Wound healing and scar wars

Instructive microenvironments in skin wound healing: Biomaterials as signal releasing platforms

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Abstract:

Skin wound healing aims to repair and restore tissue through a multistage process that involves different cells and signaling molecules that regulate the cellular response and the dynamic remodeling of the extracellular matrix. Nowadays, several therapies that combine biomolecule signals (growth factors and cytokines) and cells are being proposed. However, a lack of reliable evidence of their efficacy, together with associated issues such as high costs, a lack of standardization, no scalable processes, and storage and regulatory issues, are hampering their application. *In situ* tissue regeneration appears to be a feasible strategy that uses the body's own capacity for regeneration by mobilizing host endogenous stem cells or tissue-specific progenitor cells to the wound site to promote repair and regeneration. The aim is to engineer instructive systems to regulate the spatio-temporal delivery of proper signaling based on the biological mechanisms of the different events that occur in the host microenvironment. This review describes the current state of the different signal cues used in wound healing and skin regeneration, and their combination with biomaterial supports to create instructive microenvironments for wound healing.

Keywords: instructive biomaterials, skin regeneration, wound healing, signaling release, *in situ* tissue engineering.

1. Introduction

Skin wound healing is a complex hierarchical process that entails a multistep progression of phases. After an injury, different cell types arrive at the wound to stop the bleeding and rid the site of potential infectious organisms, dead cells and debris. Poor wound healing after a trauma or acute injury and chronic disease affects millions of people around the world[1]. It is estimated that millions of people suffer from chronic

wounds, a serious clinical and economic problem for national health services, which will only get worse owing to the ageing population and age-associated diseases such as diabetes.

Healing can follow two different mechanisms: regeneration or repair[2]. The sequence of events after skin injury has been extensively studied and involves many cell types and signals to induce wound healing. These stimuli promote the arrival of progenitor cells to the site that will start the regeneration of the damaged tissue. However, this process, which is inherent and highly orchestrated during development, loses its effectiveness in the adult body[3], resulting in disorganized extracellular matrix (ECM) commonly known as a scar. The mechanisms that leads adult skin tissue healing to a scar are poorly understood[4].

The possibility of using biomaterials as platforms to generate stimuli that can promote cell activity related to skin regeneration is a strategy that is receiving more attention thanks to its versatility. Biomaterials are a powerful tool to modify the host microenvironment due to their properties obtained through the fabrication process. Surface chemistry, topography, mechanical properties and degradation products combined or not with biological signals can promote an efficient regeneration. They are multistimuli, not limited to biochemical signals. Consequently, they can induce cell recruitment and activate a highly controlled self-secretion of growth factors (GFs) and cytokines that stimulates the production and organization of the ECM. In this review, we look at the specific stimuli that are required for efficient wound healing and explore how biomaterials can trigger such signals through the stimulation of specific cell mechanisms that aid the body to self-heal.

2. Skin wound healing

2.1. General description of the skin tissue

The skin is our largest and most extensive human body's organ and develops myriad functions that allow the body to maintain homeostasis and be protected from the environment and are essential for life. It acts as a wall to toxic compounds and physical entities, avoiding dehydration and participating In the regulation of the body's temperature [5]. Skin includes different sensors and activates different polyaminoacids with antibacterial properties to avoid infections. Skin also produces and activates several hormones, neuropeptides and cytokines, whose functions and effects are disseminate in the whole body, such as vitamin D, melatonin and sex steroids[6].

Anatomically, the skin is structured in three principal levels: the epidermis, the dermis and the hypodermis, and contains the ECM, cells (see **figure 1**) and other structures known as skin appendages which comprise mechanic and temperature sensors, sweat and sebaceous glands, hair and nails [7].

The epidermis is the thin, stratified most external layer that constitutes the chemical and physical barrier between the exterior environment and the internal body. It contains different cell populations including keratinocytes, Langerhans cells, Merkel cells and melanocytes. *Keratinocytes*, 80% of the total population of the epidermis, are by far the most abundant and are responsible for the stratified structure of the epidermis[8]. *Melanocytes* are found in the *stratum basale*, but their dendrites reach keratinocytes in the stratum spinosum. Their primary function is to produce melanin, a pigment that absorbs UV radiation, protecting keratinocytes from its harmful effects[5]. *Langerhans cells*, scattered in the stratum spinosum, constitutes the first line of immunologic defence in the skin. They attack and engulf foreign materials and

serve as antigen-presenting cells[5]. Finally, *Merkell cells* are found directly above the basement membrane in the *stratum basale*, and specialize in the perception of light touch[9].

The epidermis is substructured in strata that contain keratinocytes in different stages of differentiation, becoming corneocytes in a process called keratinization. As soon as keratinocytes differentiate from the cells in the *stratum basale,* the process begins. Cell-to-cell connection with desmosomes provides the epidermis with a tension-resistant structure capable of supporting shear forces. As keratinocytes ascend through the stratum granulosum, degradation of their nuclei and organelles are enzymatically promoted and keratin is clustered [5]. In the *stratum corneum*, cells become flattened and die. Nonetheless, cells are so tightly bonded to each other in this layer that water evaporation is prevented and the skin is kept hydrated[10]. In addition, the disulphide bonds created in keratin increase the mechanical resistance to this layer. However, cells are expulsed of the skin due to the loss of the intercellular desmosomal connections[5].

No matter what its thickness, which ranges from 50µm in the eyelids to 1.5 mm on the palms and soles, the epidermis lacks a direct blood supply [11]. In the underlying dermis, it depends on diffusion for the supply of nutrients and elimination of residual waste through the epidermal basement membrane[11], which is a semipermeable thin tissue formed by ECM that separates the epidermis from the dermal connective tissue[12]. Components of the basement membrane include collagen type IV, laminin, nidogen and perlecan [13].

The dermis is the fibrous and elastic tissue located beneath the epidermis. Its thickness can range from 300µm on the eyelid and penis to about 6mm on the soles and palms, constituting a thicker layer than the epidermis [9,14]. ECM, or connective tissue, produced by fibroblasts are the main components of the dermis[8].

Different structures can be found within the dermis: from blood vessels and nerves, which provide nutritional support and sensation respectively, to lymph vessels, small quantities of striated muscle, and several appendages like sweat and sebaceous glands, hair follicles[5]. In addition, different cells from the immune system are located in in or travel through the dermis, such as dendritic cells and leukocytes[15,16].

The connective tissue which composes the dermis is made of collagen (70%), providing strength and toughness; elastin fibers (less than 1%) maintaining normal elastic and flexible features; and proteoglycans that provide hydration and viscosity. The connective tissue undergoes constant remodelling by calcium and zinc-dependent proteolytic enzymes identified as matrix metalloproteinases (MMPs) produced by multiple cell types such as fibroblasts, keratinocytes, neutrophils and mast cells[17]. These enzymes participate in multiple processes in the skin[18]. Depending on their target and domain organization , they are classified into different groups[8] (**table 1**).

The dermis is composed of two layers:

- The thin, superficial papillary dermis (flowing connective tissue including capillaries, elastic fibers, reticular fibers, and non-organized collagen fibers, basically collagen III).

- The thicker, deeper reticular dermis (compact connective tissue including larger blood vessels, cross-linked elastic fibers, and well-organized fiber bundles of collagen III and I that run parallel to the skin surface[8]).

Fibroblasts are the most abundant cell type in the dermis and they maintain the different components of the ECM, including collagen, elastin and proteoglycans[19]. In addition, they secrete various GFs including the transforming growth factor beta (TGF-β), the cytokines such as tumour necrosis factor-alpha (TNF-α), and the MMPs, which directly affect keratinocyte proliferation and differentiation, and ECM formation. Overall, fibroblasts play a crucial role in the remodelling of the tissue and wound healing processes and participate as well in the pathogenesis of connective tissue disorders[19].

The hypodermis or subcutaneous tissue lies between the dermis and muscle, and its thickness varies from person to person[5]. It provides insulation from the cold, serves as an energy reservoir, protects deep tissues from trauma, and even acts as an endocrine organ by participating in the synthesis of oestrone and leptin[5,8]. The main component of the hypodermis are adipocytes structured into lobules separated by a the septa, which is a fibrous connective tissue that contains nerve connetions, lymphatic vessels and a rich microvascular network that provides oxygenation and nutrient exchange[5].

The skin appendages of the skin include hair follicles, nails, sebaceous glands, sweat glands, mammary glands, and ceruminous glands[10]. Hair follicles are generated by basal cells in the basement membrane and, apart from palms and soles, they can be found all around the body. They contribute to maintaining body temperature and perceiving touch sensation[10]. Nails are also composed of keratinized, flattened, dead cells[8]. Sebaceous glands, located at the base of the hair follicle, secrete an oily substance known as sebum, which lubricates and waterproofs the skin and hair[9]. Sweat glands secrete sweat to the surface of the skin[5], and mammary and ceruminous glands are modified sweat glands that produce milk and cerumen respectively[10].

2.2. The healing process

The skin is the body's first defensive wall against agressions from the external environment. When this barrier is disrupted, whether through injury or disease, survival chances decrease. To re-establish the skin's anatomic continuity and functions, it has the ability to heal through dynamic and highly regulated processes that begin directly after wounding. Healing can occur through two different mechanisms: regeneration or repair[2]. While regeneration achieves a full healing of the original tissue, repair derives in the development of a scar[2]. Scars are of poorer quality than the original tissue, since they are weaker and lack skin appendages or specialized cells like melanocytes, but enough to act as barrier. Unfortunately, human skin regeneration only happens in early gestation foetuses and mucosal surfaces [20]. In adults, the main mechanism by which skin heals is repair [2]. Many studies have tried to deal with the comparison between foetal scar-free and adult scarring mechanisms with a low success and few clinical outcomes to be translated to adult skin tissue repair. The reason seems to rely on the inherent differences between ECMs and signalling pathways[4].

Normal wound healing in adults involves the interplay of multiple cell types, GFs, cytokines, and a balanced pool of metal ions such as calcium, zinc and magnesium[20,21]. The healing process, summarized in **figure 1 and in figure 2,** progresses through four phases that overlap in time and space: haemostasis, inflammation, proliferation, and remodelling with the participation of many different types of cells [20,22].

Straight after injury, bleeding occurs, flushing out antigens and/or bacteria from the injury[23] (**figure 1 and figure 2**). The phase of haemostasis, which takes place during the first hours after the injury[20], is the process by which the bleeding stops due to platelet aggregation and plug formation, resulting in the formation of a temporal fibrin matrix. This provisional scaffold allows the migration of cells to the injured site and acts as a reservoir of GFs[20]. In addition, the factors released by the platelets trigger a vasoconstriction process and act as chemotactic agents for the recruitment of leukocytes in the wound, thus initiating the inflammatory response[24]. Notice the myriad amount of signals released by cells and how they interplay to advance the healing process (**figure 2**).

Inflammation begins minutes after injury, when neutrophils reach the wound site and clean the wound of foreign debris and bacteria, releasing chemotactic agents that amplify the immune response such as interleukin 1-beta (IL-1), interleukin 6 (IL-6) and tumour necrosis factor-α (TNF-α), Monocytes are then recruited to the wound site and mature into macrophages, which continue the cleaning process and release essential factors to progress the healing process, such as insulin-like growth factor 1 (IGF-1) and transforming growth factors α and β (TGF-α and TGF-β).[24]. Macrophages, by releasing these signaling factors, perform a very important role in the gradual pathway from the inflammation to the proliferation phase [20].

The proliferation phase starts 24-48 hours after injury when the first fibroblasts arrive at the site, chemoattracted by the factors delivered by macrophages and in the fibrin clot[25]. Once in the clot, fibroblasts proliferate and produce MMPs and ECM components including collagen type III, hyaluronic acid (HA) and fibronectin, transforming the clot into a new matrix of connective tissue[26]. During this stage other important events such as re-epithelialization, neovascularization, and formation of granulation tissue, take place [25].

Re-epithelialization is the process by which the new epidermis is created. Due to loss of contact inhibition and the GFs released in the wound bed, keratinocytes and fibroblasts are stimulated to migrate and proliferate from the wound periphery into the newly formed ECM[24].Stem cells from the hair follicle bulge also participate in the epidermal restoration by differentiating into epidermal progenitor cells[27]. Thanks to this process, the basement membrane and the different epidermal layers are re-established, but the hair follicles and other appendages of the skin usually do not regenerate[20].

Neovascularization involves the growth of new capillaries through angiogenesis in the injured site and, despite having a principal role in the proliferation stage, it is actually initiated immediately after tissue injury[28]. Angiogenesis is the growth of new vasculature from already existing blood vessels that can provide enough nutrients, oxygen and immune cells, and remove residues from the wound bed, which is indispensable for successful recovery[29] and maintenance of the tissue[20].

About 4 days after injury, the connective tissue produced by fibroblasts progresses towards the formation of a rudimentary tissue called granulation tissue. A vascular network, fibroblasts, inflammatory cells, and lymphatic vessels[20,23] invade this tissue, made of loosely organized collagen bundles and other ECM components. The numerous blood vessels present in this tissue provides it with its characteristic red or deep pink colour, which indicates the proper progression of healing.

The final phase of the healing process implies the contraction and remodeling of granulation tissue and restructuratio in a mature scar [20]. This process begins about 2-3 weeks after injury and can last for more

than two years [2,30]. Fibroblasts differentiate into myofibroblasts, which, thanks to their high contractile capacity, cause wound contraction. Myofibroblasts contain high amounts of alpha-smooth muscle actin (α-SMA)[20]. Mechanical tensions experienced by fibroblasts in the granulation tissue, together with factors such as the platelet-derived growth factor (PDGF) and TGF-α, are known to trigger fibroblast differentiation into myofibroblasts [31]. Simultaneously with wound contraction, tissue remodelling takes place: MMPs, tissue inhibitors of metalloproteinases and fibroblasts allow the replacement of collagen type III to collagen type I, which has a much higher tensile strength, producing a more cross-linked matrix[20]. During this phase, there is also regression of the vascular network and cell population, returning to levels close to uninjured skin[24]. Although skin continuity is restored, the scar that this process generates has 30% less mechanical strength than healthy tissue[20].

2.3. Types of skin wounds

Wounds can be classified according to multiple parameters. Nevertheless, the most important factors for their evaluation are the aetiology, or causing agent, of the wound, the depth of the injury, and the duration of the healing process[32]. The way in which skin injury occurs totally determines how the wound will heal[32]. Following this criteria, wounds are classified as incised, shearing, crushing, burns and contaminated[32].

Depending on the number of skin layers affected by the injury, wounds are referred to as superficial wounds, partial thickness wounds and full-thickness wounds. If only the epidermis is affected, the wound is termed superficial[23]. Lesions that reach both the epidermis and the deeper dermal layers that affect blood vessels, sweat glands and hair follicles are considered partial thickness wounds[23]. Finally, when the injury also reaches the hypodermis or deeper tissues, then they are considered full-thickness wounds[23].

Alternatively, the wounds may be acute or chronic if the classification criteria focuses on the duration of the healing process. When wounds are able to progress through the different stages of the healing process they are considered acute. On the other hand, if healing is not achieved within 3 months of injury, they are classified as chronic[23,32]. Many different underlying causes can trigger the chronicity of wounds. The Wound Healing Society categorizes chronic wounds into 4 groups: arterial insufficiency ulcers, diabetic foot ulcers (DFU), pressure ulcers and venous ulcers[33]. Despite their different causative aetiologies, these wounds present some common traits that impair their healing, such as a prolonged inflammatory phase, increased levels of MMPs and poorly vascularized tissue. All this prevents the wound from forming granulation tissue and achieving re-epithelialization[30,34,35]. DFU, for example, are always accompanied by a prolonged hypoxia that can induce insufficient perfusion and angiogenesis[36].

3. Therapies based on the microenvironment modification and stimulation

3.1. Skin wound microenvironment

The microenvironment of a wound in the skin can be assigned to the external environment immediately neighboring to the surface of a wound and the internal compartment below the surface of the wound but adjacent to it. Treatment methods such as pH, temperature, modification of partial pressure of carbon dioxide (pCO_2) or oxygen (pO_2), microbe or hydration can directly alter the characteristics of the external microenvironment of the wound and indirectly affect the internal microenvironment of the wound (cells and ECM) (**Figure 3**). If healing mechanisms are well known, the new therapies can be focused to induce

microenvironments that promote wound healing.

Regarding the pH, Keratinocyte´s secretions naturally maintain the healthy skin's pH normally between 4 and 6 [37], and consequently, the environment becomes more acidic during the different steps of an acute wound healing. Researchers infer that the acidic medium aids to minimize the risk of infection and promotes the production of granulation tissue through the stimulation of the migration and proliferation of fibroblasts and keratinocytes [38,39]. On the other hand, chronic wounds are associated with a more alkaline environment[40], which promotes the invasion of bacteria and formation of biofilms compromising the healing of the wound [40].

Temperature is a key aspect in wound healing and depends both on the external ambient temperature and the blood flow. The blood flow is regulated by vasoactive molecules modulating the size of the vessels depending on the need. Vasodilation increases temperature in an acute wound to augment the amount of nutrients and oxygen arriving to the injury. Most chronic wounds usually occur in lower extremities, but not limited to, where the disordered blood supply and oxygenation leads to a temperature decreases in 5°C [41]. Heat-radiation-assisted dressings allows to increase the capillary perfusion and the oxygen pressure [42,43].

O_2 is crucial for the metabolism of the cell. The sub-epidermal microvasculature allows the release of O_2 to the epidermis [44]. In undamaged skin, the pO_2 decreases from 5% in deep layers to 1% in outer ones [45], and pO_2 drops dramatically when the capillaries are damaged and disrupted. A pO_2 of 18% is the optimal level for human keratinocytes to grow *in vitro,* which are extremely affected by pO_2 changes [46]. A migration test demonstrated that young keratinocytes migrate faster when the pO_2 is lower than 18%, while older ones migrate faster when it is higher, suggesting that the ideal oxygen pressure depends on the age of the patient. Furthermore, cells can tolerate and survive during anaerobic conditions and metabolism, increasing the concentration of lactate, acidificating the environment and activating hypoxia-inducible factors (HIF), such as nitric oxide or angiogenic GFs /vascular endothelial growth factor – VEGF), which up-regulate vasodilatory factors[47]. Hypoxia stimulates some healing pathways. Therefore, many therapies base their strategy on increasing the pO_2 in the wound bed. Examples are the continuous diffusion of oxygen therapy (CDO), topical oxygen therapy (TOT) and the hyperbaric oxygen therapy (HBOT)[48], which is considered to enhance ulcers healing only in the short term[49].

pCO_2 regulates wound healing cell signalling independently of either O_2 or pH. After an injury, healing increases pCO_2 from about 65 to 75 mmHg during the second week [50]. It means that the CO_2 is not properly eliminated due to the limited vasculature and the CO_2 is accumulated because of the associated increase of O_2 consumption. Independently of pH, innate immunity-related gens are inhibited by a high pCO_2 [51]. As well, proliferation of endothelial cells increased when were exposed to low pO_2 and a high pCO_2 [52].

The skin is colonized by a myriad of microbes (the external "microbiome"), which in most cases are commensal and cause little harm to the host. Some of them collaborate to obtain mutualism effects[53,54], but others can induce undesired infections. The microenvironment and the body site highly influence the type of bacteria found. Sebaceous areas, for example, have a lower density of bacteria than dry and wet areas. Their main function is to collaborate with the immune system, and they are responsible for the production of most of our skin chemistry[55]. Furthermore, fungi and viruses inhabit the human skin ecosystem [54].

Regarding the hydration, skin blocks the evaporation of fluids due to its inherent low permeability. The loss of water is normally described as the water vapour transmission rate (WVTR). In undamaged skin the WVTR is generally about 8.5 $g \cdot m^{-2} \cdot h^{-1}$ [56], but after a traumatic damage, the breakage of the skin barrier results into a sudden increase in the WVTR, with a release of fluids and electrolytes. for example, burns and ulcers can reach levels of WVTRs tenfold higher than in healthy skin [56]. Phenomenons of water evaporation consume calories and make the wound cooler [57]. Dressings and wound chambers can decrease fluid loss and modulate the humidity of the external wound microenvironment. The speed of the healing and the quality of the final tissue is improved by a wet or moist microenvironment [58].

The internal wound microenvironment includes skin cells and endogenous stem cells, as well as the ECM, which is a dynamic source of signals, guide and support for cellular activity[59]. Fibroblasts are the most abundant cell type in the dermis and are crucial supporting the normal healing process. they participate in important processes such as breaking down the clot made of fibrin, forming the granulation tissue, regulate angiogenesis, assisting re-epithelization and creating the new ECM and collagenic structures to support the cells involved in the wound healing and contraction[25,60,61].

Endogenous stem cells play an important role in the well-coordinated cell-signaling cascades [62]. There are stem cells niches in the three skin layers, but cells are mainly in the epidermis. The main types are the the sebaceous gland, the basal layer of interfollicular epithelium and thehair follicle bulge. These cells are responsible of the maintenance and regulation of the epithelial stratification, hair follicle growth and wound healing process throughout life [63]. They have common traits as expression of Keratin 5 and 14, intermediate filament proteins that have been recently demonstrated to actively participate in the maintenance of cell proliferation potential in the basal layer of stratified epithelia with which they are intimately associated [64]. During differentiation, they migrate and leave their niche. This migration is guided by the basal lamina components, such as laminin and integrins involved in cell features as polarity, adhesion, survival, proliferation and migration [65]. Some clinical research has demonstrated that the topical application of stem cells have improved chronic wound healing. However, the stem cell engraftment had a low efficiency in the long term (only 2,5% after 4 weeks) but proangiogenic factors were detected in the wounds treated with MSC from bone marrow [66]. These findings are reinforced with the results obtained in preclinical studies where production of paracrine factors is the major mechanism by which stem cells improve the repair [67,68].

These paracrine factors, the cytokines involved in the skin morphogenetic signalling, are the skin morphogenetic signalling and they are fundamental to understand the healing process. Cytokines mediate all these events and regulate cell activity from the injury to the final healed tissue. They regulate cell migration, proliferation, ECM synthesis and remodelling. The possibility of their controlled administration to promote healing and accelerate the repair of acute and chronic ulcers has attracted many biologists and clinicians. From the biomaterials side, the possibility of linking these signals to the materials is an interesting approach to develop new devices and constructs to promote a friendly environment to repair the wound. **Table 2** presents a list of the principal GFs and cytokines involved in the healing process, the cells that produce them and their principal functions (PDGF, TGF-beta and VEGF being the most implicated in the different wound healing steps). These factors showed to promote the recruiting of cells like platelets and neutrophils, stimulate fibroblast proliferation and ECM production, and promote neovascularization.

3.2. Current therapies and principal issues

Among the myriad strategies that are applied in the therapies of chronic wounds, from negative pressure therapy to compression therapy, this section will focus mainly on bioactive dressings, tissue engineered substitutes and other topically applied products.

Dressings are materials placed in direct contact with the damaged skin and are always used to help in the recovery of chronic wounds. Considering the high impact of this type of injury in developed societies, the wide variety of products available for their treatment is not surprising[97]. The global wound dressing market, valued at US$4.82 billion in 2016[98], represents an important part of the pharmaceutical and medical market. Dressings have evolved from simple materials that cover the wound to advanced care systems that keep the wound protected from the environment while providing optimal moisture for the healing process[23].

Advanced products have appeared in the last 20 years to address the lack of healing capabilities of regular dressings to treat chronic ulcers and burns. Living skin equivalents and products with bioactive properties have become available. These are generally able to actively stimulate the healing process through administration of GFs or cells to the injury[23].

GFs mainly stimulate fibroblasts and keratinocytes via transmembrane glycoproteins – e.g. the recombinant human platelet-derived growth factor (rhPDGF). Steed *et al*. studied the effect on diabetic foot ulcers (DFUs) of rhPDGF in series of 118 patients [99]. Patients receiving rhPDGF for up to 20 weeks showed a significant better wound closure. Consequently, FDA approved therapies including rhPDGF for diabetic ulcers, which as becaplermin (the trade name is Regranex®). However, this treatment has some concerns, as patients that have or have had cancer cannot use it, and for all patients no more than three tubes should be applied[100]. Other products that include GFs are Fiblast® which includes recombinant human bFGF for decubitus, skin ulcers and burns[101]. Recombinant human EGF can also be found commercially in products like Heberprot-P®, Regen-D™, and Easyef®. A summary of the principal commercially available advanced products can be found in **table 3.**

3.3. Current trends in tissue engineering in skin wound healing

Natural or artificial living skin equivalents are composed of a scaffold and cells, mainly keratinocytes and/or allogenic fibroblasts. The design of architectures that provide cellular and structural components have resulted in the apparition of several products in the market [132]. These living skin equivalents can be classified as dermal or epidermal composites.

Dermal composites consist of scaffolds and viable human skin fibroblasts. In 1998, the FDA approved the first skin equivalent: Apligraf® (Organogenesis Inc.) [133]. In 2001 the FDA approved Dermagraft® *(*Organogenesis Inc.) [134] to be applied in non-healing DFUs, in which the fibroblasts are cultured onto a bioresorbable polyglactin (conventional suture material) mesh scaffold instead of type I collagen such as Apligraf®. In DFUs, it has demonstrated increase the rate of wound closure[135].

Epidermal-dermal composites are bioengineered substitutes formed of dermal cells (fibroblasts and/or stem cells), human epidermal cells (keratinocytes) and components of the extracellular matrix. Epidermal

products consist of cultured autologous keratinocytes delivered either in liquid suspension, cell sheets, or through biomaterials as cell carriers. The isolation of keratinocytes from a donor and their expansion to obtain a minimum number of cells for therapeutic purposes is critical for epidermal substitutes. The different strategies used for epidermal substitutes depend on the cell culture technique, the cell differentiation process and epithelial organization, cell transfer to the patient, and the potential use of scaffolds or substrates to offer a better cell culture and delivery, which can be synthetic or natural[136–138].

Acellular Derived Scaffolds are either animal- or human-derived, or synthetic. In the former, cells are removed during the manufacturing process [139]. Animal- or human-derived scaffolds are commonly used because of their intrinsic properties, biocompatibility and bioactivity. Moreover, they mimic the structural, biochemical and biomechanical functions of the ECM. The bioactive polymers most commonly used in the design of acellular matrices are collagen and polysaccharides like chitosan, alginate or hyaluronic acid.

4. Biomaterials for signalling release platforms: *In situ* tissue engineering

The use of natural and artificial 3D architectures as scaffold for as a support, with or without cells, is one of the most used strategies in tissue engineering [140,141]. Although this strategy has been validated and has provided relevant advances in the field, cell-based therapies have a limited pathway due to the availability of tissue from the donor. The adverse immunologic response from allogenic and xenogeneic cell sources are a risk added to the potential fungal, bacterial, prion or viral contamination from the donor to the host [142]. A valid alternative is the use of progenitor or stem cells from the own host, even it needs *ex vivo* procedures as well [143]. Nowadays, the advances in the different strategies in regenerative medicine, but particularly in tissue engineering, have introduce the possibility of the *in situ* recruiting host cell/progenitor cells or tissue-specific progenitor cells at the wound site. This strategy eliminates ex vivo cell manipulation and, therefore, provides a more efficient alternative. It success depends on the efficiency of the introduced signalling to recruit cells into the implanted biomaterial scaffold and to induce the proper phenotypic modification to these cells into specific cell lineages to obtain a functional regenerated tissue (**figure 3**). The target-specific scaffold should act as a template and be designed to "instruct" the fate of the recruited cells to proliferate and differentiate into the desired tissue cells [144]. The guiding of these host cells to create a well-integrated functional new structure can be performed by the sustained release of bioactive molecules from the implanted device [145]. In addition, a proper combination of bioactive molecules and the scaffold would offer a suitable microenvironment that would allow an efficient cellular specification inside the scaffold.

Biomaterial scaffolds are crucial for *in situ* tissue regeneration and should include exceptional features that can tune cell behaviours and then promote the growth of new tissue. General requirements for scaffolding systems, independent of the targeted tissue biodegradability and temporal structural support keeping a reasonable biological stability. In order to produce a biofunctional niche of host stem cells[146], the internal morphology must guarantee a permeability that allows a functional vascularization after implantation; a macroporous architecture to promote the colonization of the different cells recruited; and the establishment of different biochemical and biophysical signals to promote cell migration, proliferation and differentiation. Furthermore, the scaffolds should be able to deal with inflammation, minimizing the formation of fibrotic tissue; and the differentiation of the recruited cells should be controlled by introducing tissue-specific signals within the scaffold.

Cells from the host will be recruited by the scaffolds, by means of stimuli that activates stem cell niches and mobilize these cells through the circulation towards the wound.

There are many strategies to introduce efficient signalling to enhance or speed up the skin healing process through the *in situ* tissue engineering approach. Many of them involve the use of biomaterials, mainly polymers. In this section, several approaches combining polymeric materials and signals are reviewed.

4.1. Mechanical and ultrasonic signalling

Passive and active mechanical stimulation of the skin can introduce different signalling in the microenvironment and modify the tissue behaviour, triggering different biochemical pathways. In fact, when a tissue is actively deformed, the spatial conformation of ECM components changes, as does cell interaction with their neighbours and the ECM. Some of the signalling can be revealed, and cell communication affected. Chemotactic peptides such as the monocyte chemoattractant protein-1 (MCP-1/CCL2), normally linked to glycosaminoglycans (GAG) from the ECM, can be easily released to the media and detected by endothelial cells or leukocytes[147], speeding up inflammation and tissue healing. Regarding passive stimulation, it was suggested that the controlled stimuli of the mechanical stiffness of a scaffold could promote the expression and synthesis of the VEGF receptor 2 (VEGFR2)[148].

Special attention should be paid to negative pressure wound therapy (NPWT), a practise that has attracted many dermatologists during the last years [149–152]. Apart from eliminating exudation by evaporation, it deforms the ECM, stimulating several events involved in angiogenesis, inflammation and cell proliferation[149,153–158]. However, NPWT mechanisms and results are still unclear and several meta-analysis did not find enough evidences to confirm the associated enhance in the healing[158]. Thus, it there is no strong evidence for the efficiency of NPWT, apart from some very specific uses in healing post-operative foot ulcers and wounds in the foot in patients with diabetes mellitus compared with humid wound dressings[159–164].

Fibroblasts are the most important cells in the process of ECM remodelling in skin, control of wound contraction, and scar formation. During healing, skin is subject to various mechanical and other-stimuli such as compression, tension, torsion, shear stress, etc. These stimuli favour the expression of α-SMA involved in the cytoskeleton of fibroblasts[165] as one of the agents leading to the contraction of the wound.

Fibroblasts can be subject to low and high mechanical stress, and they adapt by modifying their phenotype[166,167]:

- Fibroblasts exposed to low intensity forces have a dendritic morphology (quiescent), sometimes leading to apoptosis, and do not produce ECM components; nor do they promote cell migration. There is a decrease of the extracellular signal-regulated kinases (ERKs) signalling and less expression of GF receptors.
- Fibroblasts suffering high intense mechanical forces tend to show a lamellar morphology, actively produce collagen and other ECM components (eg. fibrillary fibronectin organization), the cytoskeleton is reorganized and the focal adhesions, cell migration and proliferation are promoted.

Other effects of mechanical signalling in skin tissue regeneration can be found in **table 4.**

5.2. Physical signaling

Surface nanotopography is an important contributor to cell signalling. The introduction of different nanoscale features (e.g. ridges, grooves, steps or cliffs) facilitates cell attachment generally[178]. The minimization of the healing scar maintaining an acceptable high wound closure rate has been one of the main aims to focus the research in the introduction of bioactive nanotopography in the scaffold in wound healing[179]. For example, migration to enhance the closure of the wound was enhanced by the design of different polystyrene nanogroove patterns that allowed to correlate with adhesion as well [180]. Fibroblasts migration during wound healing was also affected by the orientation and density of nanogrooves (**figure 4c**) [179]. To determine the cell migration speed, researchers fabricated and contrasted different patterns of few micrometers and a few hundred nanometers in size, as well. They observed that the geometry of the nanotopography could affect the speed of the cell migration, their division, the production of ECM and the rate of proliferation [179]. Kim et al. used and modified a polyurethane acrylate polymer by UV-assisted capillary force lithography to produce nanodesigned grooved matrices coated with gelatine for skin regeneration purposes. They observed that the migration, in contrast to proliferation, of the tested hMSCs was significantly higher on nanogrooved matrices than the flat control at certain groove distances[181] (**figure 4a**).

As well, nanotechnology offers excellent tools for the introduction of nanotopographic signalling in biomaterials and to properly mimic the ECM and the cellular microenvironment[182]. Modifying the surface and 3D structure at the nanoscale is feasible, and nanomaterials can be combined within a larger guiding template or scaffold. Modulation of scaffolds can be approached through top-down or bottom-up strategies[183,184]. 3D self-assembled hydrogels offer one of the best ways to nanostructure an ECM having the closest morphologies to a bioinspired ECM. Self-assembled monolayers (SAMs), amphiphilic block copolymers amphipathic peptides, layer-by-layer (LbL) assembly or DNA templating are some examples found in the literature[185–189] (see **figure 4d**).

Electrospinning is a feasible way to introduce active nanotopography in a 2.5D manner for skin regeneration, and is maybe the cheapest and most efficient technique to produce ECM-like scaffolds made of submicronic and nanometric polymeric fibers (**figure 4e, 4f and 4e**) [190–195]. Part of the skin tissue is generally characterized and represented by a mesh of random collagen fibers that match with non-woven electrospun fibrous mats[178]. Actually, collagen fibers crosslinked using the glutaraldehyde method have been one of the better approaches in mimicking skin[196]. However, there are still some doubts: firstly, whether collagen maintains its nature in the presence of some halogen organic solvents[197]; secondly, the toxicity and efficiency of current crosslinking methods for amino acid-based polymeric fibers produced by electrospinning[198]; and thirdly, the size of the pores does not generally allow a cell to migrate unless there is a further remodeling of the architecture. Combined electrospun nanofibrous scaffolds of PLGA/silk fibroin had a relevant participation in the healing of diabetic wounds by the promotion of the fibroblast adhesion and proliferation [199]. Same method was used by Patel et al., but in this case they linked ECM proteins and GFs on the surface of aligned poly(L-lactide) (PLLA) nanofibers, with the purpose of simulating a real ECM physically and biochemically. Nanofibers were able to induce the neurite outgrowth. It is a relevant result considering that nerve regeneration in the skin is nowadays a big challenge in skin tissue healing. Aligned fibers were able to enhance the cell migration compared to randomly oriented ones and the fixed biochemical factors on the surface aided to promote the neurite outgrowth as well, but they were less efficient in terms of cell migration[200]. Dendrimer structures (nanosized highly branched polymers) have also been engineered in form of nanofibers providing an improvement in the inflammatory properties in wound healing[201]. Nanofibers of gelatine-dendrimer were treated with polyethylene glycol

(PEG) to produce effcient semi-interpenetrating networks (sIPNs), also including silver as antibacterial agent[202].

Dimensionality is one of the main concerns nowadays in skin wound healing. Current *in vitro* analyses are mainly carried out in 2D cultures of homogeneous populations of cell monolayers, by which researchers obtain the majority of their knowledge related to cell behaviour[203]. This has led to many imperfections and artefacts, especially when growing fibroblasts over rigid surfaces, which induced differences in terms of adhesion, migration and cytoskeletal evolution, proliferation, maturation and cell signalling compared to a more biomimetic 3D environment[204–209]. These aspects are not ideal for epithelial cells, and a 3D scenario that allows the polarization, differentiation and formation of duct-like structures is required [210,211]. As a result, the big differences between *in vitro* and *in vivo* led scientists to be more focused on *in vivo* analyses.
Among the many processes available for creating 3D environments, the most successful ones are summarized in the following section. Special mention goes to 3D bioprinting [212–215] and decellularized scaffolds[216–218], which offer promising potential in terms of signalling modulation and biomimetism.
In terms of macrodimensionality, 3D additive manufacturing techniques represent some of the most promising and flexible methods to process artificial skin tissue for wounds because of their versatility, reproducibility and low-cost [219,220]. Bioprinting, stereolithography, photolithography, and direct polymer extrusion are the most common techniques used in acellular 3D printing [221]. 3D bioprinting, in fact, allows the direct writing, mechanical stability and custom positioning of cell-laden hydrogel strands of several microns. Different layers and levels can be written once optimized the proper bioink. Decellularized tissue-based scaffolds allow the direct use of skin tissue, maintaining native organization and chemical complexity, as a skin substitute for further regeneration. Printing skin tissue involves natural or artificial hydrogels, sometimes combined with degradable polyesters as support[222]. Other feasible alternatives, such as multi-layered constructs – co-cultures of fibroblasts and keratinocytes embedded in collagen hydrogel (**figure 4b**) [223,224] – were also used as skin tissue grafts. 3D bioprinted skin tissue was one of the first proofs of concept for skin regeneration, even printing skin directly over a wound [222]. Another strategy involving fibroblasts and keratinocytes was developed by Cubo et al. where they successfully deposited the cells embedded in a scaffold made by a combination of plasma, fibrinogen and $CaCl_2$ [212]. Similar success was achieved when alternated layers of fibrin/collagen and thrombin were combined with human MSCs and human amniotic fluid-derived stem cells, and directly printed over mouse wound sites[225]. This study is interesting, as they quantified concentrations of the different GFs involved in the healing process, which correlated with fast, efficient wound coverage and closure. In terms of 3D printing with *ex-situ* cell incorporation and culture, Killat et al. developed skin substitutes for burns or reconstructive surgery by culturing both murine embryonic fibroblasts and epidermal keratinocytes onto previously printed 3D bovine collagen-elastin matrix Matriderm®[226], with good tissue functionality. Results showed a complete re-epithelialisation over a distance of 1.28 mm, and angiogenesis was efficient.

Melting electrospinning is an additive manufacturing technique that offers an alternative in terms of microdeposition, as it can position fibers over a micron in size to create controlled textured scaffolds, but only for low melting-temperature polymers such as polycaprolactone (**figure 4g**) [227,228]. Altogether, these options are leading to a change of scenario towards versatile conformations and possibilities such as core-shell fibers, emulsion or positioned fibers with modulated macroporosity and mechanical resistance[229].
Finally, electrical stimulation can accelerate the healing in skin tissue, as cell migration is essential for wound healing. Re-epithelialization failure can be the result of insufficient migration and proliferation in

the skinand is featured in chronic wounds in old people, decubitus and venous stasis ulcers [230]. Electric fields have recently been proposed as a feasible way to speed wound healing by the increasing proliferation, guiding[231] and angiogenesis[232]. Electric fields seem to stimulate the increase of the expression of several signals and receptors such as VEGF, EG receptors, integrins, V-ATPase H^+ pump, and PI3 kinase/Pten (Phosphoinositide 3-kinases/phosphatase and tensin homolog)[230,233–238].

5.3. **Chemical and biochemical signalling**

The introduction of bioactive factors in the search of more effective recovery has been widely studied[76,194,229,239–243], as bioactive factors have significant biological activity *in vivo* in wound repair and regeneration. This section will focus on the use of GFs and nucleic acids, but for the sake of simplicity, antibiotics and bactericides will not be covered[244].

Developments in tissue regeneration have mainly relied on the application of cells and GFs in combination with biomaterials. Using GFs and cells prevents the broad application of these therapies due to complications in scalability, lack of standardization, side effects, high cost, storage and regulatory issues[101,245,246]. Moreover, the effectiveness and safety of the delivery of GFs has not been fully demonstrated because their sensitivity and instability. GFs half-lives in serum are in the range of minutes[247]. Therefore, their control of the release rate and profile and avoid the initial release burst is cruzial for the proper healing of the wound[248]. Furthermore, natural barriers and elimination mechanisms, such as the *stratum corneum* or exudation, should be also considered, as these can make it difficult to reach the minimum therapeutic contact time needed between GFs and cells in the wound bed. This necessitates high dosage and numerous applications during the therapy[249]. Apart from the associated high costs, this solution can induce serious side effects, including tumorigenesis[250]. Therefore, there is a need to circumvent such drawbacks by adopting new strategies and approaches for tissue repair. The fact that cells naturally produce and regulate these GFs, cytokines and other signals should be taken into account.

Various combinations of GFs and dermal substitutes have been considered as a possible pathway for the wide application of factors in an effective form. So far, many efforts have been invested to the introduction of different stimuli for skin regeneration, for example, the delivery of bFGF, VEGF, and PDGF combined with scaffolds[248,251–253]. Martino et al. showed that low dosis administration for these GFs was efficient and the cost and the side effects minimized.[254]

Different strategies have been recently used to improve the function of the different produced architectures involving the incorporation of GFs [255–257]. A short summary of the most commonly used GFs in wound healing and tissue engineering is shown in **table 5**. For a more complete description of the application of GFs in wound healing, readers are directed to reference [101].

Complementary, gene therapy is an excellent alternative as cDNA is a DNA copy synthesized from the target mRNA that encodes a specific protein through reverse transcription. cDNAs encoding has been delivered using nonviral vectors to control several wound healing steps, including positive charged polymers and liposomes, and naked plasmids, for peptides (e.g., LL-37,[293] secretoneurin [294]) and GFs (e.g., VEGF,[295] keratinocyte growth factor (KGF) [296]).

One tested possibility is the combination of a biomimetic skin tissue equivalent with gene therapy to enhance the wound healing. The delivery of plasmid DNA which translates active proteins, by injection or in a loaded scaffold, can elude the problems of short half-life and the low stability of GF-associated approaches[297]. Many functionalized scaffolds through gene augmentation have been used for dermal regeneration to promote angiogenesis or inhibit scar formation[287,298]. Another option is the creation of gene delivery platforms and reservoirs for localized and sustained expression of GFs by the gene-activated matrices, which are constructs that incorporate DNA [299–301]. they can also be used to stimulate the functional regeneration of dermis nd full skin[302,303]. In addition, nanofibers impregnated with nucleic acids enhanced tissue regeneration and minimized scar formation in diabetic and healthy wounds[283]. Alternatively, RNAi therapy allows the silencing of some gene expression by precisely targeting overexpressed biomolecules or enzymes such as MMPs in the environment of a chronic wound [304]. In addition, miRNAs have been shown as important angiogenic regulators[305].

5.4. Inorganic signalling

Metal ions are crucial as body metabolism catalysts, as well as forming part of the structural elements of proteins, enzymes or transcription factors[306]. It is what is known as hybrid and ion release approaches. Calcium, magnesium, zinc and copper ions gradients are well known and contribute to the normal epidermal homeostasis. The distribution of other minority trace metals is not well defined, unfortunately[307,308]. Furthermore, the distribution of metal ions is specific to the observed area of the skin.

In the skin, extracellular calcium plays a vital role both in healthy skin maintenance and in the healing process. and has attracted the attention of several research groups. Calcium is the fifth most abundant atom and the most common mineral ion in the body[309], and participates in many relevant metabolic pathways. 99% of the calcium in the body is found in the bones, acting as the ion reservoir, but we also find calcium in the cells, blood, and body fluids. It is implicated in many physiological processes due to its capacity to alter electrostatic fields and the conformation of proteins[310].

Calcium is a very well known second messenger in signal transduction[309,311]. Many extracellular signals trigger intracellular reaction cascades that lead to the activation of different calcium channels from the plasma membrane, or from intracellular reservoirs inducing sudden spikes of calcium in the cytoplasm [311]. This is the origin of processes such as cardiac muscle contraction, nerve excitation, cell migration, apoptosis and proliferation [309]. A huge amount of the cell's energy is reserved for maintaining differences in calcium concentration between the outer free calcium ion (~2mM) and the inner one (~100nM)[310].

Calcium also acts as a primary signal, since extracellular calcium can bind to transmembrane receptors activating intracellular cascades that modulate cell behaviour[312]. Kobilka and Deulpi described how calcium can bind to G protein transmembrane receptors to initiate multiple signalling cascades, playing a relevant role in healthy skin and wound healing[313]. CaSR is the most studied G-protein coupled receptor that senses increases of extracellular calcium[314]. This receptor is expressed by many different cell lineages and is involved in myriad of functions[315]. In the skin, calcium is crucial for the differentiation of keratinocytes in the healthy epidermis, as a co-factor in the coagulation cascade, and is implicated in the healing process[307]. Release of calcium to the wound bed definitely improves healing[316,317]. In fact, calcium has been proposed as a pro-angiogenic factor, as Ca^{2+} can be released from some calcium phosphates, inducing higher expression of VEGF in rat mesenchymal stem cells (rMSCs) and endothelial rat progenitor cells (rEPCs) through the CaSR [148]. In other words, it enables the stimulation of the cell to manage a very important GF implicated in angiogenesis.

There are other metals involved in skin healing (**see Table 6**). Studies report that some, such as silver, have antibacterial properties; silver probably has no competitor to date. Others have shown an angiogenic effect, mainly in the modification of the hypoxia-inducible factor (HIF) pathway[318,319], in contrast with the calcium angiogenesis mechanism[148]. There is a third effect in which some metals are involved: reepithelization such as Zn^{2+} and Ga^{3+} [308,320]. Finally, a gas such as NO can multitask and be an important factor in the skin healing process.

A hybrid approach involving freeze drying was used to combine silicon based glasses with chitosan and silk fibroin for dermal reconstruction[342]. *In vivo* assays in Sprague-Dawley rats were performed and showed that this combination was potentially angiogenic-enhancing for skin regeneration.
Recently Yu et al., under the hypothesis that silicon-based bioactive glasses stimulate the angiogenesis[343] of endothelial cells and increase the production of GFs in fibroblasts such as VEGF and bFGF[344], established that the early stages of healing (from day 1 to 3) are when fibroblasts mainly migrate and proliferate. It is crucial to stimulate them to promote migration at this point. Differentiation of fibroblasts into myofibroblasts would also be an advantage, which would accelerate wound healing. Angiogenesis as a crucial factor aided by a proper surface would guide the growth of granulation tissue (two to five days after implantation) that acts as a template for further neodermis tissue. Bioglasses can also stimulate the production of bFGF and its receptor, VEGF and its receptor 2 (KDR) promoting angiogenesis in human umbilical vein endothelial cells (HUVECs)[345] and in diabetic rats[346] enhancing the skin wound healing.

5.5. Introducing signals in biomaterial surfaces

One of the main challenges in wound healing is the spatio-temporal delivery of the different signals that orchestrate the regenerative process. This complex process depends on the existence of specific signals, as well as their time-dependent and spatial distributions[246].

Nanotechnology offers many tools to introduce signals in the biomimetic scaffolds such as nanocapsules, nanoparticles, nanofibers or nanosheets. Many of them involve inorganic materials, but also polymeric or natural polymers like proteins and polysaccharides[347].

In the main, two strategies have been used to control and introduce these signals onto the surface of biomaterials, either by chemical immobilization/covalent linking of the signals into or onto the matrix, or by physical encapsulation in the delivery structure. In the first case, there is high energetic and strong affinity interactions between the signal and the surface of the substrate[348], while in the second case the interaction between the different signals and the matrix is achieved through entrapment or physical adsorption[349].

On the one hand, the covalent attachment of the signals to the carrier can provide a prolonged and more controlled delivery of the bioactive signal, as well as a slower rate of degradation and internalization. Several techniques have been developed to immobilize GFs on the substrate, where these signals will be available to interact with the cells, therefore controlling cellular fate [350,351]. Moreover, several tethering strategies have been developed to control the delivery of these biologically active molecules. For a more detailed description of the available techniques for signal immobilization, readers are directed to a review of Love et al. [352]. However, there are several limitations to the covalently binding of bioactive

signals. The principal limitation is the lack of control of the specificity of the coupling site of the signal, which can lead to a loss of bioactivity during the immobilization process.

On the other hand, adsorption of the signal involves charge–charge or other secondary interactions – such as Van der Waals or hydrogen-bonding – between the signals and matrices, or indirect interaction via intermediate proteins or other biological molecules [349]; while physical encapsulation of the signals comprises the formation of particulate systems, either at the micro- or nanoscale, in which the signals are trapped during the fabrication process [76,101,246].

6. Concluding remarks

The artificial regulation of the signals in skin regeneration and wound healing is still in its infancy. It requires further studies and a stronger knowledge of the mechanisms involved in healing. Biological environments are highly complex, dynamic and multifaceted, and cell–material crosstalk should be considered a priority[59]. In addition, we have to deal with various concomitants pathophysiological factors that cause normal wound healing to fail, such as abnormal inflammations, aged tissue, infections, malnutrition, diabetes, pressure ulcers and renal impairment. GF-related therapies are a powerful alternative, but with numerous and hidden drawbacks. Cell control and managing the signalling is a promising alternative that is becoming ever more interesting. This requires a full understanding of the cell as a self-signalling factory, and how external stimuli can act as an epigenetic factor modifying and activating the endogenous repair system of the body. Hence, ion release therapies, among other organic compounds not included in this review must be taken into consideration for wound healing therapies.

As Park and co-workers noticed, a better match should be aimed for spatial-temporal events in terms of cell activity and signalling release. This implies developing strategies to implement the interface crosstalk among cells and materials to be able to produce smart biomaterials that resemble the environment and which contain the required level of complexity to mimic the ECM of the natural tissue[353]. They should also respond properly to cell events at the different stages of healing. In terms of complexity, scaffolding systems are still very simple, and far from a smart biomaterial.

Skin appendages such as nerves, hair follicles, and pigmentation, and the hosting of commensal external microbes, are being considered by some research groups in their models and future research[354].

We would suggest that researchers pay more attention to skin personalized therapies. Many cohort studies try to be as general as possible, ignoring many issues and characteristics that can lead to a successful therapy in one type of patient but not in another. Further investigation is needed to assess the reasons for these differences, for which advanced *in vitro* models will have an important role in the future due to their versatility, safety and low cost compared to *in vivo* and clinical trials.

In summary, the future of skin wound healing therapies relies on the modulation of wound microenvironments to promote a healing promoting scenario led by the host cells. In this regard, new gene therapy approaches that are being developed nowadays could also be powerful options.

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Figures:

1. Scheme representing various cellular events occurring at the different phases of wound healing bed (Hemostasis, inflammatory, proliferative, maturation and remodeling phases). The figure also indicates the external and the internal skin microenvironments. Therapies based on the modification and stimulation of these microenvironments are nowadays being investigated to improve skin wound healing. The external microenvironment includes pH, pressure, oxygen, among others, and the internal microenvironment consists of cells and the ECM.

2. Succession of overlapping phases with the associated involved cells and GFs that describes the phases of skin repair [20,22].

3. General *in situ* tissue engineering pathway showing how biomaterial should release the proper signals to , first recruit, and then be colonized by endogenous cells in order to control the cell fate to promote and enhance the self-body mechanisms involved in repair. Notice that vascularization is crucial as it allows the proper arrival of oxygen, nutrients, cells and the elimination of cell residual products.

4. Pictures from examples of how engineered dimensionality can help to control cells behavior. a1,a2 and a3 are different cellular morphologies on various nanopatterned surfaces adapted from [181] with permission. b1, b2 and b3 are different views of multi-leveled culture of printed human skin fibroblasts and keratinocytes adapted from [223] with permission. c1-c4 are immunostaining analysis of focal adhesions at 1 day culture (green: vinculin), and c5-c8 are SEM cross-sections images of fibroblasts on various nanotopographies at 1 day culture (white dotted lines show the surface nanopatterns) adapted from [179] with permission. d1-d3 are transmission electron microscopy images of self-assembled nanotubes and nanovesicles dipeptide nanostructures adapted from [188] with permission. e1 and e2 are SEM images of electrospun poly(l-lactic acid)–co-poly(3-caprolactone) (PLLCL) and collagen/PLLCL nanofibers respectively adapted from [190] with permission. f1 and f2 are fluorescence microscopy images of human dermal fibroblasts seeded over collagen functionalized electrospun fibers (f1: after 4 h;f2 after 1 day - the scale bar represents 100 μm) adapted from [192] with permission. g1-g3 are images of the surface morphology of (g1) PLA electrospun fibers, (g2-g3) nanoparticle functionalized fibers (the scale is equal to 500 nm) adapted from [355] with permission. And f1 and f2 are stereomicrosope images of a melt electrospun PCL scaffold (scale bar represents 100 μm) adapted from [228] with permission.

Tables:

1. Classification of the different matrix metalloproteinases (MMPs) proteolytic enzymes found in the skin [8].

2. Summary of the current growth factors and cytokines involved in wound and skin regeneration (families shaded).

3. Principal advanced products for skin tissue repair commercially available.

4. Summary of the different mechanical stimuli and their effect.

5. Commonly used engineered GFs and gene therapies in skin wound healing and tissue engineering.

6. List of the most common inorganic compounds and ions used in skin wound healing.