Review

Skin regeneration scaffolds: a multimodal bottom-up approach

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Skin wounds are a major social and financial burden. However, current treatments are suboptimal. The gradual comprehension of the finely orchestrated nature of intercellular communication has stimulated scientists to investigate growth factor (GF) or stem cell (SC) incorporation into suitable scaffolds for local delivery into wound beds in an attempt to accelerate healing. This review provides a critical evaluation of the status quo of current research into GF and SC therapy and subsequent future prospects, including benefits and possible long-term dangers associated with their use. Additionally, we stress the importance of a bottom-up approach in scaffold fabrication to enable controlled factor incorporation as well as production of complex scaffold micro- and nanostructures resembling that of natural extracellular matrix.

A multimodal cocktail for skin regeneration

Skin serves the onerous function of protecting our internal organs and tissues from external, potentially dangerous insults for a lifetime. Such temporal demands require not only impeccable integrity, but also mechanical strength and durability. Box 1 provides an overview of the skin's normal structure. Loss of large parts of this barrier, be it related to illness or injury, renders the individual susceptible to disability or death. The World Health Organisation (WHO) estimates that, annually, over 300 000 deaths are attributable to fire-related burn injuries with millions more suffering from the partly devastating physical and emotional consequences thereof [1]. A further 6.5 million individuals suffer from chronic skin ulcers caused by prolonged pressure, venous stasis, or diabetes mellitus [2]. A more detailed account of normal wound healing is given in Box 2. Box 3 provides details on the currently acceptable management of thermal wounds. The holistic goals of modern cutaneous wound care consist of rapid wound excision and closure with a functionally intact and aesthetically pleasing outcome. Currently available treatment options are lacking in establishing both functional and cosmetic satisfaction. Thus, combined with the burden of pain and the currently suboptimal therapy methods, the overall onus of cutaneous wounds appears vast. It seems of particular importance to dissociate oneself from the concept of aiming to replace lost skin tissue, and rather focus on promoting the regeneration of wounded tissues by stimulating the innate ability of the skin for self-renewal. Wound contracture and scarring, properties that can misleadingly be thought accountable for faster wound healing, should be avoided in terms of wound regeneration. The gradual understanding of the biological processes involved in wound healing has opened the gates for developing smart bioconstructs that actively promote tissue regeneration via

Glossary

Acidic/basic fibroblast growth factor (a/bFGF): potent mitogens and chemoattractants for vascular endothelial cells, dermal fibroblasts and epidermal keratinocytes.
Allograft: transplantation of cells, tissues, or organs from a nonidentical donor of the same species into a recipient.
Autograft: transplantation of cells, tissues, or organs from one part of the body to another in the same individual.
Chemoattractant: a chemical substance that induces a cell or organisms to migrate towards it.
Colony stimulating factor-1 (CSF-1): a haematopoietic GF involved in the proliferation, differentiation, and survival of monocytes, macrophages, and bone marrow progenitor cells.
Cosmesis: the preservation or restoration of bodily beauty.
Epidermal growth factor (EGF): implicated in keratinocyte migration, fibroblast proliferation and differentiation, and granulation tissue formation.
Human adipose-derived stem cells (hADSCs): a source of mesenchymal SCs that have shown a potential for therapeutic vascularisation due to the production of angiogenic GFs.
Hyaluronic acid (HA): a major component of human skin extracellular matrix.
Insulin-like growth factor-1 (IGF-1): a glycoprotein mainly produced in the liver but also expressed by fibroblasts. High concentrations within wound beds accelerate keratinocyte migration and proliferation.
Keratinocyte growth factor (KGF): member of the FGF family (FGF-7) and involved in epidermal keratinocyte proliferation. In 2004, human recombinant KGF (Kepivance Biovitrum, USA) was FDA approved for use in radiotherapy-induced oral mucositis in the treatment of epithelial cancer.
Mitogen: a chemical substance that stimulates cell division.
Platelet-derived growth factor (PDGF): a chemoattractant for fibroblasts, neutrophils, and monocytes, which promotes production of new ECM by fibroblasts. FDA approved (Regranex) since 1997.
Primary cutaneous anaplastic large cell lymphoma (PCALCL): a form of non-Hodgkin's lymphoma.
Transforming growth factor-β (TGF-β): a key mediator for fibroblast migration and proliferation, granulation tissue formation, and increased collagen synthesis and neovascularisation.
Tumour necrosis factor-β (TNF-β): a cytokine expressed by a multitude of cells and involved in attracting fibroblasts.
Vascular endothelial growth factor (VEGF): a key regulator in angiogenesis during physiological processes such as wound healing but also thought to nourish pathological conditions including cancer.
Wound contraction/contracture: although an integral stage in the wound healing process, prolonged contraction results in unsightly and thickened scars.

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Box 1. The skin: beyond a first glance

Skin is the outermost covering of human beings and the largest organ of the body, encompassing the entire body surface. It has a complex three-layered structure (Figure I) which, under physiological circumstances, is intrinsically self-renewable, so that a new layer of skin develops every 2–3 weeks while continuously shedding the older top layers.

The three layers (Figure I), from outermost to innermost are: (i) epidermis: multiple, ever renewing layers of keratinocytes; (ii) dermis: separated from the epidermis at the dermal–epidermal junction (basement membrane); and (iii) hypodermis: mainly made up of adipose tissue and collagen.

Skin appendages such as hair follicles, sebaceous glands and sweat glands are numerously intermingled along blood vessels, nerve endings, and pressure and touch receptors. Regional variations exist regarding skin thickness, distribution of skin appendages, and melanocyte density. Skin mainly functions as a protecting interface, physically shielding internal organs and tissues from external insults. Once the external barrier is breached, innate surveillance mechanisms set off a cell-signalling cascade to limit pain, control infection, and accelerate wound healing naturally, ultimately creating a scar. However, extensive wounds such as those associated with full-thickness burns rarely, if ever, heal spontaneously and thus require an external means of protection, be it temporary or permanent, to stimulate not only wound healing, but scarless self-regeneration. Current conventions hold that tissue-engineered skin constructs should resemble native skin both anatomically and functionally.

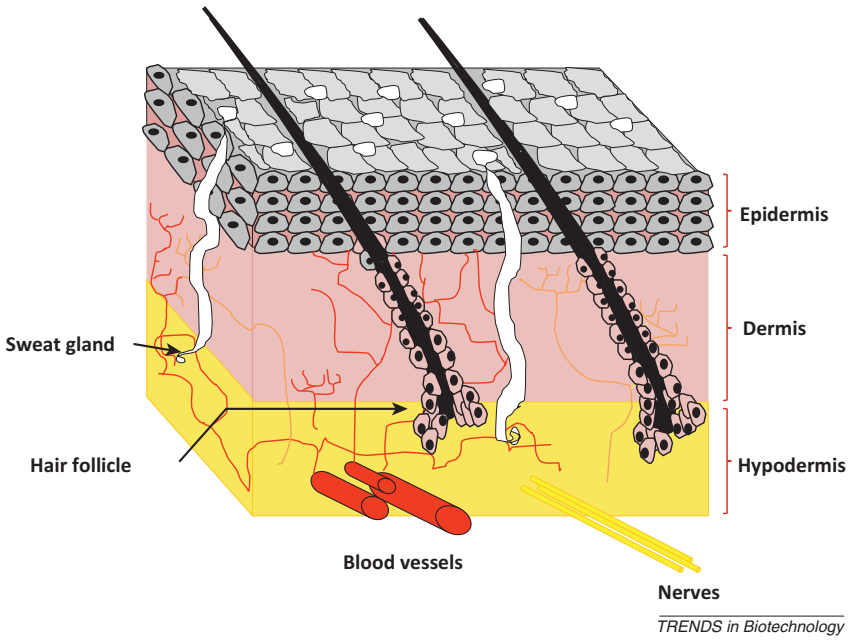

Figure I. An illustration of human skin showing the three constituting layers and skin adnexae.

appropriately engineered regeneration platforms, or scaffolds, as well as the incorporation of cell-signalling elements such as GFs and SCs. Here, we review and critically appraise current research efforts concerned with dermal regeneration scaffolds incorporating bioactive elements to promote neovascularisation and tissue regeneration. We also touch upon the most recent advances in the field of nanoscaled tissue engineering because cellular behaviour is significantly influenced by the surface nanotopography of the scaffold that promotes cellular adherence, differentiation, and proliferation, mimicking natural extracellular matrix (ECM) [3].

Current state of tissue-engineered skin substitutes

The pressing need for more suitable wound dressings has spurred on the search for alternative skin substitutes that actively promote wound regeneration. The era of dermal tissue engineering was heralded three decades ago with a bilayered and biocompatible dermal scaffold based on a bovine collagen matrix, which successfully induced the synthesis of a neodermis [1]. This novel bioconstruct revolutionised current burns practice and even today, Integra is often used as the gold standard in severe full-thickness burns that are not amenable to autograft (see Glossary) harvest, due to immediate availability, skin infiltration rates similar to that of skin allografts (85% versus 95%) and adequate cosmetic results [2].

Clinically available and novel skin substitutes can be broadly divided into epidermal, dermal, and dermoepidermal (composite) tissue-engineered constructs [4]. A multitude of choices and potential alternatives for tissue-engineering skin constructs exist and, in fact, numerous substitutes are being investigated for human usage; some of them already commercialised (Table 1).

The current lack of more sophisticated and superior skin alternatives requires a focus on regeneration rather than replacement. Current research is increasingly integrating the concept of engineering dermal scaffolds that actively promote regeneration by incorporating SCs and external GFs to recreate a favourable cellular microenvironment. SCs have been central to the field of regenerative medicine for over three decades due to an ability to induce their differentiation into any cell type with subsequent cell-specific GF and cytokine release to enhance angiogenesis [3]. This approach is motivated by the understanding that numerous cell–cell and cell–ECM cues are required to

Table 1. Characteristics of the ideal skin substitute

	Dermoepidermal substitute (composite)			Dermal substitute				Epidermal substitute		Ref
	Cadaveric skin (nonprofit skin banks)	Karoskin[a]	Apligraf[a]	Alloderm[a]	SureDerm[a]	Integra[a]	Dermagraft[a]	MySkin[a]	CellSpray[a]	
Patient safety	Potential for viral transmission Immune rejection	Potential for viral transmission Immune rejection	Potential for viral transmission	Potential for viral transmission	Potential for viral transmission	n/a[b]	Potential for viral transmission	Autologous keratinocytes are cocultured with irradiated murine cells	n/a	[2]
Scaffold degradability	Rejection rather than degradation	Rejection rather than degradation	1–2 months	Incorporates into wound bed	Incorporates into wound bed	Half-life, 30 days	Degrades by hydrolysis	<29 days	n/f	[81]
Duration of cover	Temporary	Temporary	Temporary	Permanent	Permanent	Semi-permanent	Temporary	Permanent	Permanent	[4]
Neodermis formation	Dermis revascularises and integrates into the wound bed. The epidermis is rejected 3–4 weeks post-transplantation	Dermis revascularises and integrates into the wound bed. The epidermis is rejected 3–4 weeks post-transplantation	Delivers ECM components, cytokines and GF to the wound	Repopulated by host cells, i.e., incorporates into host tissue	Repopulated by host cells, i.e., incorporates into host tissue	Neodermis formation complete in 15–20 days	Scaffolds degrade over 20–30 days. Fibroblasts simultaneously produce ECM components and GF	Only applicable in partial-thickness and graft donor side wounds, but not in full-thickness wounds	Only applicable in partial-thickness and graft donor side wounds, but not in full-thickness wounds	[82–85]
Shelf life	7–10 days if fresh. Unlimited if lyophilised	Unlimited if lyophilised	5–10 days	2 years	Up to 2 years	2 years	Up to 6 months	3 days	n/f	[4]
Cost (/cm^2) (in 2007)	donated	£0.60	£14.20	£5.90	n/f	£3.32	£7.14	n/f	n/f	[2]
Mechanical stability	Lyophilisation improves mechanical stability significantly	Lyophilisation improves mechanical stability significantly	Requires delicate handling	Stable due to presence of basement membrane.	No adverse information regarding fragility and manual handling	Easy handling	Easy handling	Easy handling and application due to a silicone support layer	Very fragile and difficult to handle	[4,86]
Scaffold vascularisation (i.e., 'take')	Cadaveric allografts take initially, i.e., vascularisation is observed, however, subsequent graft rejection requires its eventual removal	Takes initially, i.e., vascularisation is observed, however, subsequent graft rejection requires its eventual removal	Take rates depend on the type of wound and are very variable ranging from 16 to 41%	Uncertain rates of vascularisation	No adverse information regarding delayed or failed graft take	Takes relatively long time for vascularisation (10–14 days)	Take is facilitated by fibrovascular in growth and re-epithelialisation and wound closure by keratinocytes migration from wound edges	Cannot be used for full-thickness wounds as dermal component missing	Uncertain rate of take as it depends on cell–cell and cell–ECM adhesion rather than vascularisation. Higher risk of bacterial contamination leading to graft loss	[75]
No. of stages necessary for completion	Multiple because cadaveric grafts require eventual replacement	Multiple because cadaveric grafts require eventual replacement	One-stage process but needs cografting with autologous epithelial cells in full-thickness burns	One-stage process (using ultrathin split-thickness graft)	Two-stage process (using split-thickness graft)	Two-stage process (using split-thickness graft)	Two-stage process if used in burn injuries (using split-thickness graft)	Up to 12 individual applications	One-stage process	[4]

[a]Commercialised product.

[b]n/a, not applicable; n/f, not found.

achieve physiological functioning of the surrounding cells and tissues [5]. Furthermore, naturally occurring ECM components, as well as physiological molecules involved in wound healing, have dimensions in the nanometre range (1–100 nm), thus suggesting a significant and exploitable potential for 'nanoengineered' particle delivery vehicles. For example, nanoparticles could be used to create advantageous surface nanostructures or nanotopography resembling natural structures, and further seeded with SCs or functionalised with GFs [6], cytokines, and peptides [7].

Bioengineering 'smart' scaffolds for skin regeneration: a bottom-up approach

The ultimate purpose of tissue-engineered skin grafts is to enable complete and natural, albeit accelerated, wound regeneration. A 3D supporting framework should serve as a template for tissue regeneration while simultaneously preventing wound bed contraction throughout the first stages of healing [8]. The framework, or scaffold, should further serve as a platform for cellular localisation, adhesion, and differentiation, as well as guide the development of new functional tissues [9]. A focus on the multitude of different scaffold materials and fabrication techniques is beyond the scope of this article but has been extensively reviewed elsewhere [10]. Scaffold materials may be of natural, synthetic, or composite origin and engineered using a multitude of approaches including porogen leaching, electrospinning, molecular self-assembly, and phase separation. The mixing of materials of different classes to obtain composite scaffolds seems particularly promising because individual limitations of single material scaffolds (e.g., poor mechanical properties) are often overcome by the composite nature [11]. The ideal skin regeneration scaffold should actively direct tissue formation and prevent scarring. Thus, much focus has been channelled into creating suitable biomimetic surface micro- and nanostructures that can act as delivery vehicles for SCs or GFs. The synergistic tissue regenerating effects of a smart scaffold cocktail comprising (i) favourable scaffold surface patterns, (ii) GFs, and (iii) SCs have the realistic potential of overcoming current barriers and enabling fast and complete skin regeneration.

During natural wound healing, dynamic and reciprocal interactions between components of the ECM and surrounding cell-signalling molecules are responsible for the expression of GFs and cytokines. These interactions elicit cellular responses that ultimately lead to new tissue formation. Overwhelming activation of the inflammatory system and prolific recruitment of contractile cells is thought to stem from prehistoric adaptations of human skin to close irregular and often contaminated wounds as rapidly as possible, to prevent microorganism invasion and potentially lethal infections [12]. Unfortunately, this response typically leads to scar formation, often resulting in disfigurement and functional disability. Nowadays, some wounds are closed aseptically using sutures that obviate the need for a vigorous contractile response, creating more time for complete tissue regeneration. Here, external modulation of cell-signalling events via a finely tuned delivery of GFs or SCs is thought to alter the wound environment, enabling orderly regeneration. Modifying the micro- and nanoenvironment and surface architecture of the scaffold, termed nanotopography, actively influences cell migration, proliferation, and differentiation.

In the following sections, we focus on discussing incorporation of various GFs involved in wound healing into scaffolds and the possibilities of exploiting the latest SC technologies to accelerate skin regeneration. Potential advantages of cutting-edge nanoengineered scaffolds over macrosized skin substitutes as 3D stimulatory platforms for cellular growth and skin regeneration are highlighted. Overall, we support the argument that a bottom-up approach (i) enables tight control over important micro- and nanostructures within scaffold architecture; and (ii) facilitates incorporation of appropriate concentrations of bioactive factors as well as SCs.

GF functionalised skin-regeneration scaffolds

Epidermal growth factor (EGF)

EGF is implicated in keratinocyte migration, fibroblast proliferation and differentiation, and granulation tissue formation. EGF significantly enhances wound healing [13] as well as the tensile strength of the resultant ECM [14]. Prolonged exposure to EGF yielded a 200% increase in tensile strength when liposome-encapsulated EGF was delivered into murine dorsal cutaneous wounds [15]. Similarly, EGF contained within a gelatine sheet applied to dorsa of hairless dogs accelerated wound closure and healing of superficial and partial-thickness wounds [16]. Current challenges regarding the delivery of EGF at physiologically

Box 2. Normal wound healing

Wounds are defined as breaks in the continuity of the structure of the skin, usually resulting from physical trauma and leading to either temporary or terminal loss of function. Healing is a complex dynamic process that results in the restoration of anatomic continuity and function (Figure I). It consists of four distinct but overlapping entities that depend on complex and finely orchestrated cell-signalling events.

Haemostasis:
(i) Triggered post-injury by platelets within the wound bed.
(ii) Platelets interact with ECM to activate clotting cascade.
(iii) Resultant blood clot provides a platform for cell migration.

Inflammation:
(i) Triggered by PDGF and TGF-β released by platelets.
(ii) Neutrophils and monocytes are recruited to phagocytose foreign material/pathogens.
(iii) Macrophages express CSF-1, TNF-β and PDGF, which act as chemoattractants for fibroblasts.

Proliferation:
(i) PDGF, TGF-β, and ECM molecules induce fibroblasts proliferation, new ECM deposition, and integrin receptors expression for cellular recognition and adherence.

Remodelling:
(i) Crosslinking of new collagen matrix.
(ii) Fibroblast-derived enzymes including collagenases, plasminogen activator, and gelatinases create pathways through tightly woven ECM for cellular movement.
(iii) Granulation tissue is replaced with an acellular, avascular scar.

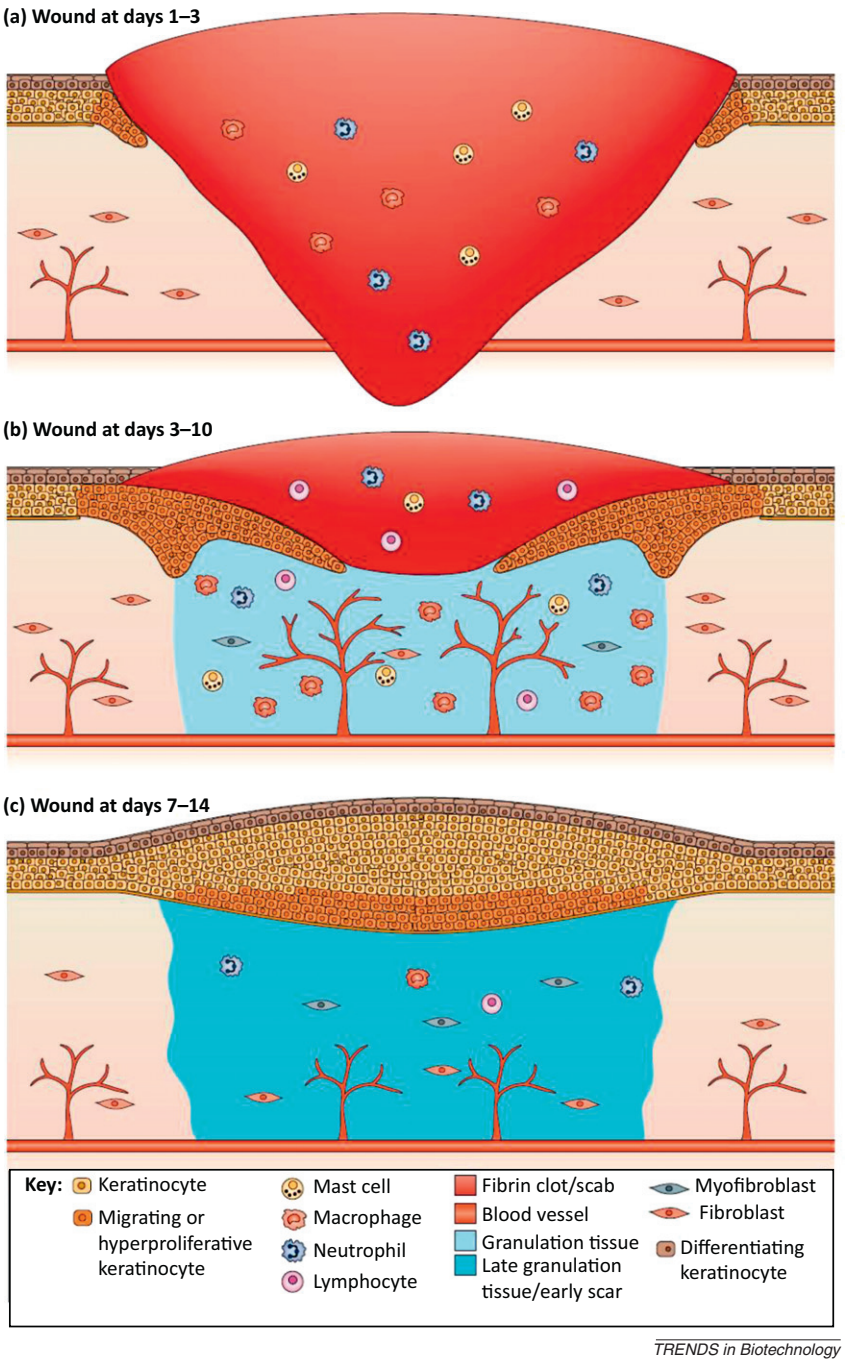

Figure I. A schematic representation of the normal wound healing process incorporating various cell-signalling molecules [80].

relevant concentrations and durations still prevail due to its rapid breakdown within the wound environment, encouraging research into effective immobilisation and delivery techniques. For example, biodegradable microspheres that contain EGF provide sustained EGF delivery and hence more effective wound healing in a rabbit dorsal skin wound model [17]. Despite such encouraging results, supplemental EGF has a mitogenic effect upon cells and has been implicated in the spread of epithelial malignancies [18]. However, the beneficial effects of EGF in the wound healing process should not be denied, thus requiring further research.

Basic fibroblast growth factor (bFGF)

FGF comprises a large family of mitogens that are actively involved in the processes of wound healing, embryonic development, angiogenesis, and tumour progression [19–21]. Both acidic fibroblast growth factor (aFGF) and bFGF are found within the wound fluid at the earliest stages of healing [22]. aFGF, like bFGF, is a potent mitogen and chemoattractant for vascular endothelial cells, dermal fibroblasts, and epidermal keratinocytes. In the initial phases of wound healing, bFGF participates by activating local macrophages and can still be identified within the healing tissue

Box 3. Management of thermal wounds

Thermal wounds have been divided into three zones of histopathological injury:
(i) Zone of coagulation (eschar): outermost area undergoes complete and irreversible necrosis and denaturation of proteins.
(ii) Zone of stasis and oedema: partially denatured proteins and slow blood flow.
(iii) Zone of hyperemia: gradually increasing blood flow.

In case of wound infection or poor perfusion, a seemingly superficial burn may in time develop into a more severe and deeper wound with necrotic areas extending into the zone of stasis.

Current treatment standards:
(i) Early and complete excision of eschar to prevent wound infection.
(ii) Full-thickness burns: wound coverage with an autologous split-thickness skin graft harvested from intact areas of the patient's skin.

If larger parts of the total body surface area have been damaged, autologous skin grafts may still be taken and then meshed in order to enlarge the size of the graft. The disadvantages of such practices are morbidity, significant pain at the donor site, and the characteristic corrugated scar as the recipient site heals. In cases of total or near-total full-thickness skin injuries, donor sites may be unavailable, necessitating the use of cadaveric skin to fulfil vital barrier functions. Cadaveric skin grafts are lyophilised, thus removing the cellular component to prevent graft immune rejection. They may either be obtained from nonprofit skin banks or purchased as for example, Karoskin. Such allografts represent temporary 'bridging' measures for immediate wound coverage in the acute stages post-injury.

Disadvantages of utilising human cadaveric skin:
(i) Donor organ shortage and limited skin bank availability.
(ii) Moral objections from the patient's or surgeon's perspective.
(iii) Risk of viral transmission from cadaveric tissues to the recipient.

There is an urgent need for fully synthetic, yet biocompatible and so-called 'smart' skin bioconstructs for the regeneration of scar-free skin.

during the remodelling phase that occurs several weeks after injury [23]. bFGF accelerates neovascularisation as shown in a rabbit ear wound healing model, in which wounds supplemented with exogenous bFGF healed significantly faster compared to untreated controls [24]. Radiation-induced cutaneous wounds created in miniature pig models treated with exogenous bFGF showed increased dermoepidermal proliferation, new blood vessel formation, higher overall mechanical skin stability, and adnexae integrity [25]. In 2001, genetically recombinant bFGF was approved for clinical use in Japan under the trade name Fiblast Spray (Kaken Pharmaceutical Co. Ltd., Tokyo, Japan). Clinical trials conducted on second-degree burn wounds in Japanese adults and children showed accelerated rates of wound healing and superior mechanical properties of the resultant scars when topical bFGF was initiated relatively soon after creation of the burn injury (1–4 days, mean 2 days) [26,27]. Despite such promising results, concerns over the carcinogenic potential of topical FGF application remain; bFGF has been identified as a major autocrine stimulant in melanoma and, in combination with UV light, is associated with tumour progression [28,29]. Further research is needed to corroborate existing evidence of beneficial effects of bFGF on wound healing and refute any associations with the progression of skin cancers.

Keratinocyte growth factor (KGF)

KGF is expressed within the dermis and hypodermis below the wound, whereas the KGF receptor is predominantly found on epithelial cells of the epidermis, suggesting a paracrine mediation of epithelial cell growth. Several animal and clinical studies have demonstrated the cytoprotective and epithelial regenerative properties of KGF [30–32]. Such favourable effects depend on several mechanisms including cell proliferation, migration, differentiation, survival, DNA repair, and detoxifying enzyme induction, which collectively act to strengthen the integrity of the epithelium [33]. During the initial 24 h of normal human wound healing, KGF expression is upregulated 100-fold and remains elevated for several days [34]. This upregulation in KGF is significantly dampened in genetically diabetic and glucocorticoid-treated mice [35,36]. In a porcine partial-thickness wound model, topical application of recombinant KGF demonstrated an accelerated rate of re-epithelialisation compared to controls not receiving KGF [37]. In a murine full-thickness wound healing model, keratinocyte proliferation and angiogenesis were significantly retarded in KGF knockouts compared to wild-type mice [38]. These results suggest a further role of KGF in the control of wound bed angiogenesis. However, the use of KGF in epithelial cancer patients raises concerns regarding potential tumourigenicity of KGF because epithelial cells express KGF receptors. Several studies have identified a potential association between the overabundance of KGF and tumour growth progression [39–41]. Further studies are warranted to examine the nature and extent of KGF involvement in tumour development.

Vascular endothelial growth factor (VEGF)

The VEGF family encompasses VEGF-A, VEGF-B, VEGF-C, VEGF-D, and PlGF. During wound healing, VEGF-A is highly expressed by keratinocytes within the wound bed to promote new blood vessel formation essential for tissue regeneration [42] and re-epithelialisation of wounds [43,44]. VEGF is the major angiogenesis-promoting GF due to its combined ability to stimulate endothelial cell proliferation and migration [45], basement membrane degradation [46], tubular and luminal structure formation [47], increased vascular permeability [48], and new vessel formation [49]. In a diabetic mouse model, minicircle plasmid DNA encoding VEGF was successfully transfected into proliferating cells within wounded tissue, resulting in a high level of VEGF expression [50]. Wound healing rates were significantly accelerated in plasmid-exposed injuries compared to those exposed to empty vehicles. Similarly, vector-mediated VEGF transfer onto experimental murine burn wounds increased angiogenesis as well as epithelial proliferation and ECM maturation [51]. Despite extensive evidence for accelerated wound healing via increased blood vessel formation, the use of VEGF must be critically appraised within the context of potential negative effects; two relatively recent studies have reported associations between chronic burn injuries and the development of primary cutaneous anaplastic large cell lymphoma (PCALCL) [52,53]. According to these reports, the growth of PCALCL is fuelled by VEGF production and secretion by atypical large lymphocytes. Furthermore, skin biopsies from the

erythematous region surrounding the PCALCL have revealed rich neovascularisation secondary to VEGF overexpression. Although the differing effects of VEGF on cutaneous wounds require a cautious approach, it seems of equal importance to investigate its beneficial effects further in order to avoid premature rejection of a potentially useful GF.

Insulin-like growth factor-1 (IGF-1)

High levels of IGF-1 found within cutaneous wounds have been shown to accelerate epidermal wound healing and inhibit apoptosis pathways [54,55], whereas reduced expression of IGF-1 is associated with retarded wound healing [56]. For example, IGF-1 receptor knockout mice (IGF-$1r^{-/-}$) exhibited skin hypotrophy with fewer and smaller hair follicles [57]. Wound healing by re-epithelialisation was accelerated by local overexpression of IGF-1, whereas no effects were observed on the underlying dermis [58]. *In vitro* studies, however, have found evidence for the stimulatory effects of IGF-1 on collagen and ECM production [59]. Similarly, wound beds of IGF-1-depleted rats had significantly lower collagen content, as measured by the amount of hydroxyproline, a major component of collagen, compared to nondepleted animals, suggesting a reduced capacity for wound healing. Subsequent IGF-1 infusion returned concentrations to near-normal levels [60]. Such contrasting evidence for a role in ECM and collagen production is indicative of a dose-dependent activation of dermal fibroblasts by IGF-1. In a porcine diabetic full-thickness excisional wound model, transplantation of IGF-1-transfected keratinocytes increased local IGF-1 expression 900-fold, leading to significantly accelerated keratinocyte migration and wound closure [61]. Wound contracture, a possible confounder in the interpretation of wound closure rates, is not thought to have significantly influenced the wound healing process, because degrees of contractions were similar in transfected and control groups. Maintenance of appropriate IGF-1 concentrations within the wound bed is significantly important because overexpression has been linked to skin hyperplasia and tumour growth [62,63].

Platelet-derived growth factor (PDGF)

PDGF is involved throughout all stages of normal wound healing. PDGF is released by degranulating platelets and activated macrophages within the wound fluid. Later in the proliferative stage, PDGF is responsible for the differentiation of fibroblasts into their contractile phenotype, the myofibroblasts which through attachments of their filopodia to components of the ECM, drag or contract the tissues together [64]. Several animal studies have demonstrated accelerated wound closure in normal and pathophysiological states when the wound bed was supplemented with exogenous PDGF [65]. In a genetically diabetic mouse model (C57BL/Ks-J-db/db), large full-thickness skin wounds ($1.5\times1.5\,cm^2$) created on the mid-back were exposed to recombinant human PDGF [66]. At 21 days, more fibroblasts, enhanced capillary formation, and accelerated wound closure were observed in treated diabetic mice compared to littermates receiving empty vehicles. Furthermore, *in vivo* blockage of PDGF receptors with the PDGFR-β inhibitor imatinib mesylate resulted in delayed wound healing, reduced wound closure, and abnormal microvascular morphology, normally observed in PDGFR-$\beta^{-/-}$ mice, thus highlighting the pivotal role of PDGF in early wound healing [67]. However, similar results could not be obtained using the same animal model, same wound dimensions, but commercially available Regranex [66]. In a recently published retrospective clinical study on the healing efficiency of supplementary PDGF on diabetic heel ulcers larger than 4 cm (an independent predictor of limb loss), some beneficial effects were observed when the standard treatment regime (distal femoral bypass surgery, partial calcanectomy, intraoperative negative pressure wound treatment) was boosted with PDGF [68]. PDGF is thought to modulate beneficially the ulcer microenvironment, thereby accelerating wound healing and closure. However, the small cohort, lack of randomisation and blinding, as well as the absence of a control population minimise the significance of these results.

Transforming growth factor-β (TGF-β)

TGF-β exists in three isoforms – TGF-β1, TGF-β2, and TGF-β3 – which are all involved in the process of wound healing. After acute injuries, TGF-β1 is highly expressed by keratinocytes, platelets, monocytes, fibroblasts, and macrophages [69]. TGF-β acts in both autocrine and paracrine manners, inducing its own synthesis by target cells and activating nearby cells to synthesise and release other GFs involved in the healing process [70]. The autocrine action of TGF-β1 by fibroblasts sustains their activity beyond the initial inflammatory stimulus [71] and is postulated to play a key role in myofibroblast differentiation [72]. TGF-β2 expression is related to wound contracture and excessive collagen deposition, but has also been demonstrated to be a causative factor in scar formation [73]. Early foetal skin is well known for its ability to regenerate wounds completely without the formation of scar tissue, which is thought to be largely associated with a significantly reduced expression of TGF-β1, coinciding with highly elevated levels of TGF-β3 [74]. As described within Box 4 on foetal wound healing, the exact underlying mechanism of action for scarless foetal wound regeneration has not been elucidated so far, leaving the respective roles of the TGF-β isoforms to be fully uncovered.

The results obtained with the use of GFs to accelerate wound healing, while encouraging on both experimental and clinical levels, must be interpreted cautiously, because the same factors are often implicated in exuberant tissue and tumour growth. More basic and preclinical research is necessary to indicate when GF concentration moves from being beneficial to becoming potentially harmful. Such ambiguous circumstances demand careful evaluation for more than one reason.

First, it is of paramount importance that exposure to inappropriate amounts of GFs is prevented due to potentially carcinogenic tendencies *in vivo*, as previously mentioned. Any such long-term adverse effects must be excluded prior to commencing clinical trials. Only a few of the numerous GFs playing their part throughout the

Box 4. Foetal wound healing

(i) Intrinsic and complete self-regeneration without scar formation attributable to different healing mechanisms.
(ii) The inflammatory response is significantly reduced with fewer differentiated inflammatory cells in early gestational foetal wounds.
(iii) Higher numbers of phagocytic cells and morphogenic factors and collagen deposition resembling that of normal skin (i.e., large bundles of ECM in a normal reticular orientation as opposed to an adult-pattern abnormal deposition of parallel bundles of mainly collagen types I and III).
(iv) Scarless healing is only possible during the first two trimesters, attributable to a finely orchestrated interplay between several factors:

a. Early reticular collagen deposition.
b. Reduced inflammatory response.
c. Higher levels of HA.
d. Altered ratios of signalling molecules and genes.

(v) During the third trimester, a transition occurs and the skin loses its ability to regenerate, resulting in the formation of fibrous scar tissues.

Previous assumptions that the sterile, GF-enriched intrauterine environment is the determining factor for scarless healing have been refuted by several seminal studies; a marsupial model for cutaneous healing demonstrated that the developing opossum retained its ability for scarless regeneration despite growing in a pouch devoid of amniotic fluid [36]. Transplantation studies on sheep models have shown that wounded adult or late foetal skin transplanted onto early-gestation foetal lambs healed with scar formation [37], whereas wounded early foetal human skin transplanted into nude mice regenerated with no scar formation [38]. This demonstrates independence from environmental factors and an intrinsic ability for early-gestational foetal skin to regenerate without scar formation.

The complete elucidation of the intrinsic foetal wound healing mechanisms would allow for the meticulous re-establishment of signalling events within an ex-foetal environment, resulting in scarless dermal regeneration in adult wounds.

Box 5. Stem cell-seeded skin regeneration scaffolds

Slow scaffold vascularisation is a critical limiting factor in skin wound regeneration due to inadequate supplies of nutrients and oxygen and a build-up of waste products within the tissues. SCs have the ability to self-renew and differentiate into lineage-specific progenies. Despite their ability to acquire any cell type of an organism, embryonic SC usage is retarded by ethical considerations. Adult SCs, on the other hand, have several advantages: (i) the ability to differentiate into several lineages within a tissue; (ii) the relative ease of access; and (iii) less stringent regulatory concerns and a higher degree of public acceptance compared to embryonic SC use. The beneficial effects of adult mesenchymal stem cells (MSCs) in wound bed neovascularisation and accelerated healing has been demonstrated in several preclinical models [87,88]. Yet, the mechanisms of action remain elusive; evidence for paracrine signalling subsists [89], although the role of MSC differentiation in wound healing is less clear, partially due to low engraftment efficiency of cells [90]. Thus, cell delivery vehicles have been applied to provide suitable cellular microenvironments and enhanced engraftment, survival and differentiation into appropriate mature cells [87] (see Figure 1 in main text). Particular emphasis is placed on the integration of human adipose derived stem cells (hADSCs) into skin regeneration scaffolds due to their accessibility and facile *in vitro* expansibility. hADSCs are a source of MSCs that have shown a potential for therapeutic vascularisation due to the production of angiogenic GFs [91]. This was demonstrated in an *in vivo* wound healing study using nude mice; two identical defects were created in the dorsal skin of mice and exposed to either collagen gel preseeded with hADSCs or empty collagen vehicles [92]. Angiogenic GF production was significantly enhanced in SC-seeded wounds compared to nonseeded wounds. Another study obtained similar results with ADSCs-impregnated microcarrier systems [93]. Despite substantial preclinical evidence for a beneficial role of SCs in the wound healing process, one should approach the clinical use of MSCs cautiously because evidence suggests a contributing role in cancer SC maintenance [94]. It remains to be seen how useful SC technology proves to be in clinical reality; the tendency to overplay the role of SCs for regenerative purposes, however, should not discourage scientists to evaluate further the substantial potential within the field of regenerative SC technology.

process of wound healing are FDA approved and clinically available for the purpose of accelerating wound repair (e.g., Regranex, Kepivance). One may argue that worries about lasting adverse effects of GF use in the context of skin wound healing may be unfounded because their application is mostly local. Furthermore, GFs are rapidly degraded into natural metabolites by the wound fluid, thus eliminating any downstream effects. Such rapid elimination from the body is, however, avoidable via encapsulation techniques, enabling GFs to linger within the wound for longer prolonging their trophic effects.

Second, determining beneficial effects on wound healing is ongoing. Selective GF treatment leads to accelerated rates of healing both in experimental and preclinical trials. Potential reasons for failure might be inappropriate routes of GF delivery or overabundance of GFs that cancel out the anticipated beneficial effects on wound healing. This, again, demonstrates the critical need to investigate the optimal GF concentrations to stimulate appropriately skin regeneration while avoiding the risk of overstimulation and tumour formation or receptor downregulation. Box 5 provides a critical discussion on the benefits and disadvantages of SC usage for tissue engineering purposes.

Nanoengineered scaffolds for a bottom-up approach to wound regeneration

Regenerative medicine aims at recuperating lost tissues by guiding cell growth and restoring original tissue architecture. This requires the presence of a scaffold because isolated cells are unable to re-establish their native structures due to a lack in extracellular guidance. Figure 1 shows a schematic representation of the ideal skin regeneration scaffold. Although various scaffolds for skin regeneration are already on the market (Table 1), their clinical implementation remains riddled with flaws, ranging from poor take rates [75] and poor cosmesis to relatively high infection rates. Efforts at increasing their effectiveness include bioactivation, that is, expanding their role from being a simple structural framework to a delivery vehicle for GFs, cytokines, or genes [76,77]. In this way, tissue regeneration can be actively promoted rather than merely passively suggested. This should, however, not discourage research into more suitable scaffold architectures, because accumulating evidence highlights the importance of biocompatible scaffold materials, appropriate pore sizes, and cell-growth-promoting surface topographies [78,79]. Although scientists agree on the importance of the extracellular microarchitecture for cell

adherence and proliferation, studies have shown an abnormally elongated phenotype when cells were grown on microfibrous materials. Cells cultured on nanofibrous structures, however, demonstrated a phenotype resembling that of cells growing within natural environments. This may be explained by the close architectural approximation of nanofibrous materials to natural ECM and collagen fibrils, which themselves exhibit nanometre dimensions. Thus, a natural cell environment can be feigned and cells guided along normal morphogenic lines. Additionally, natural ECM fibrils serve as storage vehicles for SCs and bioactive factors to regulate cell migration, proliferation, and differentiation. The bottom-up approach allows for tight control over scaffold micro- and nanoarchitecture, porosity, as well as the sequential incorporation of bioactive elements which, in turn, influence cellular interactions via the creation of a beneficial biomimetic micro- and nanoenvironment.

Concluding remarks and future perspective

The burden of cutaneous wounds is immense in both personal and financial terms. Clinically available and feasible treatment strategies are still lacking despite various skin substitutes being under thorough investigation. The emergence of nanotechnology combined with the latest SC technology and the ever-increasing appreciation of cell-signalling pathways in both adult and foetal wound healing models have opened up new avenues for precise biotechnological wound bed manipulations for accelerated healing. Cutting-edge developments within the area of tissue-engineered scaffolds lead the way into a new era of organ consciousness with the ultimate goal of tissue regeneration rather than replacement.

Approved treatment strategies for skin wounds mostly aim to replace lost tissues rather than support intrinsic self-healing mechanisms. Technological advances now grant scientists the ability to manipulate precisely scaffold materials and engineering strategies within nanometre dimensions to create nanotopographies that mimic the natural ECM. The incorporation of SCs, GFs, and nanoparticles into scaffolds promotes the intricate interplay between naturally occurring cell-signalling factors to achieve full tissue regeneration. With such fast and current developments in nanotechnology and biomedical sciences, we are continuously improving skin regeneration and repair. The future trend of regenerative medicine in

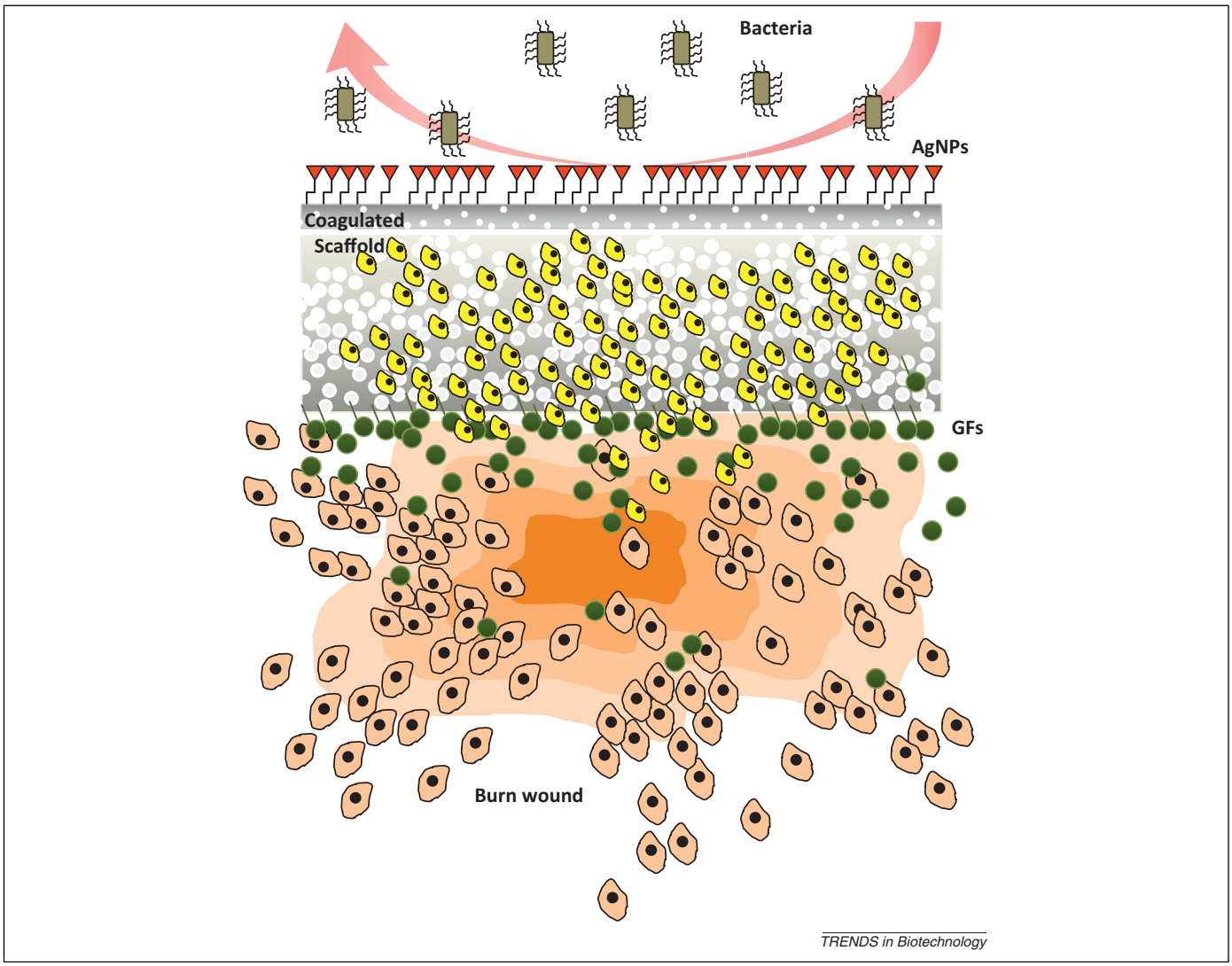

Figure 1. Schematic representation of the ideal scaffold to promote skin regeneration. Despite intense research efforts aiming at optimising available scaffolds, the proposed schematic has not been developed successfully yet. Abbreviations: AgNPs, silver nanoparticles; GFs, growth factors.

general, and tissue-engineering of skin in particular lies in: (i) the comprehension of intricate intercellular biochemical communications; (ii) the engineering of scaffold structures on a micro- and nanodimension; and (iii) the integration of GFs and SCs into such scaffolds to obtain a bioactive cocktail capable of active guidance in skin regeneration. Despite the presence of realistic benefits and dangers associated with GF or SC supplementation, ongoing research into their exploitation is fundamental if regenerative medicine is to have a future.

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Disclaimer statement

None

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