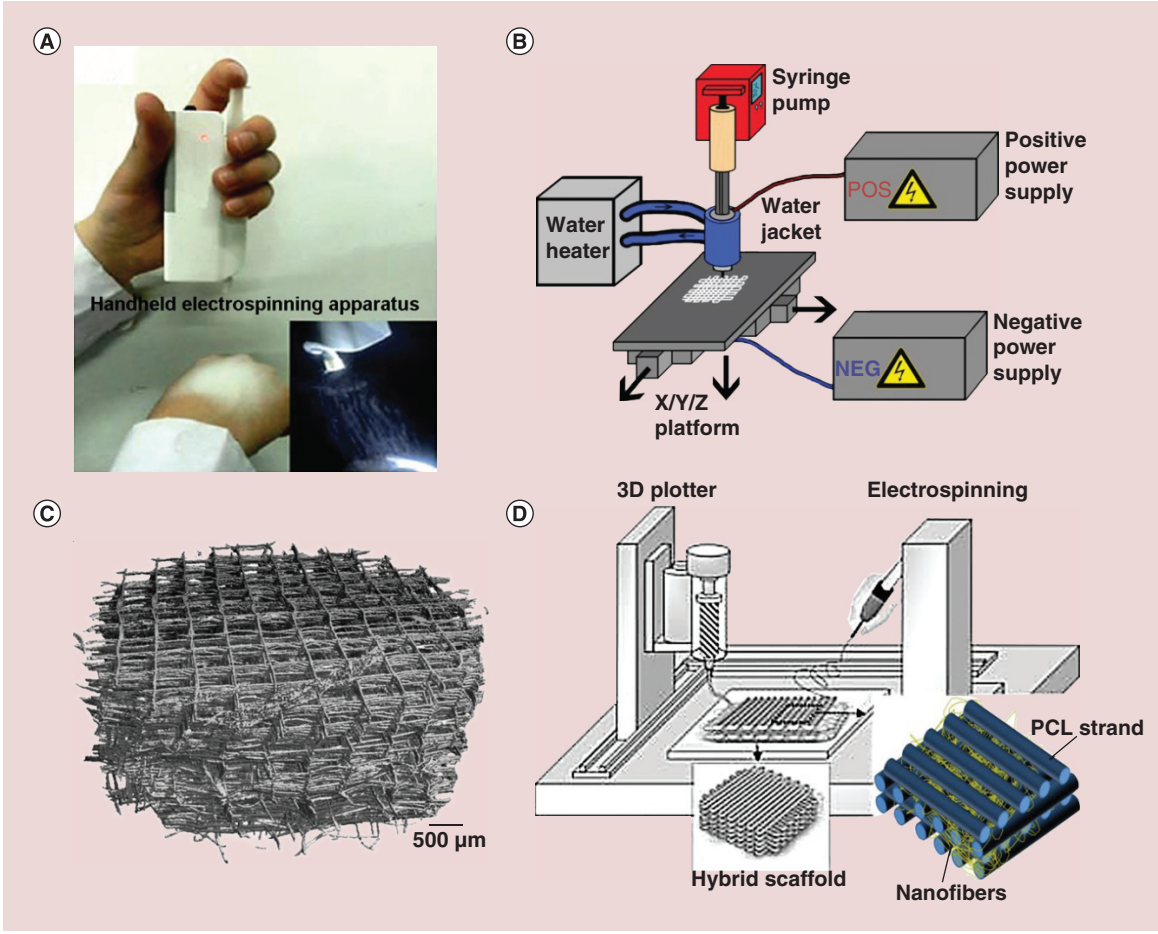

Figure 8. The recently developed new electrospinning technology which may apply to skin wound healing in the future. **(A)** Schematic of a battery-operated portable handheld electrospinning apparatus [155]. **(B)** Schematic of using a polymer melt instead of a polymer solution leads to a stabilized jet and a controlled direct writing approach for fibers' deposition [158]. **(C)** Image of micro-CT reconstruction of scaffolds generated by **(B)** setup. **(D)** Schematic of a hybrid process for fabricating 3D hierarchical scaffolds combining rapid prototyping and electrospinning [163].
CT: Computed tomography.
Reproduced with permission from [155] © American Vacuum Society, Royal Chemistry Society and Wiley (2015).
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improve cell adhesion and proliferation on these scaffolds, traditional electrospun nanofibers can be incorporated to the 3D scaffolds during construction, which is able to provide ECM-like matrix for cell attachment. Figure 8D shows the 3D plotting technique where the melt polymer is used to eject strands of microfibers to fabricate a scaffold. After a deck of microfibers is built, nanofibers are deposited on the surface before the next layer of microfibers is laid on. By this way, a hybrid scaffold consisting of microfibers and nanofibers can be achieved [163]. In addition to a cell attachment and proliferation, Moroni *et al.* reported that the hybrid scaffolds showed better ECM secretion and cell differentiation as compared with 3D scaffolds alone [164].

Conclusion & future perspective

Researchers can engineer electrospun nanofibers with compositions and structures/architectures akin to ECM in skin tissue. Electrospun nanofibers can regulate skin cell responses including proliferation, migration, differentiation and ECM deposition. These unique properties of electrospun nanofibers will allow their fabrication into sutures, wound dressings, dermal substitutes and engineered skin tissues for wound healing.

Different antimicrobial agents are incorporated into nanofiber dressings or sutures for prevention of infection; however, bacteria develop resistance to most antimicrobial agents especially antibiotics. Recently, we demonstrated that vitamin D_3-loaded electrospun nanofibers induce endogenous LL-37 antimicrobial peptide production from keratinocytes and monocytes through the vitamin D signaling pathway [165]. Such an approach could minimize surgical site/wound infection and reduce selection for multidrug resistant bacteria. Incorporation of different growth factors into nanofibers promotes wound healing. Precise control of their release from electrospun nanofibers at different times/stages may improve wound healing outcomes [166,167]. Furthermore, incorporation of electronics into nanofiber scaffolds could regulate precise release of multiple factors at different stages mimicking different phases of wound healing [168]. Combining electrospun nanofibers with electrical stimulation, mechanical stress and pulsed magnetic field could further enhance wound healing [169–171]. For example, depositing Ag and Zn on nanofiber dressings could provide an electric field to direct cell migration and improve wound healing [172]. In addition, 3D printing has demonstrated its capability in producing skin tissue constructs composed of dermal fibroblasts, keratinocytes and collagen gel through layer-by-layer [173–175]. 3D printing could combine with electrospinning to generate skin tissue constructs for promotion of wound healing. For example, electrospinning is able to produce short fibers [176], which can be used as ink for printing 3D skin tissue constructs [177,178].

Most studies use rodent models for testing the efficacy of electrospun nanofibers for wound healing; however, disparities in the healing process exist between rodents (e.g., primarily wound contraction and not subject to hypertrophic or keloid scar formation) and humans (e.g., re-epithelialization and granulation) [179]. More efforts may investigate the efficacy of electrospun nanofibers for wound healing using porcine models due to the similarities in cutaneous wound healing between humans and pigs (e.g., inflammation, proliferation, re-epithelialization and re-modeling) [179,180].

Financial & competing interests disclosure

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Executive summary

Background

- Human skin wounds are a major and rapidly growing threat to public health and the economy.
- Skin wounds may be classified as superficial, partial thickness or full thickness.

Critical features of electrospun nanofibers for wound healing

- Electrospinning technology can produce biomimetic nanofibrous materials from a wide variety of natural and synthetic polymers with biologically relevant features.
- Electrospun nanofibers provide an excellent platform for local delivery of therapeutic agents.
- The mechanical properties of electrospun nanofibers are easily altered by selecting an appropriate raw material to meet wound healing requirements.
- Electrospun nanofibers can regulate skin cell behavior via transmembrane receptors or intracellular signaling pathways.

Applications in wound healing

- Electrospun nanofibers are easily fabricated into sutures, dressings, and dermal and epidermal substitutes for use in skin wound healing.
- Researchers can combine electrospun nanofiber scaffolds with skin cells, stem cells and/or skin tissues for healing severe skin wounds.

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