

REVIEW ARTICLE

Biochemical and Biophysical Cues in Matrix Design for Chronic and Diabetic Wound Treatment

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Progress in biomaterial science and engineering and increasing knowledge in cell biology have enabled us to develop functional biomaterials providing appropriate biochemical and biophysical cues for tissue regeneration applications. Tissue regeneration is particularly important to treat chronic wounds of people with diabetes. Understanding and controlling the cellular microenvironment of the wound tissue are important to improve the wound healing process. In this study, we review different biochemical (e.g., growth factors, peptides, DNA, and RNA) and biophysical (e.g., topographical guidance, pressure, electrical stimulation, and pulsed electromagnetic field) cues providing a functional and instructive acellular matrix to heal diabetic chronic wounds. The biochemical and biophysical signals generally regulate cell–matrix interactions and cell behavior and function inducing the tissue regeneration for chronic wounds. Some technologies and devices have already been developed and used in the clinic employing biochemical and biophysical cues for wound healing applications. These technologies can be integrated with smart biomaterials to deliver therapeutic agents to the wound tissue in a precise and controllable manner. This review provides useful guidance in understanding molecular mechanisms and signals in the healing of diabetic chronic wounds and in designing instructive biomaterials to treat them.

Keywords: wound healing, dressing, diabetic, matrix, biomaterial

Introduction

TISSUE ENGINEERING WAS defined as “an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function.”¹ Although health benefits of tissue engineering are obvious, tissue engineering industry finds itself on a “roller coaster ride.”^{2–7}

After its emergence and soaring development with enthusiasm from business community in the 1990s, tissue engineering industry entered a dark period in the early 2000s, with the capital value of publicly traded tissue engineering companies reduced by 90% between 2000 and 2002.⁶ Organogenesis (Canton, MA) and Advanced Tissue Sciences (La Jolla, CA), two leading companies that brought the first commercially-produced tissue engineering products to the market, engineered skin substitutes, declared bankruptcy in 2002.⁸ This performance resulted in the reassessment of tissue engineering products, which are thought to be limited by their long preparation time, challenging quality control, complex distribution chains, and short shelf life. Fortunately, the reassessment resulted in viable products such as skin substitutes now commercially available through Organogenesis.

Recently, the focus of tissue engineering has undergone considerable evolution from replacement to regeneration *in situ* because it has been recognized that instead of recreating the complexity of living substitutes for transplantation,⁹ we can develop instructive materials to harness body’s innate power of self-repair.¹⁰ In these scenarios, the matrix not only serves as a scaffold that provides mechanical support and defines the shape of tissue constructs but also provides a multitude of complex stimuli to regulate cell behavior in tissue remodeling.

Advances in biomaterial science and engineering combined with ever-growing knowledge in cell biology led to the design of biomaterials with appropriate biochemical cues and biophysical guidance for tissue regeneration *in situ*. Moreover, complexities in the matrix design and fabrication were enabled by innovative technologies with precise control and improved reproducibility.^{11,12} As the first category of commercial tissue engineering products, wound dressings witnessed these innovations. In this study, we review recent advances in designing acellular instructive matrix for wound healing applications, under the principle of facilitating the cell–extracellular matrix (ECM) interactions with biochemical and biophysical cues to induce or support native tissue regeneration.

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Diabetic Chronic Wounds

Wound healing requires a well-orchestrated integration of biological and molecular events of cell migration and proliferation and ECM deposition and remodeling. This complex process can be described in three overlapping phases as follows: inflammation, proliferation (angiogenesis and reepithelialization), and remodeling. Transition between these phases is regulated by inflammatory mediators, growth factors, cytokines, and mechanical forces.^{13,14} However, the process can be impaired by some systemic complications, such as diabetes mellitus, and results in chronic wounds. Chronic wounds are pervasive worldwide, affecting more than 70 million people particularly in the senior population with harsh medical and social consequences.¹⁵ In particular, diabetic foot ulcer affects 15% of people with diabetes and is the leading cause of nontraumatic amputation.¹⁶

Denervation of local sympathetic nerve system is a hallmark of diabetic neuropathy and results in sensory deficits, so that the patients cannot respond to external stimuli such as pressure and heat.¹⁷ Absence of protective symptoms leads to further deformities and infection.¹⁸ Meanwhile, diabetic wound healing is challenged by vasculopathy. Vasculogenesis is limited by insufficient mobilization of circulating endothelial progenitor cells from the bone marrow¹⁹ and their impaired homing to the wound site.²⁰ Impaired endothelium function also involves a reduction of nitric oxide (NO) synthesis and increased NO degradation by excess reactive oxygen species (ROS).²¹ Moreover, excessive glycosylation of matrix proteins induced by hyperglycemia leads to basement membrane crosslinking and angiogenesis dysfunction.²² Excessive activation of matrix metalloproteinases (MMPs) such as MMP-9 also impairs cell migration and degrades critical matrix proteins and growth factors.²³

On the cellular level, the resident cells in diabetic chronic wounds are associated with abnormalities. Fibroblasts isolated from diabetic chronic ulcers are senescent with decreased responses to growth factors, including transforming growth factor- β 1 (TGF- β 1), platelet-derived growth factor (PDGF), epidermal growth factor (EGF), insulin-like growth factor-1 (IGF-1), and basic fibroblast growth factor (bFGF).²⁴⁻²⁷ Macrophages in diabetic wounds show decreased release of cytokines, including tumor necrosis factor- α (TNF- α), interleukin (IL)-1 β , and vascular endothelial growth factor (VEGF).²⁸ Attachment and migration of keratinocytes, the critical events in reepithelialization, are impaired in diabetic chronic wounds due to altered ECM composition and increased ECM degradation.^{29,30} Meanwhile, all resident cells in the wound are stressed by excessive production of ROS from macrophages and neutrophils caused by prolonged inflammation, coupled with impaired antioxidant defense in response to hyperglycemia.³¹⁻³³

Wound Dressing Matrices

Wound dressing matrices were the first category of Food and Drug Administration (FDA)-approved tissue engineering products and have embraced numerous innovations ever since. In general, wound dressings can be categorized into skin substitutes with cells and acellular dressings. Living skin substitutes consist of dressing materials hosting dermal cells and/or epidermal cells. With current techniques, epi-

dermal grafts capable of covering the entire body surface area can be generated from a 3-cm² biopsy from autologous tissue.³⁴ Mesenchymal stromal cells have also been reported to promote both normal and diabetic wound healing and clinical trials are under way.³⁵ However, commercial lesions have been hard won for living skin substitutes since they were limited by the costly and lengthy preparation, short shelf life, and difficulties in distribution.³⁶

Based on how acellular dressings interact with the native environment, they can be further grouped into bio-inert dressings and bioactive dressings. Bio-inert dressings mainly serve as a physical barrier and keep the wound environment moist. Winter first described that moisture-retaining dressings accelerated epithelialization of acute superficial wounds in pigs compared with air-exposed wounds³⁷ and similar results were later observed in human.³⁸ Moreover, some conformable bio-inert dressings can absorb exudate in the draining wounds.³⁹

A group of bioactive acellular wound dressings were developed to deliver bioactive agents to the wounds. Antibiotic drugs, including streptomycin,⁴⁰ minocycline,⁴¹ vancomycin,⁴² neomycin,⁴³ and ciprofloxacin,^{44,45} have been delivered and antiseptic agents have been applied such as iodine-releasing agents (e.g., IodozymeTM and Hyiodine[®]), silver-releasing agents (e.g., SilverSeal[®]), and chlorhexidine (e.g., BACTIGRAS[◇]).

Wound dressing scaffolds can be categorized into naturally derived and synthetic biomaterials (Table 1). In general, they each target only one aspect of the wound healing process, whereas a multifaceted approach may be preferred for efficient and complete healing. Decellularized matrices have gone through minimum biochemical modification and still preserve the native ECM microstructure, which provides topographical guidance for tissue regeneration. Decellularization products that have been approved by the FDA for diabetic wounds include OASIS[®] (derived from porcine small intestinal submucosa), MatriStem[®] (derived from urinary bladder matrix), and AmnioFix[®] (derived from amniotic membrane). Naturally derived biomaterials, including proteins (e.g., collagen) and polysaccharides (e.g., chitosan), have wide biomedical applications and some of them have been approved by FDA as well. Their extensive use has been associated with thorough characterization and a small likelihood of side effects in new applications.⁴⁶ A key advantage of naturally derived biomaterials is their general capacity to support cell attachment, proliferation, and differentiation.⁴⁷ The inherent composition and structure of naturally derived biomaterials enable their biological recognition, including cell receptor binding and cell-triggered degradation and remodeling.⁴⁷ Type I collagen is the most popular option because of its close similarity to the native ECM.^{48,49} Other naturally derived biomaterials such as chitosan,⁵⁰ cellulose,⁵¹ fibrin,⁵² gelatin,^{53,54} silk,⁵³ and alginate⁵⁵ have also been used.

In contrast, synthetic biomaterials provide precise control over material properties (e.g., biochemical composition, mechanical properties, topography, and structure), simplified purification process, and reduced possibilities of immunogenicity and pathogen transmission.⁵⁶ Rapid development of electrospinning and nanotechnology enables synthetic wound dressings with versatile nanoscale structure and function. In a recent study, Yu *et al.* synthesized an elastic, wearable crosslinked polymer layer that mimics

TABLE 1. COMMERCIALY AVAILABLE MATRICES AND DEVICES FOR DIABETIC ULCER TREATMENT

Category	Interface material	Example	Action mechanism	Advantage(s)	
ECM	Decellularized matrix	OASIS [®] (Smith & Nephew) MatriStem [®] (ACell) AmnioFix [®] (MiMedx)	Cell recruitment Tissue regrowth	Natural porous structure	
	Platelet-rich plasma	Aurix [™] (Nuo Therapeutics)	Cell recruitment Tissue regrowth	Autologous source	
	Collagen	Aongen [™] (Aeon Astron) ColActive [®] (Covalon) Integra [™] Bilayer (Integra) Integra [™] Flowable (Integra) Puracol [®] (Medline) PuraPly [™] (Organogenesis) Ultrafoam [™] (Daval)	Hemostasis Cell recruitment Exudate removal	Main component of native ECM; self-assemble into fibrillar structure	
	Gelatin	Gelfoam [™] (Pfizer)	Hemostasis	Resorbable	
	Hyaluronic acid	Hyalomatrix [®] (Fidia Anika) Jaloskin (Fidia)	Maintain moisture	Rapid absorption	
	Naturally derived biomaterials	Chitosan	Aquanova [™] (Medtrade) Chitoderm [®] (Trusetal) HemCon [®] (HemCon) NOCC [™] Hydrophilic (Kytogenics)	Hemostasis	Positive charge for electrostatic interaction
		Alginate	Maxorb [®] II (Medline) AlgiDERM [®] (Bard) AlgiSite M (Smith & Nephew) Kendall [™] (Medtronic) Kaltostat [®] (ConvaTec) Suprasorb [®] (Specialty Fibers)	Exudate removal Hemostasis	Swelling induced by Ca ²⁺ -Na ⁺ exchange
		Cellulose	Dermafill [™] (Cellulose Solutions) DURAFIBER [◇] (Smith & Nephew)	Moisturize Mechanical Support	Mechanical stability
		Carboxymethyl cellulose	REGRANEX [®] (Smith & Nephew)	PDGF-BB	Robust controlled release
		Synthetic matrix	PU	V.A.C. [®] GranuFoam [™] (KCI) RENASYS [™] Foam (Smith & Nephew)	Negative pressure
PVA	V.A.C. [®] WhiteFoam [™] (KCI)		Negative pressure		
Silicone	PICO [®] (Smith & Nephew)		Negative pressure	Semipermeability	
PEG	Tegaderm [™] Matrix (3M)		MMP inhibition	High hydrophilicity	
Polyacrylate	AcryDerm [®] (AcryMed)		Oxygen delivery	High absorbency	
PLA	Suprathel [®] (Polymedics)		Moisturize	Nontoxic biodegradation	
PGA	GORE [®] BIO-A [®] (GORE)		Cell ingrowth	Nontoxic biodegradation	
White petrolatum	SANTYL [®] (Smith & Nephew)		Enzymatic debridement	Occlusive moisturizer	

ECM, extracellular matrix; MMP, matrix metalloproteinase; PDGF, platelet-derived growth factor; PEG, polyethylene glycol; PGA, polyglycolic acid; PLA, polylactic acid; PU, polyurethane; PVA, polyvinyl alcohol.

normal youthful skin and demonstrated its application to decrease the herniation appearance of lower eyelids with rapid curing on the skin.⁵⁷

Instructive Cues to Promote Wound Healing

Because of the unmet clinical needs to treat diabetic wounds, there are growing efforts to develop matrices that are instructive and cost-effective. Recent advances in biotechnology and biomedical engineering have enabled novel approaches to mimic the wound healing microenvironment. These approaches can be generally categorized into biochemical cues and biophysical cues (Fig. 1 and Table 2).

Biochemical cues

The importance of biochemical cues on cellular behavior and tissue morphogenesis is well recognized. Biochemical properties are often considered as the first parameter in biomaterial design for wound healing applications (Fig. 1).¹⁰

Growth factors. Growth factors are potent regulators of cell activities, including migration, proliferation, differentiation, and survival. PDGF-BB as the Regranex[®] gel was the first growth factor approved by the FDA in 1997. It was used in the treatment of diabetic neuropathic ulcers.⁵⁸ Other growth factors investigated in the wound dressings include

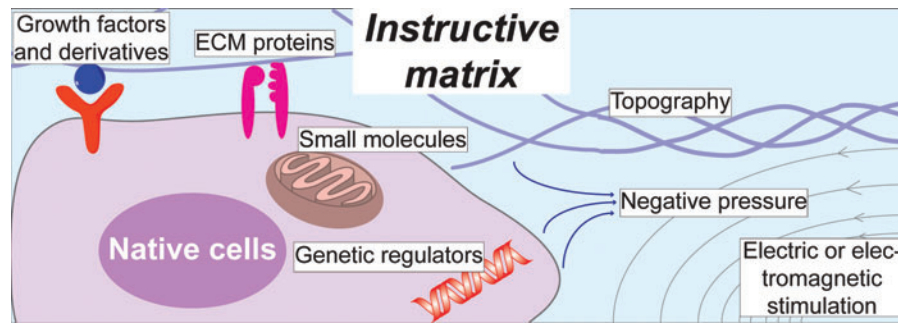


FIG. 1. Various biochemical and biophysical cues provided by matrix to regulate the native cell response. Growth factors and derivatives can interact with native cells through their specific receptors. The composition of ECM proteins is usually recognized by integrins. Small bioactive molecules, including oxygen and NO, can diffuse into the cells and mainly affect mitochondrial activities. Genetic regulators, including cDNA, miRNA, and siRNA, can be delivered by nonviral vehicles and facilitate gene transcription and translation. Electric or magnetic cues can also influence cell responses such as migration. cDNA, complementary DNA; ECM, extracellular matrix; miRNA, microRNA; NO, nitric oxide; siRNA, small interfering RNA. Color images available online at www.liebertpub.com/teb

EGF,⁵⁹ VEGF,⁶⁰ bFGF,⁶¹ and TGF- β .⁶² Conventional delivery methods often provide growth factors in the soluble form, which results in a burst release causing severe side effects such as hypotension (e.g., VEGF) and nephrotoxicity (e.g., bFGF).⁶³ Recently, novel delivery strategies have been explored and a synergistic signaling between growth factor receptors and integrin ligands was proposed,⁶⁴ which motivates immobilizing growth factors in close affinity to ECM proteins. Martino *et al.* demonstrated that growth factors delivered by this method were effective at a lower dosage,⁶⁴ thereby reducing application cost and potential side effects. Dynamic release of different growth factors with independent release profile can recapitulate the dynamic environment in developmental pathways.^{65,66} Novel immobilization methods

have been applied on instructive biomaterials to localize, enhance, and sustain growth factor bioactivities.⁶⁷⁻⁷⁰

Growth factor derivatives and peptide sequences. Peptides are short functional amino acid sequences derived from primary receptor domains of specific proteins and they bring an advantage of cost-effectiveness in scalable chemical synthesis compared to recombinant growth factor production.⁷¹ While retaining similar or higher biological potency, peptides are more stable than native growth factors, which can simplify the preparation and modification of biomaterials.¹⁰ The best characterized peptide is the integrin-binding Arg-Gly-Asp (RGD) sequence, which is found in many ECM proteins, including fibronectin, type IV collagen, and

TABLE 2. RESEARCH STRATEGIES TO IMPROVE DIABETIC WOUND HEALING

Category	Action mechanism	Example	Advantage(s)	Challenge(s)
Biochemical	Growth factor delivery	VEGF, EGF, bFGF, TGF- β	Potent regulators of three wound healing phases	Carcinogenic potential; uncontrolled release
	Growth factor derivatives	RGD sequence	Cost-effectiveness; enables cell recognition on synthetic materials	Requires covalent immobilization
	Small bioactive molecules	Oxygen (<i>commercially available</i>) Nitric oxide	Potent regulator of three phases	Short half-life
	Genetic regulators	cDNA, siRNA, microRNA	Upregulate dysfunctional genes or silence disease-causing genes	Delivery efficiency; targeted release
Biophysical	Topography	Electrospun microfibers	Accelerates cell ingrowth, for example, reepithelialization	Temporal presentation
	Negative pressure (<i>commercially available</i>)			
	Electric stimulation (<i>commercially available</i>) Electromagnetic therapy (<i>commercially available</i>)			

bFGF, basic fibroblast growth factor; cDNA, complementary DNA; EGF, epidermal growth factor; RGD, Arg-Gly-Asp; siRNA, small interfering RNA; TGF- β , transforming growth factor- β ; VEGF, vascular endothelial growth factor.

laminin.^{72–74} RGD sequence has been extensively applied in modifying synthetic biomaterials to provide cell-adhesive sites.^{56,75} Other peptides have been designed as mimics of growth factors (e.g., angiopoietin,^{76,77} VEGF,⁷⁸ and TGF- β 1⁷⁹) or as novel delivery systems.^{80,81}

Small bioactive molecules

Oxygen. The importance of oxygen in wound healing is well documented as high energy is required in the granulation tissue for cellular activities, including bacterial defense, cell proliferation, and cell migration. Oxygen level in chronic wound tissues is lower than that in normal wounds.⁸² Historically, hyperbaric oxygen therapies (HBOTs) were developed in which the body is intermittently exposed to pure oxygen in a stationary pressure chamber.⁸³ HBOT delivers the oxygen through a systematic circulation; therefore, its efficacy is limited in tissues with poor circulation. Since the late 1960s, a topical oxygen therapy has been developed, which involves applying pure oxygen with sealing around wound tissues for about 90 min, once a day at a pressure slightly above ambient atmospheric pressure.⁸⁴ More recently, oxygen-releasing wound dressings (e.g., Oxyzyme™) have been commercialized to promote wound healing by eliminating cellular hypoxia after tissue damage.⁸³ Instead of attaching a bag filled with pure oxygen, the Oxyzyme system generates oxygen by chemical reaction.

Oxygen level is an important regulator of wound healing. Once recruited to the wound site, leukocyte's bactericidal activity is positively correlated to local oxygen concentration.⁸⁵ HBOT reversed diabetic defects by mobilizing endothelial progenitor cells from the bone marrow, critical for angiogenesis.²⁰ During remodeling, fibroblast proliferation and collagen synthesis are also oxygen dependent.^{86,87} Other than directly supplying energy for the cellular metabolism, oxygen is also critical for growth factor signaling.⁸⁸ Metabolically active cells in the wound area consume large amount of oxygen and this together with interrupted blood supply contributes to a hypoxia gradient in the wound. The discovery of hypoxia-induced factor 1 α (HIF1 α) highlighted the importance of hypoxia gradient in the wound healing process⁸⁹ and stabilized HIF1 α expression, critical to improve diabetic wound healing.⁹⁰

Nitric oxide. Since the discovery of NO as an endothelial relaxing factor,⁹¹ it has been intensively studied in virtually all organs and tissues under both normal and pathological conditions.⁹² NO is generated by nitric oxide synthase (NOS) enzyme and three isoforms of NOS have been identified: two constitutively expressed isoforms (endothelial NOS [eNOS] and neuronal NOS [nNOS]) and one inducible NOS (iNOS) isoform.^{93–95} All three isoforms of NOS have been found in the skin: nNOS has been observed in keratinocytes and melanocytes^{96,97}; eNOS has been detected in basal keratinocytes, dermal fibroblasts, endothelial capillaries, and eccrine glands^{98,99}; and iNOS has been induced in keratinocytes,^{100,101} dermal fibroblasts,⁹⁸ and endothelial cells.¹⁰²

NO is involved throughout the three phases of wound healing as an important regulator of angiogenesis, inflammatory response, and collagen deposition.¹⁰³ During early inflammatory phase, NO regulates the infiltration of monocytes and neutrophils by activating pro-inflammatory cytokines

(e.g., IL-8 and TGF- β 1) and serves as a chemoattractant.^{104–106} NO promotes angiogenesis and keratinocyte migration in proliferation phase. Importantly, NO is vital for the VEGF activity as blockade of NOS prevents VEGF-induced endothelial cell proliferation.¹⁰⁷ NO also promotes endothelial cell proliferation and migration directly.¹⁰⁸ Moreover, NO has been reported to promote proliferation of keratinocytes and inhibits their apoptosis.^{109,110} During remodeling, NO predominantly regulates the collagen synthesis in fibroblasts as treatment with NO donors (e.g., dietary L-arginine or iNOS overexpression) significantly enhanced the collagen deposition in the wound.^{111–113}

Over the past two decades, NO delivery devices/vehicles have been developed to transit the therapeutic potential of NO in chronic wounds. It is extremely difficult to handle NO as a gas molecule owing to its instability and its oxidation potential to the toxic nitrogen dioxide molecule. Therefore, various vehicles such as S-Nitrosothiols,^{114,115} diazeniumdiolates,^{116,117} and nanoparticles^{113,118,119} have been used to deliver NO and, thereby, improve the wound healing process.

Genetic regulators. Genetic therapies were originally developed from successful delivery of genes based on viral vectors.¹²⁰ However, use of viral vectors in humans faces safety challenges such as immune reactions. Therefore, nonviral vectors have been developed to deliver genetic regulators, including naked DNA¹²¹ and RNA interference.¹²² Efficient delivery and targeted release are the main challenges for genetic therapies and recent advances such as electroporation have significantly contributed to address them.¹²³ In this study, we review recent advances in genetic regulators delivered by nonviral vectors with a focus on wound healing applications.

Complementary DNA. Complementary DNA (cDNA) is a DNA copy synthesized from the target mRNA that encodes a specific protein through reverse transcription. Nonviral vectors, including cationic polymers, cationic liposomes, and naked plasmids, have been used to deliver cDNAs encoding for peptides (e.g., LL-37,¹²⁴ secretoneurin¹²⁵) and growth factors (e.g., VEGF,¹²⁶ keratinocyte growth factor¹²⁷) to regulate different wound healing phases. Sonic hedgehog is a prototypical morphogen that plays essential roles during the embryonic development and promotes angiogenesis in postnatal tissue remodeling.¹²⁸ Asai *et al.* demonstrated that topical sonic hedgehog gene vector accelerated wound healing in diabetic mice with promoted microvascular remodeling.¹²⁹ Park *et al.* delivered sonic hedgehog DNA intradermally as polyplexes formed with cationic poly(β -amino esters) and reported improved angiogenesis and wound healing.¹³⁰

The safety of cDNA delivery using naked plasmids has been demonstrated in a number of recent clinical studies using intramuscular injections of hepatocyte growth factor (HGF) DNA plasmids to treat critical limb ischemia.^{131–134} Importantly, local delivery of HGF plasmids did not cause peripheral edema and did not increase systemic HGF protein level.¹³² Therapeutic benefits, including reduced pain, increased ankle-brachial index, and improved wound healing, were observed in these studies and some ischemic ulcers healed completely.^{131–134}

cDNA transfection usually results in transient expression of exogenous genes; therefore, methods have been developed to prolong their expression. Kulkarni *et al.* encapsulated lipoplexes carrying eNOS encoding gene in fibrin microspheres and formed a “fibrin in fibrin” temporal release system.¹³⁵ Electrospun fibers have been immobilized with plasmids to sustainably deliver bFGF¹³⁶ and EGF.¹³⁷

Small interfering RNA. While cDNA transfection enables the production of a functional protein, small interfering RNAs (siRNAs) can silence disease-causing genes by complimentary binding to mRNA sequences of corresponding gene targets. Therefore, cDNA and siRNA can serve as controls for each other in recovery experiments.^{138,139} More importantly, siRNA posttranscriptional modifications have been applied to achieve transient local ablation of malignant genes at different phases of wound healing.

Chen *et al.* identified INT6/eIF3e as a regulator of HIF2 α activity and reported that siRNA-Int6 promoted angiogenesis by accumulating HIF2 α and downstream transcription of angiogenic factors in a hypoxia-independent manner.¹⁴⁰ Using a previously developed agarose matrix, Nguyen *et al.* achieved a near-complete local knockdown of p53 and accelerated wound healing in diabetic mice with upregulated vasculogenic cytokines such as VEGF and SDF-1.¹⁴¹ Wetterau *et al.* delivered prolyl hydroxylase domain 2 (PHD2) siRNA with agarose matrix in diabetic mice and the suppression of PHD2 increased the expression of HIF1 α and angiogenic regulators.¹⁴²

Remodeling phase is a therapeutic target because dysregulated remodeling results in hypertrophic and unaesthetic scars. Lee *et al.* first delivered Smad3 siRNA to inhibit skin fibrosis induced by radiation.¹⁴³ Similarly, Wang *et al.* inhibited TGF- β /Smad signaling by applying TGF- β 1 receptor siRNA and reported reduced hypertrophic scarring.¹⁴⁴ To sustain the release of these TGF- β /Smad targeting siRNAs, delivery matrices, including trimethyl chitosan¹⁴⁵ and pressure-sensitive hydrogels,¹⁴⁶ were developed. MMP-responsive nanofiber matrix was developed for diabetic wound remodeling to control the release of MMP-2 siRNA, which restored the wound recovery rate.¹⁴⁷

More recently, Charafeddine *et al.* developed nanoparticles encapsulating siRNA to deplete Fidgetin-like 2 *in vivo*, which accelerated cell migration due to increased directional motility.¹⁴⁸ In another study, Randeria *et al.* demonstrated the delivery of siRNA in spherical nucleic acid-gold nanoparticles to suppress ganglioside-monosialic acid 3 synthase, a critical mediator of insulin resistance, reversing impaired diabetic wound healing.¹⁴⁹

MicroRNA. MicroRNAs (miRNAs) are a major group of noncoding RNAs and are important regulators of gene expression by posttranscriptional regulation. They are small and endogenously formed repressors that usually bind to the 3'-untranslated region of target mRNAs. Importantly, their interactions are noncomplimentary and a single miRNA can regulate multiple mRNAs simultaneously while a single mRNA can be regulated by various miRNAs.¹⁵⁰ Recent studies suggested that miRNAs play important roles in wound healing, including regulating angiogenesis, reepithelialization, and wound remodeling.^{151,152}

Angiogenesis is critical for initiating wound healing and miRNAs have been shown as important angiogenic regulators.¹⁵³ In diabetic wounds, Chan *et al.* showed that downregulation of miR-200b promoted angiogenesis and accelerated healing by desilencing GATA binding protein 2 and VEGF receptor 2.¹⁵⁴ In another study, they described that downregulation of miR-199a-5p promoted angiogenesis both *in vitro* and *in vivo* by inducing Ets-1 and MMP-1 expression.¹⁵⁵

Reepithelialization has been a target for miRNA interventions as well. Biswas *et al.* reported that hypoxia induced miR-210 expression and downregulated the cell-cycle regulatory protein E2F3, resulting in impaired wound closure with limited keratinocyte proliferation.¹⁵⁶ miR-21 was reported to be induced by TGF- β ¹⁵⁷ and promote keratinocyte migration *in vitro* and reepithelialization *in vivo*.^{158,159} However, Pastar *et al.* reported contradicting results showing that miR-21 inhibits wound healing with suppressed leptin receptor signaling.¹⁶⁰ miR-203 was also reported as an important regulator of mRNAs responsible for keratinocyte proliferation and migration such as RAN and RAPH1.¹⁶¹ Sundaram *et al.* identified miR-198 as regulatory switch in controlling multiple genes to facilitate reepithelialization.¹⁶² Li *et al.* identified miR-31 as another key regulator to promote keratinocyte proliferation and migration targeting epithelial membrane protein 1 (EMP-1).¹⁶³ In another study, they identified miR-132 as a regulator to facilitate transition from inflammation to proliferation phase and its implication in chronic wounds, which are stalled at the inflammation phase.¹⁶⁴

Remodeling is critically related to scar formation that can be caused by dysregulated collagen production and remodeling.¹⁶⁵ MiR-29 was identified as a key regulator of collagen expression in systemic sclerosis¹⁶⁶ and collagen deposit in skin fibroblasts.¹⁶⁷ Yang *et al.* reported that downregulation of miR-155 at wound sites did not accelerate wound closure, but led to a reduced fibrosis with less collagen and α -smooth muscle actin expression.¹⁶⁸

Biophysical cues

There is growing recognition that biophysical cues are essential regulators of cell-matrix interactions.¹⁶⁹ Besides intrinsic physical properties of the matrix, different instructive biophysical cues have been applied to promote tissue regeneration. Specifically, in this study, we present biophysical cues, including topographical guidance, negative pressure, electrical stimulation, and electromagnetic therapy, which have been applied in promoting wound healing.

Topographical guidance. Advances in micro- and nanoscale fabrications significantly contributed to the growing recognition of topographical guidance on cell behavior.¹⁶⁹ Specifically, micropatterned surfaces with arrayed grooves or cell-recognizable adhesion sites have been utilized to guide skin cell migration *in vitro*, mimicking reepithelialization *in vivo*.¹⁷⁰⁻¹⁷² Using photolithography, Kim *et al.* made nanogrooves with various spacing distances and found an optimal spacing ratio to promote fibroblasts migration, proliferation, and ECM deposition.¹⁷¹ Marmaras *et al.* patterned grooves of 1- μ m width and 0.6- μ m height and applied it apically to guide dermal fibroblast

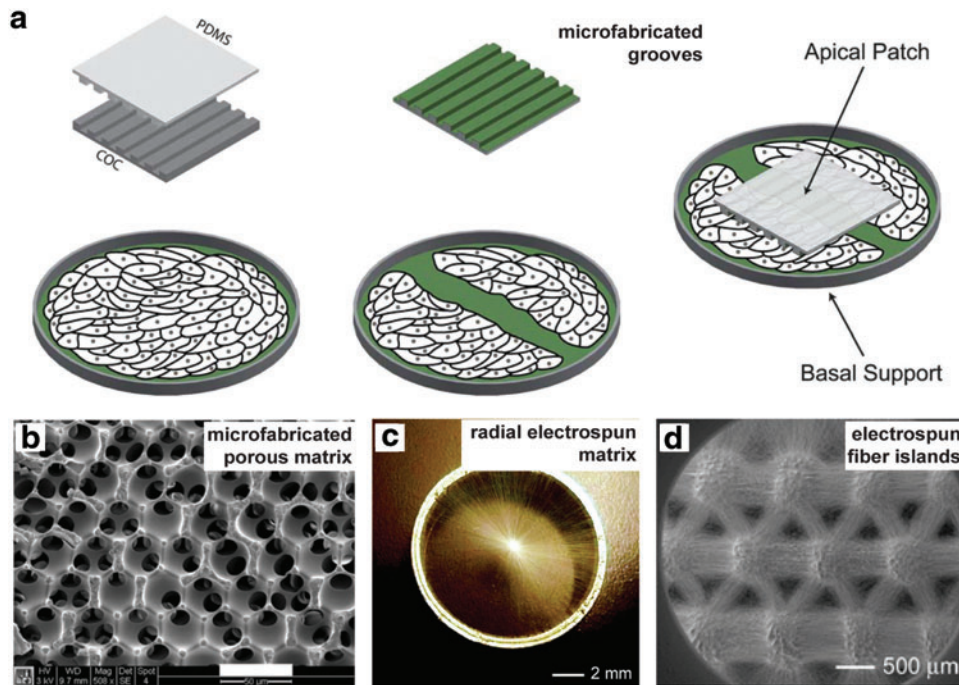


FIG. 2. Engineered bio-materials using topographical guidance. **(a)** Schematic of wound treatment using a microgrooved PDMS patch. **(b)** Porous polymer matrix fabricated by sphere templating. **(c)** Electrospun fibers aligned in radial orientation. **(d)** Microisland arrays of electrospun nanofibers. Reproduced with the permission from Refs.^{170,177,222,223} Copyrights 2012 Royal Society of Chemistry, 2012 John Wiley and Sons, 2010 American Chemical Society, and 2010 John Wiley and Sons, respectively. PDMS, polydimethylsiloxane. Color images available online at www.liebertpub.com/teb

migration¹⁷⁰ (Fig. 2a). Fu *et al.* developed ultrafine fibrous network on top of polycaprolactone/collagen nanofibrous matrices by collagen coating and observed phenotype change and increased motility of keratinocytes.¹⁷² It is of note that cell spreading and migration depend on both feature size and the spacing between.

It has been well documented that porous biomaterials better integrate with native tissue compared with the same material fabricated into a solid form.¹⁷³⁻¹⁷⁵ Parkinson and Rea reported that porous anodic aluminum oxide membranes significantly improved the healing of burn injuries in pigs.¹⁷⁶ To investigate the effect of pore sizes, Marshall *et al.* developed a spherical templating method and fabricated constructs where every pore was in the same size and uniform interconnectivity (Fig. 2b).¹⁷⁷ By varying the pore sizes from

10- to 160- μm and investigating their integration and healing *in vivo*, the optimum healing characterized with maximum vascular density was obtained with 35- μm pores regardless of the polymer composition or implantation site.¹⁷⁷ By comparing the phenotype of macrophages on the surfaces of implants, they found activation polarization shift toward the M2 phenotype on sphere-templated implants.¹⁷⁸ This provides possible explanation for the improved healing and was further proved *in vitro*, where the monocyte/macrophage activation was significantly higher on porous surfaces.¹⁷⁹

Negative pressure. Topical negative pressure (TNP) therapy is a long established practice as an adjunctive therapy for chronic wounds since 1980s.¹⁸⁰ In 1993, Argenta and colleagues developed the V.A.C.[®] therapy system that

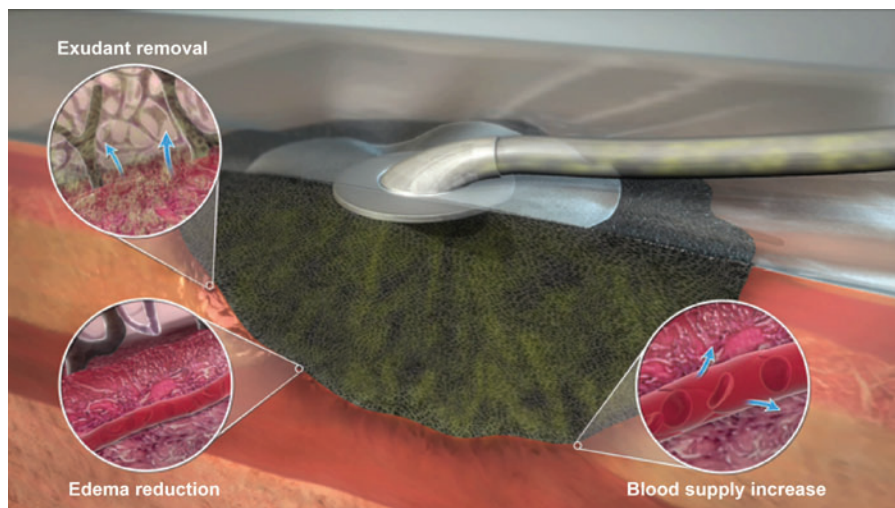


FIG. 3. A commercially available vacuum-assisted wound closure device (KCI's proprietary V.A.C.[®] Therapy System). The macrostrain assists in the excessive fluid removal (*arrows* indicate exudant flow), tissue edema reduction, and blood flow optimization (*arrows* indicate vessel dilatation). Courtesy of KCI, an Acelity Company. © 2015 KCI. Color images available online at www.liebertpub.com/teb

generates uniform subatmospheric pressure using a proprietary reticulated foam customized into the shape of wound, a semi-permeable film to seal and maintain moisture, and proprietary track pad and tubing (Fig. 3).^{181,182} An intermittent therapy has been observed to achieve better healing outcomes compared with continuous negative pressure application.¹⁸² Although there is no standard protocol yet, a negative pressure of 125 mm Hg has been suggested as the baseline setting for all wounds and lower negative pressure needs to be tailored for soft tissue to minimize potential ischemic effects.¹⁸³

Mechanisms that can be attributed to the benefits of TNP therapy have been investigated in basic research. The best described mechanisms are an increase in blood flow,^{182,184} the promotion of angiogenesis,¹⁸⁵ and granulation tissue formation.¹⁸⁶ Moreover, the local negative pressure induces deformation of ECM and generates microstrain on cells, which can promote cell proliferation and migration. Based on computer simulation, the strain levels the cells experience are comparable to the strain levels that promote cell proliferation *in vitro*.¹⁸⁷ Cyclic strain further promotes keratinocyte proliferation and migration¹⁸⁸ and this can be attributed to the superior outcome of intermittent TNP therapy compared with continuous treatment.

Electric stimulation. Electrical stimulation offers a safe, affordable, and facile approach for regulating cell behavior and function.¹⁸⁹ Electrical stimulation is a biomimicry tool in tissue regeneration representing bioelectrical signals in the body and has been widely used in wound healing applications^{190,191} and in the clinics.¹⁹² Endogenous electric fields naturally arise after wounding and keratinocytes migrate in response to it, leading to wound closure. An external electrical stimulation aims to enhance the effect of naturally occurring electric fields on wound healing process. Electrical stimulation increased fibroblast proliferation and collagen synthesis both *in vitro*^{193,194} and *in vivo*.^{195,196} Moreover, electrical stimulation induced keratinocyte migration and differentiation *in vitro*.¹⁹⁷ Major clinical trials have used pulsatile waveforms or pulsed stimulations¹⁹⁸ and found wound healing depended on the stimulation parameters (e.g., current type, frequency, amplitude, and duration).¹⁹⁹ High frequency electric fields (>1000 Hz) better promote wound healing compared with low frequency ones (1–1000 Hz) because they can create polarity in the wound.

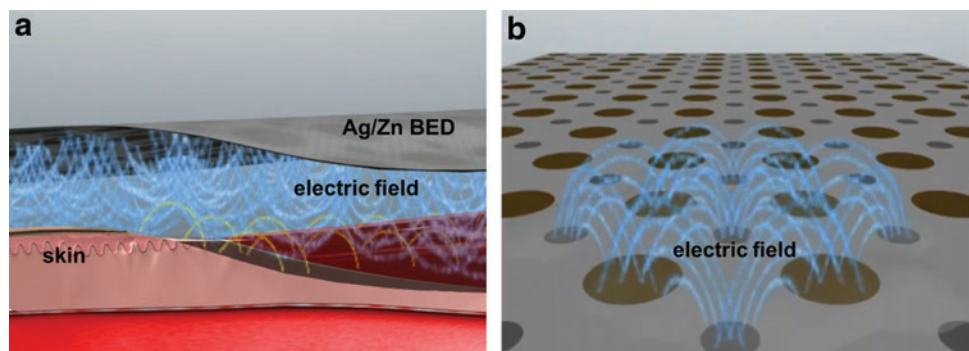
Several studies used electrical stimulation to treat diabetic wounds. Electroporation technique has been successfully

used to accelerate diabetic chronic wound healing.^{200,201} In another study, an electrostimulation system (Fenzian™) was used for a pilot study of 21 diabetic patients with chronic ulcers. Results showed wound area and diameter decreased only in the patients who received the stimulation.²⁰² A bioelectric dressing that generates physiologic levels of microcurrent (2–10 μ A) (Procellera®) (Fig. 4) has been reported to accelerate wound healing by promoting re-epithelialization.²⁰³ In another work, a wireless device was used to apply electrical stimulation to treat diabetic foot ulcers²⁰⁴ by applying microcurrents to the wound surface noninvasively with reduced risk of infection.

Recent advances in material science and microfabrication technologies enabled us to produce smart bioelectric dressing devices with imbedded electronics for local, accurate, controllable electrical signal delivery.²⁰⁵ Such devices can be used for the delivery of therapeutic agents (e.g., antibiotic drugs and RNAs) to wounds.²⁰⁶ Smart and flexible bioelectronics within the device can sense and monitor physiological parameters in wound area (e.g., pH and temperature) and release therapeutic agents through electrical signals. It is hoped that such technologies would tackle current problems of chronic wound healing and play a major role in the treatment of patients with diabetic wounds.

Pulsed electromagnetic therapy. Pulsed electromagnetic therapy provides a facile, noninvasive, and safe approach for wound treatment.²⁰⁷ A major trust for clinical applications of pulsed electromagnetic therapy was derived from its successful application in treating chronically broken bones.²⁰⁸ *In vitro* studies showed that electromagnetic field affects keratinocytes and fibroblasts by upregulating a variety of genes that modulate inflammatory response.²⁰⁹ Interaction of pulsed electromagnetic field with endogenous electric fields resulted in upregulation of multiple growth factors and NO, which regulated cell migration and angiogenesis.¹⁹² Pulsed electromagnetic field increased the tensile strength of diabetic wounds in rat models.²¹⁰ The treatment significantly accelerated wound closure and reepithelialization after 10 days. A large variety of devices and protocols have been proposed and used in electromagnetic therapy, including the RecoveryRx® medical device (Fig. 5).²¹¹ Next steps would be computerized control of electromagnetic field and real-time monitoring and assessment of its biological effects to achieve personalized treatments.

FIG. 4. Schematic diagram of design, application (a), and electric fields (b) generated by Procellera® bioelectric dressing. Courtesy of Banerjee *et al.*²⁰³ Color images available online at www.liebertpub.com/teb



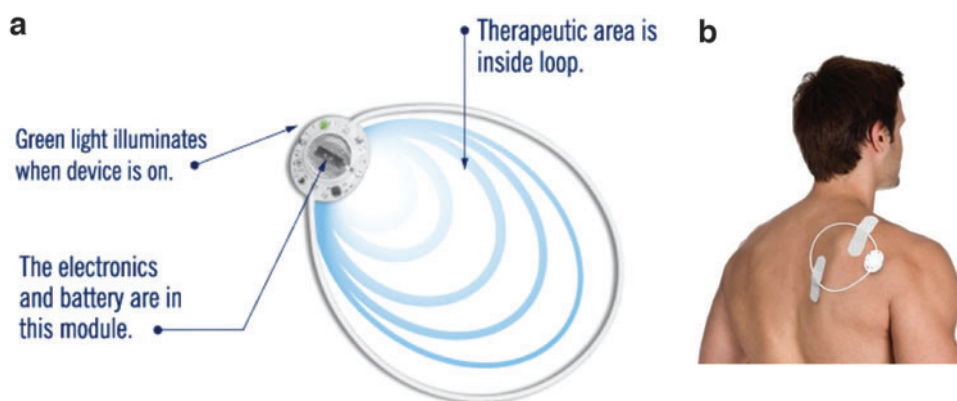


FIG. 5. Diagram of the design and magnetic field generated by RecoveryRx® medical device (a) and its application on human body (b). Color images available online at www.liebertpub.com/teb

Microfabrication Technologies to Provide Biochemical and Biophysical Cues in Matrices

Recent advances in top-down and down-top microfabrication technologies have increased the versatility and resolution of these techniques in biomedicine.²¹² Microfabrication technologies are able to provide biochemical and biophysical cues within biological structures mimicking the structure and function of ECM. Such biochemical and biophysical cues can regulate cell adhesion, migration, proliferation, differentiation, and ultimately, tissue morphogenesis and function. Therefore, microfabrication technologies have widely been used in different tissue regeneration applications and other biological studies.²¹³ In this study, we review and discuss commonly used microfabrication technologies (i.e., electrospinning, microfluidics, molecular self-assembly, and phase separation techniques) in providing biochemical and biophysical signals in wound healing matrices.

Electrospinning is a widely used microfabrication technique by which polymeric micro- and nanofibers can be made from the polymer melt or solution using electric field. The technology has a long history of development and was first proposed to produce electrospun fibers in the late 1930s.²¹⁴ Nowadays, electrospinning technology has found numerous applications in science and technology ranging from biomedicine,²¹⁵ fabrication of functional composites,²¹⁶ filtration,²¹⁷ energy,²¹⁸ and electronics.²¹⁹

In particular, electrospinning is a powerful technique to provide structures similar to the collagen structure in the ECM. For example, Rho *et al.* fabricated biodegradable electrospun fibers of type I collagen and used them as the wound dressing in rat models.²²⁰ Electrospun nanofibers with different topographic features as random, aligned, nanoporous, or yarn fibers have been developed for skin tissue engineering.²²¹ More recently, using a collector composed of a central point electrode and a peripheral ring electrode, Xie *et al.* generated radially aligned electrospun nanofibers (Fig. 2c).²²² In the subsequent studies, they generated microisland arrays of nanofibers with uniaxially aligned nanofibers in between (Fig. 2d)²²³ and demonstrated that the nanoscale topographical guidance promoted skin cell migration *in vitro* and reepithelialization *in vivo*.²²⁴

Microfluidics is defined as the manipulation of small amounts of fluids in microchannels.²²⁵ Microfluidics has influenced a wide range of research fields and applications,

such as chemical synthesis,²²⁶ biology,²²⁷ optics,²²⁸ and information technology.²²⁹ The use of microfluidic systems in wound dressing matrices is primarily due to capillary or vessel formation within the matrices.²³⁰ Such microfluidic systems can lead to the fabrication of large skin substitutes with well-controlled pore structures. Chin *et al.* used a microfluidic bioreactor in producing porous glycosaminoglycan-collagen gels as an artificial skin substitute. They showed that migration of fibroblasts was enhanced within the fabricated collagen because of high porosity of structures.²³¹ Zheng *et al.* fabricated a microfluidic system of alginate and collagen and used it to study vascular cell invasion in an animal wound model.²³² The system enhanced the host tissue invasion and blood vessel formation compared with uniform collagen material.

The microfluidic template can further be developed to include heterogeneous and biologically relevant structures with high fidelity in pore size and interconnectivity, which modulate the host tissue response and formation of functional blood vessels. As a novel and alternative approach, three-dimensional (3D) printing can be used to fabricate microfluidic systems within biomaterials for wound healing applications.^{233–235} Three-dimensional bioprinting technology is able to provide mechanically strong, scalable, and structurally integrated vascularized systems within biomaterials guiding cells to promote microvascularization.

Molecular self-assembly is defined as the autonomous assembly and organization of components initiating from the molecular level to final structure or state without external intervention.²³⁶ Molecular self-assembly approach can be used to fabricate hydrogels of therapeutic agents.²³⁷ Therapeutic agents in hydrogel forms can be self-deliverable or delivered using external stimuli. For example, Yang *et al.* fabricated supramolecular hydrogels based on D-Glucosamine (a natural compound in cartilage with significant role in wound healing) for wound healing applications.²³⁸ The self-assembly of peptide-based nanofibers with EGF promoted wound healing in human tissue models.⁸⁰ Diverse self-assembly techniques²³⁹ enable us to fabricate functional biomaterials as the wound dressings with tunable size, structure, and properties.

Phase separation does not require any specific equipment and is able to produce 3D and porous materials. The process is composed of five major steps as follows: material dissolution in suitable solvent, phase gelation and separation, solvent extraction from the gel, and freeze drying the

remaining material.²⁴⁰ The material porosity can be varied by changing the solvent. Siafaka *et al.* prepared sponges of chitosan with 2-hydroxyethyl acrylate through phase separation technique. The sponges loaded with levofloxacin as the wound dressing showed a great inhibitory effect on bacteria growth.²⁴¹ Mi *et al.* also used a phase separation technique in the preparation of chitosan films containing antibacterial agents as the wound dressing.²⁴² The porosity of films ensured a sustained release of antibacterial agents. In addition, porous films allowed oxygen permeability and controlled moisture of wound area and drainage of wound exudates. Phase separation technique stands as a common approach to fabricate polymeric membranes as the wound dressing.²⁴³ However, these techniques may be time consuming and cannot be considered as completely green because of solvent residue in fabricated materials.

Concluding Remarks

Wound dressings were among the first tissue engineering products to be approved and their development was founded on wound healing biology. There is a relatively mature understanding of cell biology and cell–matrix interactions in the wound microenvironment. Commercialization of the wound dressings based on living cells was difficult and there is a consensus now that in the design of wound dressings for clinical use, the products must be user-friendly and cost-effective.

Acellular wound healing products enabled by novel technologies have been developed to deliver instructive cues to stimulate or restore the native regeneration potential and serve as promising alternatives to skin substitutes. Although numerous matrices and devices have been investigated in basic research or tested in clinical studies, optimal wound treatment still requires substantial improvement and joint effort from different fields, including biomaterial science, gene therapy, microfabrication technology, and electrical and electromagnetic engineering. Moreover, the implementation of these novel technologies and devices for clinical use requires further interdisciplinary collaboration and education of healthcare workers.

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Authors' Contributions

All authors have made contributions by writing the article.

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