Review

The Wound Microenvironment and Recent Therapeutic Advances to Treat Wounds

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Abstract: Based on its large surface area and covering the whole human body, the skin body's largest organ and its main function is protection. Injuries and wound healing involving the skin offer valuable lessons shared with and of relevance to other organ systems and the diseases that impact them. Arguably the most complex human body process, wound healing is a multifaceted process that involves multiple cells and the extracellular matrix (ECM), with each component playing a specific role in the different stages of the healing process. Importantly, studies indicate that cells with stem cell-like properties are present within many of the human tissues and play key roles in case of tissue and cellular injury. Cell-to-cell and cell-to-ECM interactions are salient in wound healing subsequent to an injury. Microenvironment related factors and the variations therein including hypoxia or the abundance of oxygen, the presence/absence of growth factors and cytokines add to the complexity of the wound healing process and impact cell function and result in compromised or enhanced wound healing. This expert review critically examines the advances in biochemical and analytical tools that are enable the analysis of numerous cells and molecules within the wound microenvironment, revealing great cellular heterogeneity as well as novel molecular targets of importance to enhance wound healing. In a broader angle, we emphasize the ways in which wound healing is significant in the search for perfect skin after injury and in many common complex human diseases including cancer. In all, wound healing is a centrepiece of integrative biology research and applications in medicine as well as dermatology as discussed in this review.

Keywords: wound healing; inflammation; microenvironment; stem cells; extracellular matrix; hypoxia; growth factors

1. Introduction

In terms of surface area, the skin is the largest human organ that plays major functions including protecting organs and tissues from environmental insults, infections and injuries [1, 2]. The cost of maintaining the skin and its treatment in case of injury is significantly huge and is a major consideration in the health sector [3, 4]. The global increase in diabetes cases as well as cases of sickle cell disease has resulted in many patients experiencing chronic non-healing wounds with no effective therapies available [5, 6]. Novel, innovative, and effective therapies are desperately needed for wound healing.

One major complexity associated with wound healing is the involvement of several cells and the need for coordination of cellular function through the various stages of wound healing. The three layers of the skin serve different purposes, with the top layer the epidermis mainly functioning to shield the human body tissues and organs from the direct effects of environmental insults [7]. Glands as well as hair follicles present in the dermis help to moisten the skin as well as sensing danger, respectively. Below the epidermis is the dermis, a skin layer with huge amounts of ECM proteins as well as blood vessels [8]. The main functions of the dermis are the provision of nutrients and physical strength to the skin. Below the dermis is the subcutaneous tissue which acts as a reservoir of energy

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as well as providing growth factors and cytokines to the upper layers of the skin [9, 10]. Several immune cells are present in each of the skin layers and provide immunity to the skin [11]. In case of skin damage, immune cells and a multitude of other cells mobilise and coordinate to bring about healing [12]. The several stages of wound healing occur in a staggered sequence, with different cells taking part in different stages.

Fibrin clot formation by activated platelets is one of the first processes following skin damage [13]. Blood flow is restricted by the clot and several immune cells anchor on the clot upon reaching the wound [14]. Possible bacterial infection of the wound is taken care of via recruited neutrophils [15]. Following injury, cells such as monocytes and dendritic cells migrate to the wound to provide immunity [16]. Besides the provision of immunity against infections, immune cells are also involved in the removal of damaged tissues and debris from the wound site [17].

Once the wound site has been cleared of damaged tissues and cells, new blood vessels start to form via the process of angiogenesis. Enhanced proliferation as well as branching of endothelial cell leading to formation of new blood vessels is accompanied by activation of pericytes needed for anchorage of the formed vessels [18]. Reports suggest that both pericytes and mesenchymal stem cells (MSCs) enhance the wound healing via participation in angiogenesis and their differentiation [19, 20]. In addition, bone marrow derived stem cells have been identified at wound sites where they participate in the formation of new blood vessels [21]. Most cells involved in formation of the vasculature are of perivascular origin, with reports indicating that new blood vessels regress without the involvement of the perivascular [22, 23]. Fibroblasts near the wound site can differentiate into myofibroblasts and are involved in the synthesis of ECM as well as formation of granulation tissue within the clot [24]. Reports indicate the involvement of stem cells in addition to de-differentiated epithelial cells in re-epithelialization [25, 26]. These stem cells are tissue-specific stem cells that display great heterogeneity and are present in the sweat glands as well as hair follicles [18, 27]. Stromal cells present within the skin layers also display great heterogeneity and contribute towards wound healing via release of growth factors and cytokines [28, 29]. Data indicating the influence of inflammatory cells on wound healing has led to increased attention being given to these cells [30, 31].

Whilst wound healing in embryos and before birth restores the skin to its original state as it is a regenerative process, adult wound healing is more of a restoring barrier function and some of the original tensile strength [32, 33]. Thus, in adults a scar is formed rather than 'perfect skin'. In cases of too much scarring, keloids are formed [34, 35]. To understand scarring, aberrant wound healing as well as chronic wounds it is important to focus on cells as well as cell-matrix interactions [36]. In many ways, our understanding of wound healing has been shaped by animal model studies due to the ease with which wounds can be established in animals [37, 38]. Whilst animal models have led to significant knowledge on wound healing, these models do not recapitulate human skin in many ways. For example, the human skin is not as elastic as rodent skin [39, 40]. In addition, human skin is attached to underlying structures and healing involves re-epithelialization as well as formation of granulation tissue [41]. Recent strategies for studying human skin wound healing include the use of organotypic cultures as well as *ex vivo* human skin cultures [42, 43]. Murine models of wound healing require the use of silicone stents which can allow wound healing to progress via re-epithelialization and granulation tissue formation [44, 45].

This review is an analysis of the role played by various factors within the wound, from cells, ECM, and biomolecules such as growth factors and cytokines. Importantly, this review article discusses novel and innovative strategies for wound healing and how the skin regenerative process can be accelerated. These strategies include the use of growth factor- and cell-therapy and ECM alterations that may provide therapeutic effect and enhance the wound healing process.

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2. The Wound Healing Process

The first step towards wound healing is the stopping of bleeding and closure of damaged blood vessels [46]. This process is referred to as haemostasis. The constriction of blood vessel walls following skin injury brings about a stop to the bleeding (Figure 1). This is followed by primary and secondary haemostasis, processes that overlap and are linked to each other [47]. Whilst platelets are not activated and not attached to blood vessels before injury, injury causes platelets to aggregate causing a plug formation [48]. In secondary haemostasis, soluble hepatocyte-produced fibrinogen undergoes conversion and forms fibrin strands that are insoluble [49]. The meshwork of fibrin strands together with the platelet plug form the thrombus which functions to prevent further bleeding and provides anchorage for other cells within the wound microenvironment [50]. The thrombus also functions to tether and release various growth factors and cytokines needed for wound healing [51].

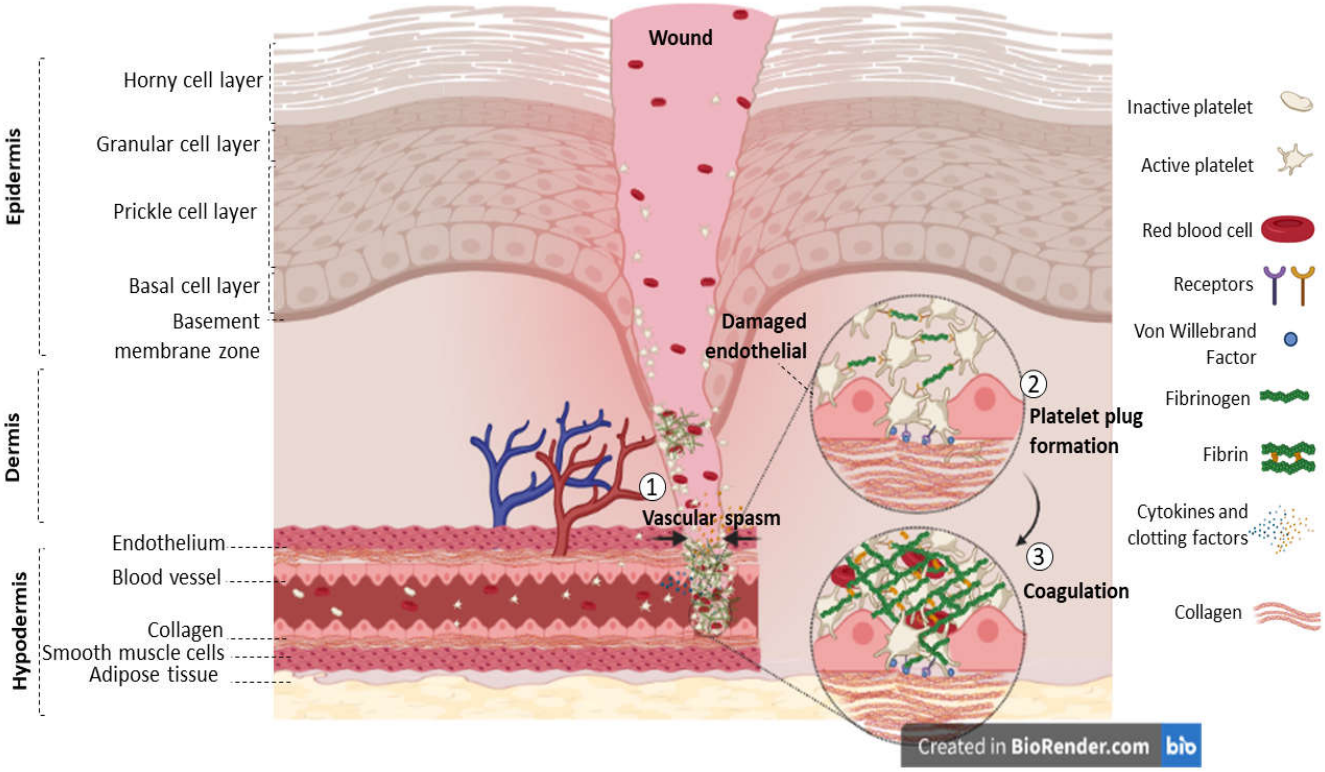

Figure 1. Injury to the skin cause cells to release various factors that induce the constriction of blood vessels, leading to a stop to bleeding.

In order to reduce bleeding after injury, blood vessels undergo constriction via contracture of smooth muscles (Figure 1) [52]. When the endothelium is damaged it releases several vasoconstrictors including endothelin and these facilitate the closure of ruptured vasculature [53]. Damaged or injured cells also release various prostaglandins and catecholamines that function to control the process of vasoconstriction [54]. Mesenchymal cells such as vascular smooth muscle cells are also activated by cytokines and growth factors including PDGF causing blood vessel constriction [55]. Over time, the lack of oxygen can cause bleeding to resume as muscles relax. Coagulation activation through the action of many mediators controls the process of vasoconstriction in the long term (Figure 1) [56].

The term platelets was derived from the word 'little plates' as these cells were so small they could only be seen under the microscope and resemble the 'plate shape' due to invaginations of the cell membrane [57, 58]. Platelets function in many cellular processes such as angiogenesis and thrombosis. Injury causing rupture of blood vessels results in

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activation of platelets and their attachment to tissues at the wound site, where the cells participate in the formation of a blood clot [58]. Platelet cells are found within close proximity of endothelial cells. The endothelial cells produce prostacyclin and heparin-like glycosaminoglycans that act to prevent platelet cells from being activated and aggregation [59].

Exposure of the matrix below the endothelial layer after injury and blood vessel damage results in platelets binding to the matrix via the G protein-coupled receptors [60]. This causes integrin activation and the enhanced binding of platelet cells to other platelets and matrix [61, 62]. Platelets bind to fibrinogen, collagen as well as fibronectin via integrins through the RGD sequences [63]. Integrins involved in this attachment include $\alpha 2\beta 1$ and $\alpha IIb\beta 3$ [64]. Attachment of platelets to ECM proteins activates several signaling cascades resulting in increased actin levels within the cytoplasm. For example, it has been shown that filamentous actin increases about 2-fold when platelets are activated, causing the platelets to change their conformation to a round shape which further changes to a flat shape containing lamellipodia and pseudopodia [65, 66]. Activated platelets bind strongly to the ECM via integrins and this aid in sealing any ruptured blood vessels [58, 67].

The flat shaped platelets have increased surface area for binding as well as exchange and release of biomolecules and substances via granular activity [59]. Among the secreted biomolecules are integrins needed for attachment to ECM proteins as well as activating substances such as serotonin and histamine [68, 69]. Platelet aggregation is also caused by the involvement of several glycoproteins in attachment of platelets to the ECM. For example, glycoprotein VI and glycoprotein Ib-IX-V are involved in the binding of platelets to collagen and Von Willebrand factor (vWF), respectively [70]. These two surface receptors are responsible for platelets binding to the ECM within the sub-endothelial layer [70]. Other biomolecules and substances released by platelets that aid in aggregation and binding to the ECM include thromboxane A2 and calcium ions [71]. Overall, platelet attachment to the ECM in addition to the action of released compounds and substances causes the formation of the platelet plug which stops bleeding and seals the ruptured vessels.

In addition, platelets embedded within the plug synthesize and release several factors that are key in different phases of wound healing. These factors are continuously released over time and when required [72, 73]. Importantly, platelets release increased amounts of factors including TGF--β, EGF and PDGF. Other including fibroblasts and endothelial cells also release several growth factors and cytokines and these in turn have both auto- and paracrine effects on all cells present [73, 74]. Several reports link proper wound healing to the release and presence of these growth factors and cytokines [59, 73, 74]. It is for this reason that cells such as MScs have been suggested as a cell therapy for wound healing [19, 20]. Furthermore, plasma has also been suggested as a therapeutic treatment for wounds [75, 76]. Reports indicate that MSCs release a higher amount of growth factors and cytokines compared to other cells and therefore suitable for use as cell therapy [77, 78]. It is important to note that the use of both MScs and platelet-rich plasma for wound treatment has produced mixed results [79, 80]. The mixed results can be attributed to, among other reasons, the source, and methods of preparation of plasma as well as the mesenchymal stem cells. In addition, both MSCs and platelets show cellular heterogeneity [81, 82]. Within the thrombus, activated platelets are found near the centre whilst less-activated platelets are found at the edges [83]. The importance of having several cell types within the wound microenvironment is illustrated by reports indicating that even the absence of platelets does not cause defects in wound healing [84-86]. Activation of factor X, leading to prothrombin being converted to thrombin results in formation of fibrin from fibrinogen cleavage [13, 52, 59, 87, 88]. Fibrin is then crosslinked via the action of factor XIII and fibrin attach to the platelet plug forming the thrombus. The thrombus main function is to be a scaffolding through which cells infiltrate the wound and participate in the wound healing process [89, 90].

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3. Inflammation and Immune Cells within the Wound Microenvironment

The first cellular response to injury is to activate several processes including the calcium waves as well as the generation of reactive oxygen species [91, 92]. Reports indicate that cells within the wound microenvironment increase cytoplasmic calcium ion levels [91]. Injured cells release increased amounts of DNA, ECM proteins, chemokines and hydrogen peroxide leading to the recruitment of immune cells including neutrophils [93]. Several pieces of evidence show that chemokines can promote angiogenesis [94, 95]. Chemokines bind to the G protein-coupled receptors and this activates several signaling cascades involved in the movement of cells in response to chemokines levels [96]. Cleaved fibrin within the wound microenvironment in addition to growth factors promote and regulate chemokines production over time [97]. Importantly, the release of hydrogen peroxide as well as migration of immune cells into the wound microenvironment suppress infections whilst promoting blood vessels formation [98, 99]. Among the immune cells recruited to the wound are mast cells. Mast cells are granulocytes that release several factors including cytokines and proteases [100]. The release of these cytokines and proteases enhances the recruitment of inflammatory and other cells to the wound site [101]. Reports indicate the lack of mast cells within the wound microenvironment results in reduced wound healing and immune cells recruitment [102, 103].

The release of DNA, lipids, hydrogen peroxide and chemokines by injured cells cause neutrophils to hone in on the injured site [104, 105]. Within hours of injury, neutrophils constitute above half of the cellular component of the wound [106, 107]. Neutrophils detect chemokines and other factors released by injured cells via several surface receptors for example integrins, GPCRs and Fc receptors [106, 108]. The function of neutrophils as ‘early arrivals’ at the wound site is to prevent infections via phagocytosis and release of toxic granules (Figure 2) [106, 107]. Within toxic primary granules are antimicrobial agents including cathepsins G, azurocidin and lysozyme that destroy bacteria [15, 109, 110]. Secondary granules contain matrix metalloproteases, lactoferrin and collagenase-2 [110]. Neutrophils also release secretory vesicles containing cell surface receptors including integrins and cytokine receptors [109].

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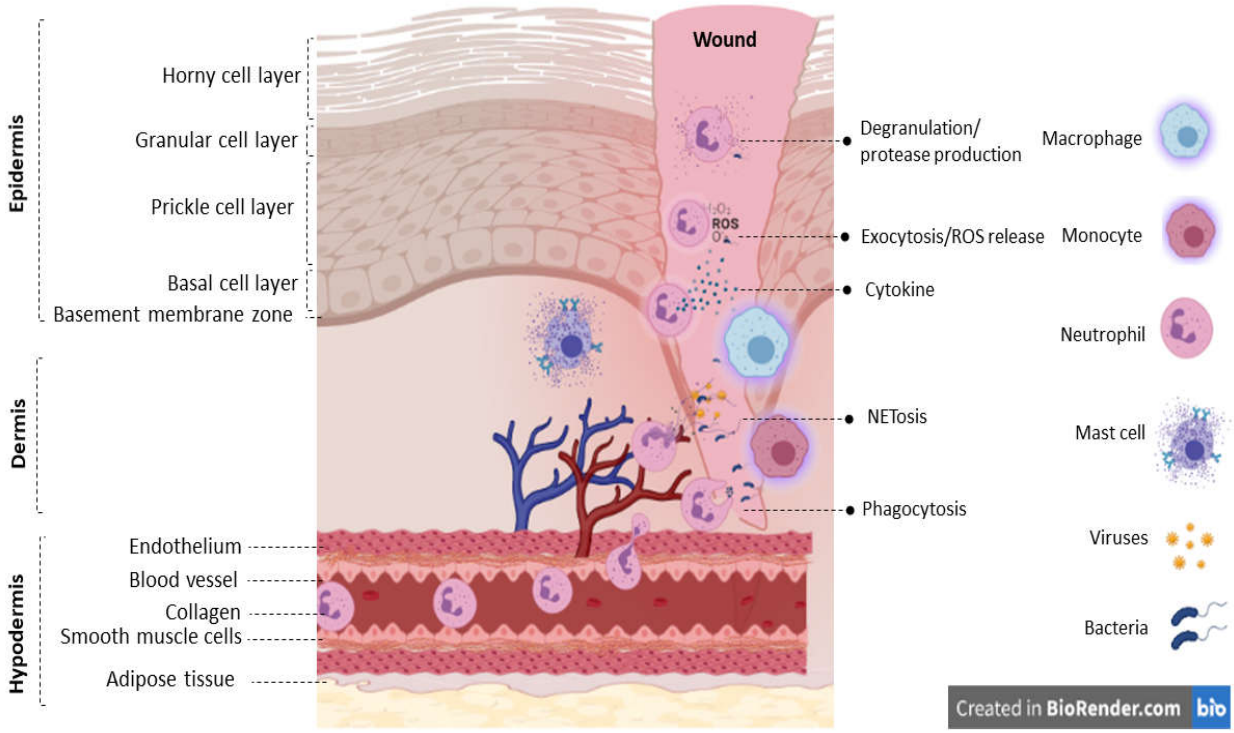

Figure 2. The function of neutrophils as 'early arrivals' at the wound site is to prevent infections via phagocytosis and release of toxic granules. Neutrophils are involved in fighting pathogens via the release of enzymes from granules as well as production of neutrophil extracellular traps. Chromatin filaments are extended out of cells into the wound microenvironment/wound and these contain proteases on them.

Toxic granules contain several proteases including protease 3 and cathepsins G that can break down the ECM as well as the basement membrane, allowing migration of multiple cell types to migrate to the wound site [15, 111]. Proteases can activate matrix metalloproteases and together can break down ECM proteins such as collagens, fibronectin and vitronectin [15, 109, 110]. Belaaouaj and colleagues demonstrated that the lack of neutrophil elastase results in a lack of bacterial clearance in mice [112]. Enhanced levels of enzymes production by neutrophils on the other hand can lead to tissue destruction as well as blunt blood vessels formation [113]. Extracellular traps from neutrophils also help in the elimination of infectious agents via proteases attached to chromatin filaments (Figure 2) [114]. The extracellular traps are release via the action of enzymes such as elastase which rupture the neutrophil membrane in the process resulting in the death of the cell [114]. Neutrophil extracellular traps can also be released via vesicles meaning the neutrophils can further be involved in processes such as phagocytosis [115]. Reports indicate that neutrophils show phagocytic stages similar to that of macrophages although neutrophils have special receptors for the identification of particular pathogens [115, 116]. Neutrophils bind to antigens via the use of Fc receptors and integrins. The clustering of both Fc receptors and integrins leads to the activation of various downstream signaling cascades [117, 118]. The plasma membrane forms a 'phagocytic cup' that engulfs the pathogens, forms an early phagosome (Figure 2). The early phagosome fuses with granules containing proteases involved in destruction of pathogens [117, 119]. Once infection is cleared, neutrophils undergo apoptosis or necrosis and are removed through the action of macrophages [120]. If neutrophils are not cleared after infection, they can lyse resulting in release of cytotoxic biomolecules and causes tissue damage (Figure 2) [120, 121]. Resolution of inflammation can only happen when all neutrophils are cleared. Reports indicate that neutrophils can also enter circulation and travel to distant sites [122, 123].

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Macrophages express CD45 as well as Cd11b as common markers in both humans and mice [124, 125]. In addition, macrophages found in mice express F480 whilst human macrophages display lack of CD66B expression [124, 125]. Macrophages have been shown to migrate towards wound sites where they protect against infections [126]. In addition, monocytes can be recruited from the bone marrow to the wound site where they are converted into macrophages [127-129]. Indeed, several studies have demonstrated both macrophages and monocytes plasticity during wound healing and tissue regeneration [129, 130]. Tissue damage or injury likely activates local macrophages to proliferate so as to increase in numbers [131]. Importantly, monocytes can differentiate into macrophages and thus increases the number of macrophages found within the wound microenvironment [132, 133]. Recruitment of monocytes to the wound site is achieved via stromal derived factor 1 and other hypoxia-linked factors [134]. Macrophages also synthesise the factor monocyte chemoattractant protein (MCP-1) which can attract monocytes to the wound site [135]. Wounds lacking in macrophage numbers demonstrate increased levels of neutrophils as well as decreased ECM synthesis and release of growth factors [136, 137]. The net effect is reduced wound healing. In contrast, increased macrophages within the wound site is linked to enhanced wound healing or regeneration of damaged tissues [138]. Macrophages expressing several factors including interleukin-6 are found during wound repair and healing and are called M1 macrophages [139, 140]. These macrophages are pro-inflammatory and kill pathogens via the action of reactive oxygen species within phagosomes [141, 142]. Migration of pro-inflammatory macrophages is facilitated by the action of MMPs that digest various ECM proteins, creating highways through which they move [143]. Small fragments of ECM also act to stimulate the immune cells including macrophages to promote inflammation [144]. Old neutrophils are also removed through the action of macrophages within the wound site.

As soon as the inflammatory stage of wound healing is resolved, macrophages transition from being pro-inflammatory to become the M2 macrophages that are anti-inflammatory [145]. M2 macrophages are known to promote new blood vessels formation and express Tie2 [146, 147]. Macrophages that promote vascular system formation do so via fusing endothelial vessels as well as connecting them to systemic vasculature [146, 148]. Pro-angiogenic macrophages release VEGF which is required during angiogenesis [136, 149]. Similar phenotypes have been observed in pro-angiogenic macrophages and in endothelial cells [150].

The proliferation stage of wound healing involves interactions between macrophages and fibroblasts (Figure 3). Specifically, macrophages have been shown to activate normal fibroblasts into myofibroblasts with the concomitant increase in ECM proteins synthesis [151, 152]. Several reports also indicate that macrophages can transdifferentiate or convert into fibroblasts through activation of TGF/Smad 3 signaling [153, 154]. Macrophages that transdifferentiate or are converted into fibrocytes have been implicated in scar formation as a result of increased collagen deposition within the wound site [155, 156]. The fluctuating conditions of the wound have been suggested to play a part in the plasticity of the cells within it including macrophages and fibroblasts [157, 158]. After re-epithelization of the wound, 'phagocytic' macrophages, also known as M2c macrophages, release several enzymes to remove the excess ECM proteins and other cells that are no longer needed for sealing of the wound [159, 160]. Skin fibrosis, the occurrence of excessive ECM proteins and cells, occurs if M2c macrophages are not able to clear the wound site of excessive ECM proteins and cells [161]. Impairment in macrophage function as well as in numbers of macrophages present within a wound has been associated with keloids as well as scars [155, 162]. Furthermore, aberrant interactions between macrophages and other cells within the wound can result in fibrosis. For example, aberrant interactions between macrophages and fibroblasts can cause scar formation [163, 164]. In diabetic wounds macrophage delay the expression of chemokines and the consequential delay in recruitment of monocyte and macrophage activation causes neutrophils to linger around for a long time within the wound microenvironment [165]. As reported by Maruyama and colleagues dysfunctioning macrophages do not release growth factors in a normal way [166]. In

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addition, debris and ECM protein breakdown products remain within the wound leading to proliferation delays [167]. Ultimately, a chronic inflammatory environment occur within the wound microenvironment, characterized by elevated levels of macrophages and apoptotic cells [165].

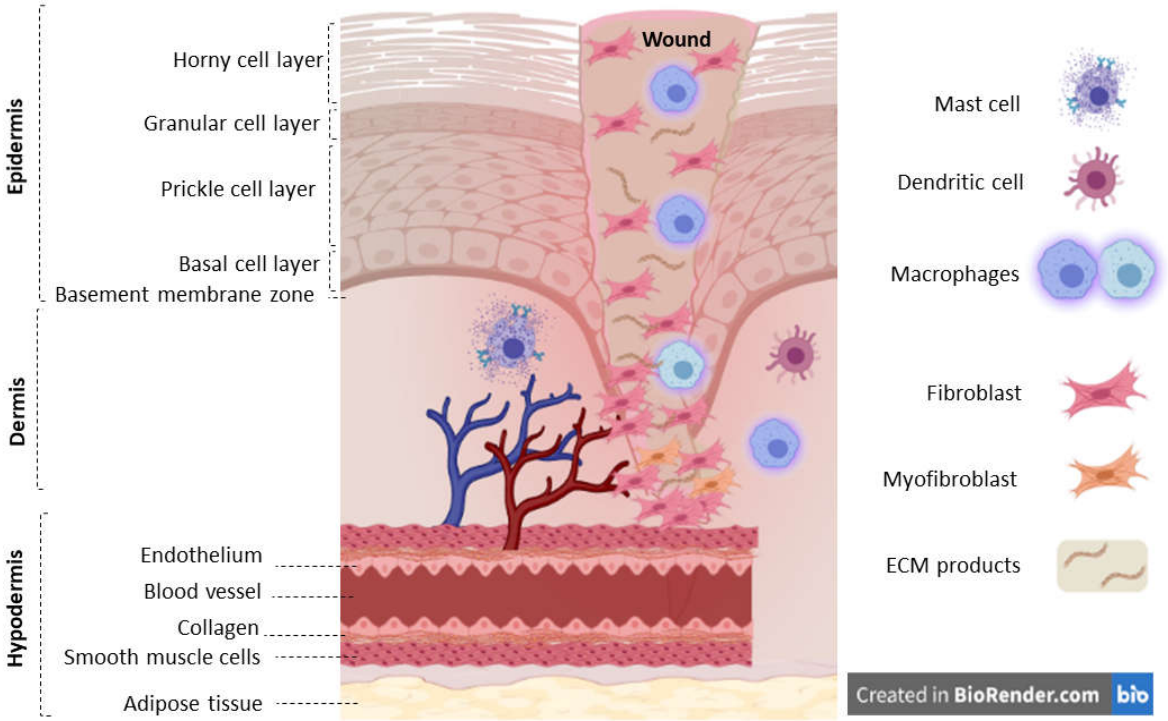

Figure 3. The proliferation stage of wound healing involves interactions between macrophages and fibroblasts. Specifically, macrophages have been shown to activate normal fibroblasts into myofibroblasts with the concomitant increase in ECM proteins synthesis.

Ehrlich reported in his doctoral thesis in 1978 of progenitor cells from the bone marrow, which were coined as mast cells, that differentiate within the connective tissue into mature mast cells [168, 169]. Well known for protecting against helminths infestation, data on the role played by mast cells in wound healing is not clear [170]. Perhaps their main function has been revealed as prevention of infections during wound healing [171, 172]. In addition, through synthesis and release of various growth factors as well as enzymes including tryptase, mast cells can degrade the ECM allowing immune cells to be recruited to the wound site [173]. Mast cell-derived histamine influences both fibroblast and keratinocyte proliferation thereby participating in re-epithelialization and wound contraction [174]. Large numbers of mast cells have been shown to contribute to skin fibrosis as well as in scarring [175]. Wulff and colleagues demonstrated that mast cell lysate is able to induce scarring in fetal wound healing [176]. Cellular heterogeneity is now a constant characteristic of most cells and different subsets of mast cells have been observed [177, 178]. A contributing factor to mast cell heterogeneity, indeed for all cells within the wound microenvironment, is the constant remodelling of the wound as it undergoes different phases [179-182].

Mostly known for priming T-cell functions, dendritic cells found within the epidermis are also called Langerhans cells after the scientist Paul Langerhans discovered the cells whilst working on skin tissue [183]. Ralph Steinman in 1973 identified and referred to the cells as dermal dendritic cells [184, 185]. Due to similarities with macrophages in terms of being phagocytic and the presence of common surface markers, dendritic cells have been suggested to represent a type of macrophages [186]. However, macrophages primarily function to remove cells and ECM debris as well as pathogens, whilst dendritic

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cells are primarily antigen presenting cells [187-189]. Through the recognition of DNA, peptides, and other material from dead cells via the use of clec9A receptors, dendritic cells activate CD8+ T cells to eliminate the dead cells [190, 191]. Viral recognition by dendritic cells occurs via the use of TLR3 receptors which binds to double stranded RNA [192, 193].

The epidermal Langerhans cells originated from myeloid progenitors and are known to survey the tissue for the presence of pathogens [194-196]. Continuous interaction with keratinocytes is required for Langerhans cell maintenance and once an infection occurs, they decrease the expression of E-cadherin so they can migrate via the dermis into the lymph nodes. Within the lymph nodes, Langerhans cells activate the adaptive response involving T cells [189, 197]. Circulating dendritic cells, also referred to as plasmacytoid dendritic cells, can also be recruited to the wound site in case of an infection [198, 199]. The plasmacytoid dendritic cells release various cytokines including interferon-alpha and beta (IFN-α and IFN–β) in the event of an injury and their absence has been shown to prevent wound re-epithelialization [198, 200]. The isolation and characterization of dendritic cells is hampered by the lack specific markers. Currently, whilst CD11c is the common marker used for their isolation, other cells such as T cells and some macrophages also express this marker [201, 202]. Another marker is CD103. However, several other cells such as epithelial lymphocytes and T cells [203, 204]. Advances in technology have seen the isolation and analysis of single cells within the skin and the wound microenvironment and this has revealed great knowledge about both dendritic cells and other immune cells [205-207].

Within the skin layers are $\gamma\delta$+ T cells and the $\alpha\beta$+ T cells [208]. Most studies focus on dendritic epidermal T cells (DETCs) which are residents of the basal layer of the epidermis and release various growth factors involved in wound re-epithelialization [209, 210]. Studies on animals demonstrate that the lack of dendritic epidermal T cells can delay wound healing [211, 212].

Dendritic epidermal T cells can be activated by ligands such as CD100 released by wounded keratinocytes [211, 213]. Dendritic epidermal T cells change their morphology from a dendritic structure to a round morphology and start releasing various factors including KGF-1 as well as insulin growth factor-1 leading to enhanced keratinocyte proliferation within the wound microenvironment [212, 214, 215]. In the case of keratinocytes proliferation being reduced and less hyaluronan deposition, wound closure is delayed [212, 215]. Aberrant interactions between keratinocytes and dendritic epidermal T cells can lead to delayed wound healing in mice models [211, 216]. In case of an infection, $\gamma\delta$ T cells of the dermis recruit neutrophils and also activate CD4+ T cells to proliferate in order to clear the infection [217-219]. Dermal T cell-derived cytokines including IL17 and TNF-α play key roles in recruitment of neutrophils and infection clearance [210, 220]. A subset of dermal T cells is known to activate dendritic cells [218]. $\alpha\beta$+ T cells can be found within circulation as well as within the skin [192]. The $\alpha\beta$+ T cells can be CD4+ cells, CD8+ cells as well as regulatory T cell subsets [192]. CD4+ cells can be found in both the dermis and the epidermis in the event of an infection but will return to the epidermis once the infection is resolved [221].

Skin fibrosis has been linked with dysfunctional T cells [222, 223]. T cells within the skin demonstrate great heterogeneity and the role played by each subset in wound healing is still to be elucidated [224-226]. Of the different T cells present in skin, dendritic epidermal T cells are the most studied yet difficult to isolate [227, 228].

4. Granulation tissue and Endothelial Cells in Wound Healing

Nascent connective tissue, referred to as granulation tissue, is formed as wound healing progresses through the proliferative phase. As described by John Hunter and Alexis Carrel, the granulation tissue undergoes several changes during the proliferative phase of wound healing [229, 230]. Granulation tissue is made up of ECM proteins and proteoglycans that form a scaffold, allowing other cells and blood vessels to anchor and grow [231]. As wound healing progress and the wound microenvironment is remodeled, the

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connective tissue over time replace the granulation tissue [231]. Cellular proliferation, formation of the connective tissue and regeneration of damaged tissue require constant supply of nutrients and oxygen, emphasizing the need for new blood vessels formation [231, 232]. Angiogenesis, driven by the release of growth factors such as VEGF, results in vascular networks formation [233-236]. The assumption that endothelial precursor cells were responsible for vessel formation in adults was shown to be not true [237, 238]. Microvascular resident endothelial cells are activated to proliferate and migrate by hypoxic conditions and in the process degrade ECM proteins within the connective tissue. Eventually, endothelial cells form cell-cell junctions and form new capillaries (Figure 4) [233].

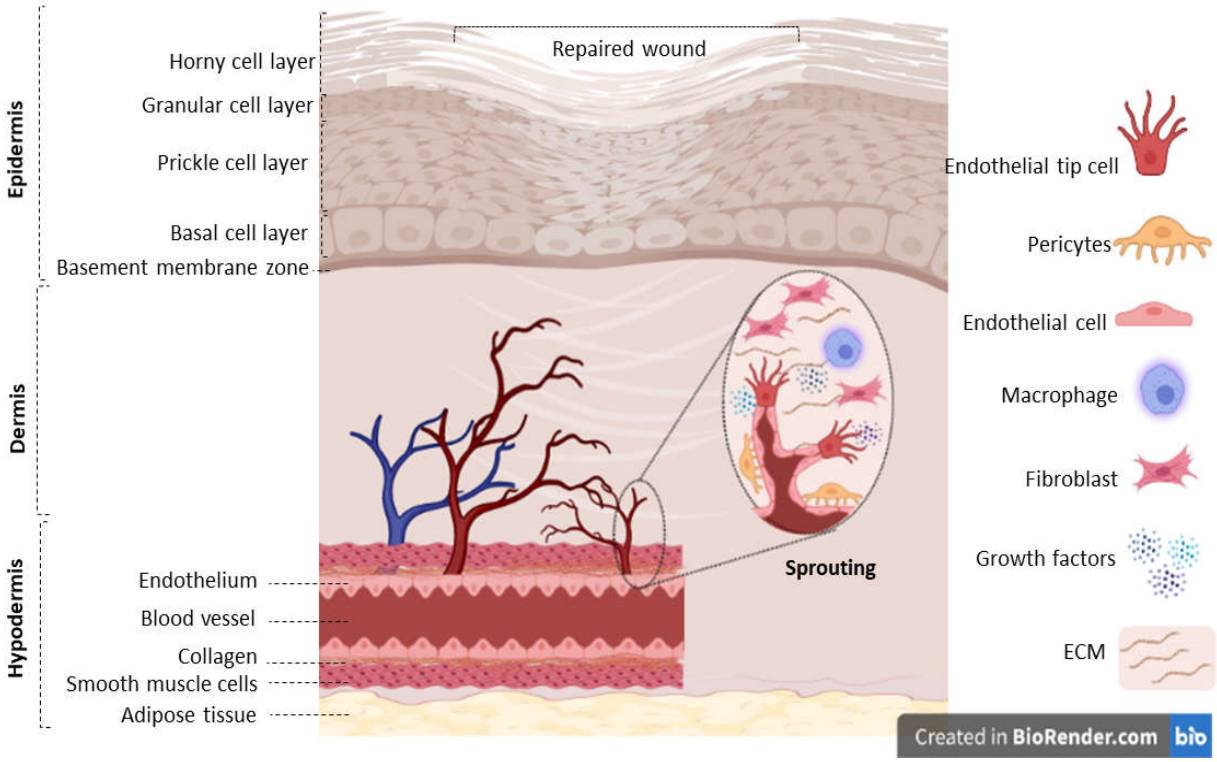

Figure 4. Formation of new blood vessels during wound healing via the sprouting of endothelial cells at the tip to form capillaries. Endothelial cells respond to various macrophage- or epidermal cell-derived growth factors including VEGF.

Endothelial cells lining blood vessel surfaces are responsible for forming new blood vessels. Growth factors and the hypoxic conditions within the wound microenvironment drive endothelial cell activation and migration within the clot. This migration and sprouting of endothelial cells in the direction of angiogenic factors, including VEGF, result in formation of new blood vessels [239, 240]. Great heterogeneity is displayed by endothelial cells, with some cells taking the leading role in migrating and thus occupies the tip of the new vessels being formed [240]. Trailing endothelial cells make up the bulk of the newly formed vessel. Sprouting endothelial cells eventually become tubules [241, 242]. Various signaling cascades have been shown to influence endothelial cell migration and involvement in tubule formation [243, 244]. Several reports demonstrated the involvement of angiogenic growth factors in activating the Notch cascade [240, 245-247]. Enhanced Notch signaling is known to activate endothelial cells. As reviewed elsewhere, chemokines are crucial to endothelial cell activation and vessel formation during wound healing [248-250].

The cell surface of endothelial cells under normal circumstances do not allow platelets and other immune cells to bind, but once the tissue is wounded, endothelial cells have been shown to express selectins that allow leukocytes to bind to their surface [251, 252]. For example, endothelial cells have been shown to express receptors for P-selectin and E-selectin leading to leukocyte adhesion [251, 253]. P-selectin and E- selectin knockout has

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a negative effect on wound healing [254, 255]. Several integrins expressed on endothelial surfaces, including those for collagen and fibrin and fibronectin, play key roles during angiogenesis [239]. Activated endothelial cells forming new vessels express enhanced levels of $\alpha v\beta 3$ integrin receptors that allow the cells to move within the wound microenvironment that is rich in ECM proteins [256, 257].

Contribution of pericytes and fibroblasts to wound healing

Pericytes were first named so by Zimmermann around 1923 as cells that are found on the axis of blood vessels, having been discovered earlier by Rouget in 1873 [258, 259]. One of the key roles of pericytes is the maintenance of the vasculature integrity during the different phases of wound healing (Figure 4) [260]. In addition, pericytes participate in controlling blood flow to and from different tissues, making them very important in wound healing [260, 261]. Currently it is difficult to distinguish pericytes from several cells found within the perivascular space such as mesenchymal stem cells, smooth cells, and fibroblasts [238, 262]. Several reports have suggested that both pericytes and MScs are of the same origin [263, 264]. This is further complicated by the lack of definitive markers for pericytes and the lack of evidence of their origins [265-267]. For example, only subsets of pericytes can express alpha-smooth muscle actin (α-SMA) [266, 268]. Structurally, pericytes are large cells able to fold around endothelial cells due to their elongated cell membrane [269, 270]. As reviewed elsewhere, pericytes are heterogeneous cells that express various surface markers including desmin and nestin [267, 271]. Advances in technologies including the use of artificial intelligence, microfluidic systems and single cell technologies would soon delineate their origin and most importantly their many functions in wound healing and skin regeneration.

Fibroblasts have been shown to be central to the integrity of the connective tissue of almost all bodily organs through the synthesis and laying of the extracellular matrix [272, 273]. Fibroblasts can exist in an inactivated state or can be activated by various factors including growth factors as well as cytokines. In addition, inactivated fibroblasts display great heterogeneity/plasticity, and this has a huge impact on the contributions of fibroblasts to wound healing. This fibroblast heterogeneity is displayed in terms of growth factor release, ECM protein synthesis and regulation of the immune system [274]. Several reports have indicated that the origin of fibroblasts is of multi-lineages, and this contributes to the different functions attributed to these cells [275, 276]. For example, skin fibroblasts come from the neural crest and other fibroblasts found in the ventral body skin are of lateral plate mesodermal origin [86, 277]. Driskell and colleagues demonstrate that a particular subset of fibroblasts is responsible for determining the structure of the dermis during skin development [278]. What determines if fibroblasts behave is a particular way include factors such the position in certain tissues as well as their orientation in respect to the epidermis. The activation of the Wnt/B-catenin cascade by dermal fibroblasts at hair follicle base is key for hair follicle development [279]. Dermal fibroblasts can also be induced into myofibroblasts; themselves can form dermal adipocytes, preventing scar formation in the process [280]. Several reports indicate that activated fibroblasts, demonstrated by α-SMA expression can originate from MSCs [281, 282].

Wound healing involves wound microenvironment contraction, which increases the wound stiffness. Wound cells especially fibroblasts attach to ECM proteins including collagen and fibronectin, pulling fibrils in the process and increasing wound contraction [283]. The enhanced deposition of ECM proteins to strengthen the tissue leads to fibroblasts being converted to myofibroblasts, as determined by α-SMA expression (Figure 7) [283, 284]. For example, vitronectin, osteopontin and hyaluronan have been implicated in fibroblasts conversion to myofibroblasts [285, 286]. In the granulation tissue, growth factors and MMPs have been shown to play important roles in the transition of fibroblasts to myofibroblasts [287, 288]. It has been suggested that only a small subpopulation of local fibroblasts can convert into myofibroblasts during the process of wound healing, but several reports including our own show that MScs can be converted to activated fibroblasts

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given the right microenvironmental signals [281, 289, 290]. Normal fibroblasts go through several transient stages via proto-fibroblasts and eventually into myofibroblasts through the action of TGF-B and interactions with ECM components such as fibronectin [291]. The progression of wound healing and the eventual restoration of tissue integrity at the wound site results in myofibroblasts being cleared from the site.

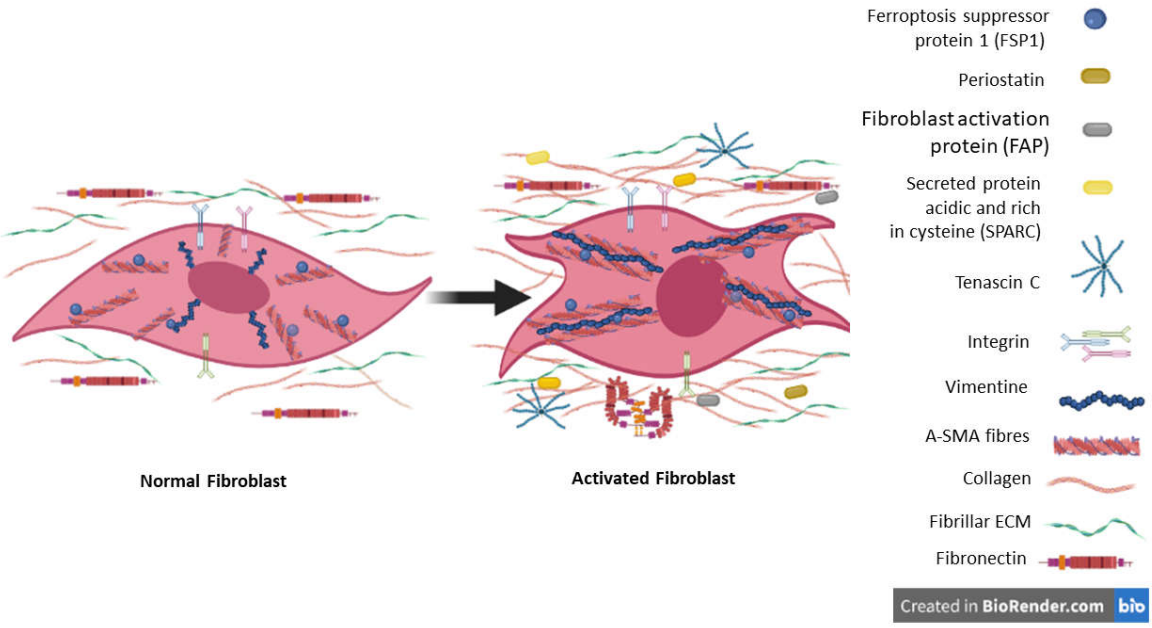

Figure 5. Normal fibroblasts are activated into myofibroblasts, a transient cell type, involved in synthesis of large amounts of ECM and growth factors and attach to fibronectin leading to wound contraction.

5. Formation of new epithelial layer

The keratinocytes of the epithelium within the epidermis are connected via desmosomes, cell to cell junctions, necessary to form a barrier which can protect the human body from various insults such as extreme temperatures and infections [7]. The lowest layer of the epithelium is sometimes called the basal layer and is in contact with the basement membrane. The topmost layer is the stratum corneum which is exposed to the external environment and is always in constant state of renewal and shedding. Two extra layers of the epithelium are the granular layer and the spinous layer [292, 293]. Several other components present within the epidermis include melanocyte stem cells, immune cells, and hair follicles. Melanocyte stem cells present within the epidermis as well as in hair follicles express high levels of K14 within the basal layer and are in constant flux between proliferating and differentiating. Support for the presence of melanocyte stem cells comes from experiments demonstrating stem cell movement from outside the wound to the centre of the wound giving rise to differentiated cells [294]. Sebaceous glands also found within the epidermis are always undergoing changes, similar to hair follicles undergoing growth and degeneration.

In the event of an injury, keratinocytes at the wound site dissociate from each other as well as from the basal lamina and spread to close the open wound. Integrins on the cell surface of keratinocytes participates in activation of several cascades including the MEK-ERK signaling leading to enhanced proliferation of keratinocytes as well as immune activation [295]. Decreased or impaired integrin function has been linked to a diminished wound healing process [296].

In order to close the wound injury, keratinocytes must proliferate and migrate, and this is driven by several factors including the transcription factor Slug [297]. Furthermore, growth factors including TGF-B, FGF2 and EGF induce keratinocytes to express

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keratins K6 and K16 that are important for migration [298]. Migrating keratinocytes express proteolytic enzymes to create 'highways' through the plug-in order to migrate. Keratinocytes continue to interact with other cells within the wound such as macrophages and fibroblasts, in addition to releasing cytokines that have a stimulating effect on the macrophages and fibroblasts [211, 299]. Keratinocyte-derived VEGF is also involved in promoting angiogenesis [300].

Injury to the skin impact cellular proliferation as well as activation and this is true for melanocytes [301]. Melanocytes are the cells responsible for melanin production and have been reported to be derived from dendritic cells. Melanin is the skin pigment that functions to protect the skin from the effects of ultra-violet light in addition to reactive oxygen species [302]. Melanocytes are mainly located within the inter-follicular epidermis as well as hair follicles [303]. Special stem cells found within the hair follicle bulge called melanocytes stem cells maintain the melanocyte population in addition to releasing factors that impacts on melanocytes behaviour [304, 305]. When injury occurs, melanocytes stem cells leave the bulge within the hair follicle into the injured tissue, divide and differentiate into melanocytes found in several regions including the new epithelial layer within days [306, 307]. Melanocytes within the actual healing tissue do not express high levels melanin, in contrast to adjacent melanin in the un-injured tissue [308].

In case of injury, melanocyte stem cells are mainly differentiating into melanocytes and not maintaining their numbers. Thus, over time, melanocyte stem cells can be depleted [307]. Melanocytes also interact with other cells during the process of wound healing. For example, it has been shown that fibroblasts within the injured tissue interact with melanocytes and are activated by several factors from melanocytes to produce ECM proteins [309, 310]. In turn, fibroblasts also release several factors including cytokines to influence melanocytes behaviour [310]. Currently, very few studies have focussed on the interactions of fibroblasts and melanocytes.

6. Maturation of newly formed tissue

Remodelling of wounded tissue as well as tissue maturation goes beyond just closure of the wound and involves regular deposition of ECM and the retreating of blood vessels from the surface of the tissue [56]. As wound healing progresses, there is a change in the collagen found within the granular tissue from collagen III to collagen I [276, 311]. As reviewed by Gurtner and colleagues, the replacement of collagen III by collagen I is caused by increased synthesis of collagen I as well as degradation of collagen III [231]. Degradation of collagen III and other ECM proteins during tissue remodelling is done by MMPs synthesised and released by different fibroblasts within the wound site [312, 313]. As remodelling progresses, TIMPs can start to inhibit ECM degradation by blocking the activity of MMPs. The balance between MMPs and TIMPs is critical to wound healing and maturation of tissue following injury [314, 315]. Ultimately, a large percentage of the fibroblasts within the wound site undergo cellular apoptosis as the healing process comes to an end [316]. It has been reported that fibroblasts can avoid being 'cleared' from the matured wound site via the expression of CD47 which prevents macrophages from phagocytosing the fibroblasts and also degraded ECM remnants [163]. Continual deposition of ECM proteins can cause hypertrophic scars.

New blood vessels formed during the process of wound healing are initially without tight cell-cell adhesions and therefore allow blood and other biomolecules out into the wound microenvironment [50]. As the wound heals and mature tissue is formed, blood vessels undergo major changes resulting in formation of stable blood vessels that are maintained by quiescent endothelial cells [317].

7. Wound Healing Disorders

Each constituent member of the wound microenvironment can easily influence the process of wound healing, from cells, ECM, and factors. Globally, millions of dollars are spent each year trying to find cures for disorders associated with wound healing. For

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example, fibrosis and scarring are major health challenges requiring innovative and novel treatment strategies. Excessive laying of the ECM proteins during wound healing by fibroblasts can result in keloids affecting the quality of life of patients [318]. Increased predisposition to the development of keloids appears to be genetic, with many people in regions such as Africa and Asia suffering from keloids more than persons from other regions [319, 320]. Furthermore, specific body areas including the chest, arms can easily develop keloids than other regions of the body.

Injuries from accidents, burns and trauma can result in excessive scarring causing major dysfunction of human organs or tissues as well as affecting the nervous system [321-323]. Hypertrophic scars demonstrate vascularized tissue within which are found inflammatory cells and fibroblasts [324, 325]. Both fibroblasts and inflammatory cells deposit huge amounts of ECM proteins in a disorganized fashion [326]. There are currently limited treatment options available for deep injuries and burns and the resulting hypertrophic scars [327]. Several mechanotransduction signaling pathways have been implicated in the development of wound healing disorders such as keloids and hypertrophic scars. One of the most studied pathways is the focal adhesion kinase-integrin cascade. Skin cells including fibroblasts express FAK which is highly expressed in cells responding to mechanical cues [328, 329]. Fibroblasts and keratinocytes are the main cells within the skin and associated signaling therefore is critical in wound healing. Activation of FAK in fibroblasts for example, occurs during wound healing in response to mechanical cues and this can cause hypertrophic scars [155, 330]. Downstream of the FAK are several components and these include the phosphatidylinositol 3-linase (PI3K)-Akt and the MEK-ERK cascade [331, 332]. On the other hand, downregulation of FAK signaling is associated with non-healing, clearly demonstrating the role FAK plays in wound healing [333, 334]. Downregulation in FAK signaling can be caused by degradation of FAK, as is the case in diabetic wounds with the consequential delay in wound healing [335].

Several studies have illustrated that fibroblasts can respond to various cues by remodelling the ECM within their microenvironment. For example, Driskell and colleagues demonstrated that a special subset of fibroblasts was responsible for the formation of scars rich in several ECM proteins and without hair follicles [278]. In addition, Rinkevich and colleagues demonstrated that presence of special mouse fibroblasts responsible for the synthesis of the connective tissue as well as wound healing [289]. When placed under mechanical strain keratinocytes are known to influence the levels of ECM-degrading enzymes such as MMPs [334, 336]. Further studies are required to understand fibroblasts-keratinocytes interactions in wound healing, especially the release of growth factors and cytokines. Florin and colleagues demonstrated that factors released by fibroblasts play a key role in enhancing proliferation of keratinocytes [337]. Besides primary cells including fibroblasts and keratinocytes in the skin, other cells such as T cells and macrophages can be recruited to the skin with or without an injury. The full relationship between these cells and fibroblasts and keratinocytes is still under intense investigations.

Several conditions are associated with non-healing wounds from ulcers, diabetes, aging and vascular diseases and these ultimately leads to discomfort and low quality of life [338]. Many strategies aimed at enhancing wound healing are limited in their effectiveness and at most are short-lived [339, 340]. Contributing to the ineffective therapies and treatments is the lack of understanding of the mechanisms that promote non-healing wounds and other skin pathological conditions. In most cases, skin disorders and non-healing wounds are caused by many factors coupled to an 'enabling microenvironment' that promote aberrant outcomes. For example, in diabetic patients, growth factor release is impaired, and this causes a chronic inflammatory environment [341, 342]. Furthermore, a delay in macrophage activation as well as monocytes recruitment to the wound site causes cellular debris to persist within the wound microenvironment, contributing to the development of an inflammatory environment [167, 343]. Chronic inflammation into several stages of wound healing causes the wound not to heal [343]. Within the diabetic wound microenvironment is also low levels of growth factors needed for blood vessel formation as well as elevated levels of reactive oxygen species that affect the function of endothelial

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cells [74, 344, 345]. Reports indicate that cells present within the diabetic wound including fibroblasts, MSCs and keratinocytes demonstrate impaired functions [341, 346, 347]. Recent reports also show that the skin microbiome plays a key role in determining the outcomes of wounds as some bacteria can have negative effects to wound healing [348-350]. Bacterial presence within diabetic wounds for example can have negative effects to wound healing [351-353]. Growth of bacteria and the formation of biofilms on the wound can lead to many complications including resistance to antibiotics [354, 355].

Overall, the wound microenvironment has emerged as an important player in wound healing in addition to cells, the ECM, and biomolecules. Efforts to 'return' the wound microenvironment to its natural state may reverse the aberrant cellular functions and chronic inflammation usually observed in non-healing wounds [356-358]. Many novel strategies and products used for non-healing wounds are limited in their effectiveness [359-363].

8. Advances in Therapeutic Strategies for Wound Healing

Skin grafting from either the same patient or from other sources is one of the ways to enhance wound healing or treat wounds in medicine. In many cases, the graft itself does not survive due to insufficient skin tissue and therefore lacking in proper integration to the skin [364, 365]. In case of grafts from another individual, immune rejection is a major problem, leaving many patients with no alternative but to turn to immune-inert alternatives such as matrix-based and cell therapies [365-367]. Whilst these new and advanced therapies are promising it is important to note that they need further research and regulatory approvals [368].

Extracellular matrices can be used to induce the regeneration of the dermis. The ECM can provide the scaffolding needed for new dermis to form as well as be embedded with cells that can release growth factors and cytokines needed for dermis regeneration [369, 370]. Growth factors and cytokines help in recruiting several cells from the surrounding tissues and stimulating synthesis of new ECM proteins [370-372]. The properties of the scaffold used in inducing dermal regeneration are determined by the methods used during decellularization and any further manipulations such as cross-linking. In most cases native scaffolds lacking in chemical modifications perform better in terms of inducing wound healing and angiogenesis within the wound microenvironment [373-375]. Several skin substitutes are available and have been approved by the FDA and these include Integra Dermal Regeneration Template which is used mostly for burn injuries. Diabetic foot ulcers can be treated with Omnigraft. These skin substitutes act as temporal epidermis and degrade over time to be replaced by the newly synthesized tissue. Local cells adjacent to the wound enter the wound microenvironment and begin to synthesise new ECM proteins and release growth factors needed for formation of new vessels [376]. A silicone layer functions mainly to control the amount of moisture within the wound microenvironment and also provides a base onto which new tissue is formed [377, 378]. Reports indicate that the use of skin substitutes results in favourable outcomes in patients with burn injuries [379-382]. As Integra products are mostly crosslinked, a foreign body reaction ensures and may limit ECM proteins deposition [383].

A dehydrated human amnion membrane allograft from MiMedx Group, referred to as Epifix, is derived from the placenta and is composed of a connective tissue matrix and a basement membrane with various growth factors and cytokines present [384-386]. In addition, reports indicate that various ECM proteins as well; as tissue inhibitor of metalloproteinases are present [387, 388]. Reports show that Epiflex can enhance wound healing compared to various commonly used methods [389, 390]. Several clinical trials have shown the efficacy of Epiflex in the treatment of even resistant non-healing wounds [390-393]. A wound matrix referred to as OASIS, derived from porcine intestine and consisting of collagen and fibronectin can be used for the treatment of ulcers [394, 395]. This wound matrix also contains various growth factors and is readily available and has a comparative long shelf life to other biomaterials. An acellular dermal allograft referred to as Alloderm

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derived from cadaveric skin is mainly composed of collagens and basement membrane [396-398]. The components of Alloderm allow for the formation of new tissue similar in structure and function to the native tissue and thus avoid an immune response [398-400]. Both a freeze-dried as well as injectable formulas of Alloderm exists [397].

8.1. Growth Factor-Based Treatment of Wounds

Most advances in wound healing treatments have been based on the use of growth factors and skin grafting but this has not significantly reduced the occurrence of chronic wounds. One caveat of using growth factor-based therapies is the fact that exogenous addition of growth factors requires that they be replenished constantly, and this makes if expensive [401-403]. In simple terms, growth factors are involved in signaling cascades and therefore influence various cellular processes. Several other factors contribute to make the use of growth factors in wound healing difficult and these include low absorption and several reported side effects [404]. Recent advances in the use of biomaterials to carry growth factors to specific tissues has resulted in improvements in their delivery and therefore effectiveness [405]. The complexity of wound healing means that different growth factors are needed at specific stages of the process, thus delivery systems must ensure that the growth factor stability is maintained until the growth factor are needed. The growth factors and other biomolecules such as cytokines are then released at a specific site and time as when needed [401]. Several products have been developed and unfortunately many products show conflicting results in trials. One product that has been approved by the FDA is Regranex Gel, containing the human platelet-derived growth factor. Several other products contain different growth factors in different amounts. For example, Heberprot consist of recombinant human epidermal growth factor and is recommended for the treatment of foot ulcers caused by diabetes. Several delivery systems have been developed for growth factors and these included nanoparticles, scaffolds, and hydrogels. Nanocomposites from biopolymers and synthetic polymers have shown immense potential in many areas of regenerative medicine and tissue engineering applications such as wound healing. Blending the polymers and nanoparticles alter the properties of the polymers so that they can be easily used in wound dressings. Currently, blended polymers have found use in wound healing and the maintenance of tissue integrity [406, 407]. These delivery systems have been excellently reviewed elsewhere and are not the focus of this review. It is safe to say that the use of growth factors and their delivery systems in wound healing is gaining ground and very promising at the moment. Safety of the growth factors and the different delivery systems must continue to be evaluated.

8.2. Cell-Based Treatment of Wounds

As cells are the source of growth factors, chemokines and cytokines during wound healing, cell-based treatment of wounds has received a great deal of attention from scientists and clinicians. With chronic wounds remaining unhealed, there is a potential of using different cell preparation, either as a supply of growth factors and other biomolecules or as a way to induce regenerate damaged tissue. One of the cell-based products available for the treatment of dermal burns is Epicel. It is made up of layers of keratinocytes supported by gauze. Results however are disappointing with several studies showing conflicting on the use of Epicel for the treatment of burn wounds. In addition, Epicel has been associated with contracture and scar formation [408]. A layer of keratinocytes, fibroblasts and the ECM protein type I collagen is used to make Apligraf. Cells release numerous factors that have been shown to enhance wound healing. The layer of cells and the collagen is only viable for a few days and is expensive to produce. Apligraf has been approved for the treatment of ulcers and enhancing the healing of surgical wounds [409, 410]. A layer of the scaffold poly (lactic-co-glycolic acid) and fibroblasts makes up the dermal substitute called Dermagraft. The scaffold can be absorbed and is seeded with the fibroblasts that secrete ECM proteins as well growth factors and cytokines [411, 412]. The FDA and the EMA have approved Dermagraft as a treatment strategy for ulcers and non-

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healing surgical wounds [413, 414]. Grafix is a cryopreserved human amnion allograft containing several cells such as mesenchymal stem cells, fibroblasts, epithelial cells, and ECM proteins [415, 416]. The cryopreservation maintains a very high cell viability and Grafix currently being used as a treatment strategy for leg ulcers, burns and chronic wounds among other conditions [417]. Various other cell-based strategies are under development for use in wound healing. The use cells to enhance wound healing faces major problems including lack of cellular engraftment due to low oxygen and nutrients levels within the wound microenvironment. To increase the possibility of cells being engrafted, Kim and colleagues, developed a biocompatible patch that formed spheroids incorporating fibroblasts in order to enhance interaction with macrophages as well as induce angiogenesis [418]. The authors demonstrated the utility of the patch versus the use of fibroblasts alone [418].

Electricity has been shown to play a crucial role in maintenance of tissue homeostasis as well as influencing various cellular processes such as wound healing. The development of skin patches that can generate electrical pulses in response to glucose has been reported [419]. Using various strategies including an animal model of skin wound healing, Kim and colleagues demonstrated that an enzymatic biofuel cell skin patch enhances the process of wound healing via induction of angiogenesis and impacting on ECM synthesis by fibroblasts [419]. Studies including the above mentioned one reveal that electricity can influence ion channels and enhance wound healing in the process.

9. Conclusion

The complexity of wound healing demonstrated by the various cells involved, the ECM proteins, the various biomolecules released into the wound microenvironment and the stage-wise progression means that there is still more information about the process that must be studied and revealed. All these parts of the wound healing process come together to form a 'wound healing microenvironment' that is dynamic and difficult to target during treatment. Even the study of single cells only determines what happens to a single cell, whereas in the wound healing microenvironment, all components interact on a temporal basis.

Most cells within the skin display great heterogeneity owing to the skin covering the whole body. In its natural state the skin has a specific set of cells that can be activated in case of injury. Important in this category are stem cells found in various parts of the skin where they are known to self-renew and to differentiate into various cells found within the skin. Whether stem cells are the source of all or even subtypes of cells is still unclear. Cellular heterogeneity has been studied especially within the epidermis. In addition, various immune cells are also found within the skin where they prevent infection or are involved in clearing germs when an injury occurs. Research is underway to understand how all these cells interact with each other as well as other external factors such as hypoxia and physical factors. The multitude of signaling cascades activated in a homeostatic skin and when injury occurs still requires determination. Furthermore, whilst literature presents a 'simplified' version of wound healing with various stages, the real process has overlapping stages. Finally, whilst both growth factor- and cell-based therapies have shown promising results with some already approved by regulatory bodies such as the FDA, the cells producing the growth factors are heterogeneous in nature. To be effective, subsets of the cells with specific properties must be used to produce growth factors and for cell therapy.

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References

1. Afaq, F., *Natural agents: cellular and molecular mechanisms of photoprotection.* Archives of biochemistry and biophysics, 2011. **508**(2): p. 144-151.
2. Panich, U., et al., *Ultraviolet radiation-induced skin aging: the role of DNA damage and oxidative stress in epidermal stem cell damage mediated skin aging.* Stem cells international, 2016. **2016**.
3. Fife, C.E. and M.J. Carter, *Wound care outcomes and associated cost among patients treated in US outpatient wound centers: data from the US wound registry.* Wounds: a compendium of clinical research and practice, 2012. **24**(1): p. 10-17.
4. Leavitt, T., et al., *Scarless wound healing: finding the right cells and signals.* Cell and tissue research, 2016. **365**(3): p. 483-493.
5. Qing, C., *The molecular biology in wound healing & non-healing wound.* Chinese Journal of Traumatology, 2017. **20**(4): p. 189-193.
6. Nussbaum, S.R., et al., *An economic evaluation of the impact, cost, and medicare policy implications of chronic nonhealing wounds.* Value in Health, 2018. **21**(1): p. 27-32.
7. Baroni, A., et al., *Structure and function of the epidermis related to barrier properties.* Clinics in dermatology, 2012. **30**(3): p. 257-262.
8. Urciuolo, F., et al., *Bioengineered skin substitutes: the role of extracellular matrix and vascularization in the healing of deep wounds.* Journal of clinical medicine, 2019. **8**(12): p. 2083.
9. Mohamed-Ali, V., et al., *Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-α, in vivo.* The Journal of clinical endocrinology & metabolism, 1997. **82**(12): p. 4196-4200.
10. Porter, S.A., et al., *Abdominal subcutaneous adipose tissue: a protective fat depot?* Diabetes care, 2009. **32**(6): p. 1068-1075.
11. Nestle, F.O., et al., *Skin immune sentinels in health and disease.* Nature Reviews Immunology, 2009. **9**(10): p. 679-691.
12. Gurtner, G.C., et al., *Wound repair and regeneration.* Nature, 2008. **453**(7193): p. 314-321.
13. Clark, R., *Fibrin is a many splendored thing.* Journal of Investigative Dermatology, 2003. **121**: p. xxi-xxii.
14. Gethin, G., *Understanding the inflammatory process in wound healing.* British journal of community nursing, 2012. **17**(Sup3): p. S17-S22.
15. Wilgus, T.A., S. Roy, and J.C. McDaniel, *Neutrophils and wound repair: positive actions and negative reactions.* Advances in wound care, 2013. **2**(7): p. 379-388.
16. Park, J.E. and A. Barbul, *Understanding the role of immune regulation in wound healing.* The American Journal of Surgery, 2004. **187**(5): p. S11-S16.
17. Davies, L.C., et al., *Distinct bone marrow-derived and tissue-resident macrophage lineages proliferate at key stages during inflammation.* Nature communications, 2013. **4**(1): p. 1-10.
18. Ansell, D.M. and A. Izeta, *Pericytes in wound healing: friend or foe?* Experimental dermatology, 2015. **24**(11): p. 833-834.
19. Wu, Y., et al., *Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis.* Stem cells, 2007. **25**(10): p. 2648-2659.
20. Chen, L., et al., *Paracrine factors of mesenchymal stem cells recruit macrophages and endothelial lineage cells and enhance wound healing.* PloS one, 2008. **3**(4): p. e1886.
21. Kosaraju, R., et al., *Adipose-derived stem cell-seeded hydrogels increase endogenous progenitor cell recruitment and neovascularization in wounds.* Tissue Engineering Part A, 2016. **22**(3-4): p. 295-305.
22. Jolivel, V., et al., *Perivascular microglia promote blood vessel disintegration in the ischemic penumbra.* Acta neuropathologica, 2015. **129**(2): p. 279-295.
23. Rajsheker, S., et al., *Crosstalk between perivascular adipose tissue and blood vessels.* Current opinion in pharmacology, 2010. **10**(2): p. 191-196.
24. Darby, I.A., et al., *Fibroblasts and myofibroblasts in wound healing.* Clinical, cosmetic and investigational dermatology, 2014. **7**: p. 301.
25. Plikus, M.V., et al. *Epithelial stem cells and implications for wound repair*. in *Seminars in cell & developmental biology*. 2012. Elsevier.
26. Nakagawa, H., et al., *Human mesenchymal stem cells successfully improve skin-substitute wound healing.* British Journal of Dermatology, 2005. **153**(1): p. 29-36.
27. Donati, G., et al., *Wounding induces dedifferentiation of epidermal Gata6+ cells and acquisition of stem cell properties.* Nature cell biology, 2017. **19**(6): p. 603-613.
28. Rennert, R.C., et al., *Microfluidic single-cell transcriptional analysis rationally identifies novel surface marker profiles to enhance cell-based therapies.* Nature communications, 2016. **7**(1): p. 1-9.
29. Atalay, S., A. Coruh, and K. Deniz, *Stromal vascular fraction improves deep partial thickness burn wound healing.* Burns, 2014. **40**(7): p. 1375-1383.
30. Wallace, H.A., B.M. Basehore, and P.M. Zito, *Wound healing phases.* 2017.
31. Szpaderska, A.M. and L.A. DiPietro, *Inflammation in surgical wound healing: friend or foe?* Surgery, 2005. **137**(5): p. 571-573.
32. Gould, L.J. and A.T. Fulton, *Wound healing in older adults.* Rhode Island Medical Journal, 2016. **99**(2): p. 34.
33. Gould, L.J., P.M. Abadir, and E.F. White-Chu, *Age, frailty, and impaired wound healing.* Principles and practice of geriatric surgery, 2020: p. 465-482.
34. Walmsley, G.G., et al., *Scarless wound healing: chasing the holy grail.* Plastic and reconstructive surgery, 2015. **135**(3): p. 907-917.
35. Bran, G.M., et al., *Keloids: current concepts of pathogenesis.* International journal of molecular medicine, 2009. **24**(3): p. 283-293.
36. Duscher, D., et al., *Mechanotransduction and fibrosis.* Journal of biomechanics, 2014. **47**(9): p. 1997-2005.
37. Perez, R. and S.C. Davis, *Relevance of animal models for wound healing.* Wounds: a compendium of clinical research and practice, 2008. **20**(1): p. 3-8.

38. Takeo, M., W. Lee, and M. Ito, *Wound healing and skin regeneration.* Cold Spring Harb Perspect Med, 2015. **5**(1): p. a023267.
39. Zomer, H.D. and A.G. Trentin, *Skin wound healing in humans and mice: Challenges in translational research.* J Dermatol Sci, 2018. **90**(1): p. 3-12.
40. Grada, A., J. Mervis, and V. Falanga, *Research Techniques Made Simple: Animal Models of Wound Healing.* J Invest Dermatol, 2018. **138**(10): p. 2095-2105.e1.
41. Pastar, I., et al., *Descriptive vs mechanistic scientific approach to study wound healing and its inhibition: Is there a value of translational research involving human subjects?* Exp Dermatol, 2018. **27**(5): p. 551-562.
42. Kim, J., et al., *Integration of the Human Dermal Mast Cell into the Organotypic Co-culture Skin Model.* Methods Mol Biol, 2020. **2163**: p. 91-107.
43. Rikken, G., H. Niehues, and E.H. van den Bogaard, *Organotypic 3D Skin Models: Human Epidermal Equivalent Cultures from Primary Keratinocytes and Immortalized Keratinocyte Cell Lines.* Methods Mol Biol, 2020. **2154**: p. 45-61.
44. Galiano, R.D., et al., *Quantitative and reproducible murine model of excisional wound healing.* Wound Repair Regen, 2004. **12**(4): p. 485-92.
45. Tkalcević, V.I., et al., *Differential evaluation of excisional non-occluded wound healing in db/db mice.* Toxicol Pathol, 2009. **37**(2): p. 183-92.
46. Skover, G., *Cellular and biochemical dynamics of wound repair. Wound environment in collagen regeneration.* Clinics in podiatric medicine and surgery, 1991. **8**(4): p. 723-756.
47. Furie, B. and B.C. Furie, *Mechanisms of thrombus formation.* New England Journal of Medicine, 2008. **359**(9): p. 938-949.
48. Periayah, M.H., A.S. Halim, and A.Z.M. Saad, *Mechanism action of platelets and crucial blood coagulation pathways in hemostasis.* International journal of hematology-oncology and stem cell research, 2017. **11**(4): p. 319.
49. Sieggreen, M., *Healing of physical wounds.* The Nursing Clinics of North America, 1987. **22**(2): p. 439-447.
50. Junker, J.P., E. Caterson, and E. Eriksson, *The microenvironment of wound healing.* Journal of Craniofacial Surgery, 2013. **24**(1): p. 12-16.
51. Briquez, P.S., J.A. Hubbell, and M.M. Martino, *Extracellular matrix-inspired growth factor delivery systems for skin wound healing.* Advances in wound care, 2015. **4**(8): p. 479-489.
52. Godo, S. and H. Shimokawa, *Endothelial functions.* Arteriosclerosis, thrombosis, and vascular biology, 2017. **37**(9): p. e108-e114.
53. Naftalin, L.W. and J.A. Yagiela, *Vasoconstrictors: indications and precautions.* Dental Clinics, 2002. **46**(4): p. 733-746.
54. Higgins, T.S., et al., *Systematic review of topical vasoconstrictors in endoscopic sinus surgery.* The Laryngoscope, 2011. **121**(2): p. 422-432.
55. Sachinidis, A., et al., *The platelet-derived growth factor isomers, PDGF-AA, PDGF-AB and PDGF-BB, induce contraction of vascular smooth muscle cells by different intracellular mechanisms.* FEBS letters, 1990. **275**(1-2): p. 95-98.
56. Teller, P. and T.K. White, *The physiology of wound healing: injury through maturation.* Perioperative Nursing Clinics, 2011. **6**(2): p. 159-170.
57. Brewer, D.B., *Max Schultze (1865), G. Bizzozero (1882) and the discovery of the platelet.* British journal of haematology, 2006. **133**(3): p. 251-258.
58. Sorrentino, S., et al., *Roll, adhere, spread and contract: structural mechanics of platelet function.* European journal of cell biology, 2015. **94**(3-4): p. 129-138.
59. Golebiewska, E.M. and A.W. Poole, *Platelet secretion: From haemostasis to wound healing and beyond.* Blood reviews, 2015. **29**(3): p. 153-162.
60. Pradhan, S., et al., *The heterotrimeric G protein Gβ1 interacts with the catalytic subunit of protein phosphatase 1 and modulates G protein–coupled receptor signaling in platelets.* Journal of Biological Chemistry, 2017. **292**(32): p. 13133-13142.
61. Watson, S.P., *Platelet activation by extracellular matrix proteins in haemostasis and thrombosis.* Current pharmaceutical design, 2009. **15**(12): p. 1358-1372.
62. Nieswandt, B., D. Varga-Szabo, and M. Elvers, *Integrins in platelet activation.* Journal of Thrombosis and Haemostasis, 2009. **7**: p. 206-209.
63. Wagner, C.L., et al., *Analysis of GPIIb/IIIa receptor number by quantification of 7E3 binding to human platelets.* 1996.
64. Santoro, S.A., *Identification of a 160,000 dalton platelet membrane protein that mediates the initial divalent cation-dependent adhesion of platelets to collagen.* Cell, 1986. **46**(6): p. 913-920.
65. Spadoni, I., et al., *A gut-vascular barrier controls the systemic dissemination of bacteria.* Science, 2015. **350**(6262): p. 830-834.
66. Fox, J.E. and D.R. Phillips, *Inhibition of actin polymerization in blood platelets by cytochalasins.* Nature, 1981. **292**(5824): p. 650-652.
67. Sandmann, R. and S. Köster, *Topographic cues reveal two distinct spreading mechanisms in blood platelets.* Scientific reports, 2016. **6**(1): p. 1-11.
68. Blair, P. and R. Flaumenhaft, *Platelet α-granules: basic biology and clinical correlates.* Blood reviews, 2009. **23**(4): p. 177-189.
69. Rendu, F. and B. Brohard-Bohn, *The platelet release reaction: granules' constituents, secretion and functions.* Platelets, 2001. **12**(5): p. 261-273.
70. Andrews, R.K., et al., *Glycoprotein Ib–IX–V.* The international journal of biochemistry & cell biology, 2003. **35**(8): p. 1170-1174.
71. Rucker, D. and A.S. Dhamoon, *Physiology, thromboxane A2.* StatPearls [Internet], 2020.
72. Senzel, L., D.V. Gnatenko, and W.F. Bahou, *The platelet proteome.* Current opinion in hematology, 2009. **16**(5): p. 329.
73. Amable, P.R., et al., *Platelet-rich plasma preparation for regenerative medicine: optimization and quantification of cytokines and growth factors.* Stem cell research & therapy, 2013. **4**(3): p. 1-13.
74. Barrientos, S., et al., *Growth factors and cytokines in wound healing.* Wound repair and regeneration, 2008. **16**(5): p. 585-601.

 doi:10.20944/preprints202211.0170.v1

75. Eppley, B.L., J.E. Woodell, and J. Higgins, *Platelet quantification and growth factor analysis from platelet-rich plasma: implications for wound healing.* Plastic and reconstructive surgery, 2004. **114**(6): p. 1502-1508.
76. Lacci, K.M. and A. Dardik, *Platelet-rich plasma: support for its use in wound healing.* The Yale journal of biology and medicine, 2010. **83**(1): p. 1.
77. Maxson, S., et al., *Concise review: role of mesenchymal stem cells in wound repair.* Stem cells translational medicine, 2012. **1**(2): p. 142-149.
78. Ma, S., et al., *Immunobiology of mesenchymal stem cells.* Cell Death & Differentiation, 2014. **21**(2): p. 216-225.
79. Wang, S., et al., *Wound dressing model of human umbilical cord mesenchymal stem cells-alginates complex promotes skin wound healing by paracrine signaling.* Stem cells international, 2016. **2016**.
80. Fernandez-Moure, J.S., et al., *Platelet-rich plasma: a biomimetic approach to enhancement of surgical wound healing.* Journal of surgical research, 2017. **207**: p. 33-44.
81. Dzobo, K., *Multipotent Human Mesenchymal Stem/Stromal Cells: An Updated Review on Historical Background, Recent Trends and Advances in their Clinical Applications.* 2021.
82. Phinney, D.G., *Functional heterogeneity of mesenchymal stem cells: implications for cell therapy.* Journal of cellular biochemistry, 2012. **113**(9): p. 2806-2812.
83. Davies, P.F., *Hemodynamic shear stress and the endothelium in cardiovascular pathophysiology.* Nature clinical practice Cardiovascular medicine, 2009. **6**(1): p. 16-26.
84. Szpaderska, A.M., et al., *The effect of thrombocytopenia on dermal wound healing.* Journal of Investigative Dermatology, 2003. **120**(6): p. 1130-1137.
85. Opneja, A., S. Kapoor, and E.X. Stavrou, *Contribution of platelets, the coagulation and fibrinolytic systems to cutaneous wound healing.* Thromb Res, 2019. **179**: p. 56-63.
86. Rodrigues, M., et al., *Wound Healing: A Cellular Perspective.* Physiol Rev, 2019. **99**(1): p. 665-706.
87. Bergmeier, W. and R.O. Hynes, *Extracellular matrix proteins in hemostasis and thrombosis.* Cold Spring Harb Perspect Biol, 2012. **4**(2).
88. Bainbridge, P., *Wound healing and the role of fibroblasts.* Journal of wound care, 2013. **22**(8).
89. Negut, I., G. Dorcioman, and V. Grumezescu, *Scaffolds for Wound Healing Applications.* Polymers (Basel), 2020. **12**(9).
90. Wilkinson, H.N. and M.J. Hardman, *Wound healing: cellular mechanisms and pathological outcomes.* Open Biol, 2020. **10**(9): p. 200223.
91. Lansdown, A.B., *Calcium: a potential central regulator in wound healing in the skin.* Wound repair and regeneration, 2002. **10**(5): p. 271-285.
92. Bryan, N., et al., *Reactive oxygen species (ROS)–a family of fate deciding molecules pivotal in constructive inflammation and wound healing.* Eur Cell Mater, 2012. **24**(249): p. e65.
93. Jaeschke, H. and T. Hasegawa, *Role of neutrophils in acute inflammatory liver injury.* Liver International, 2006. **26**(8): p. 912-919.
94. Martins-Green, M., M. Petreaca, and L. Wang, *Chemokines and their receptors are key players in the orchestra that regulates wound healing.* Advances in wound care, 2013. **2**(7): p. 327-347.
95. Yates, C.C., et al., *Lack of CXC chemokine receptor 3 signaling leads to hypertrophic and hypercellular scarring.* The American journal of pathology, 2010. **176**(4): p. 1743-1755.
96. Feugate, J.E., et al., *The cxc chemokine cCAF stimulates differentiation of fibroblasts into myofibroblasts and accelerates wound closure.* The Journal of cell biology, 2002. **156**(1): p. 161-172.
97. Szaba, F.M. and S.T. Smiley, *Roles for thrombin and fibrin (ogen) in cytokine/chemokine production and macrophage adhesion in vivo.* Blood, The Journal of the American Society of Hematology, 2002. **99**(3): p. 1053-1059.
98. van der Vliet, A. and Y.M. Janssen-Heininger, *Hydrogen peroxide as a damage signal in tissue injury and inflammation: murderer, mediator, or messenger?* Journal of cellular biochemistry, 2014. **115**(3): p. 427-435.
99. Cordeiro, J.V. and A. Jacinto, *The role of transcription-independent damage signals in the initiation of epithelial wound healing.* Nature reviews Molecular cell biology, 2013. **14**(4): p. 249-262.
100. Wulff, B.C. and T.A. Wilgus, *Mast cell activity in the healing wound: more than meets the eye?* Experimental dermatology, 2013. **22**(8): p. 507-510.
101. Bankova, L.G., et al., *Mouse mast cell proteases 4 and 5 mediate epidermal injury through disruption of tight junctions.* The Journal of Immunology, 2014. **192**(6): p. 2812-2820.
102. Nishida, K., et al., *Mast cells play role in wound healing through the ZnT2/GPR39/IL-6 axis.* Scientific reports, 2019. **9**(1): p. 1-14.
103. Komi, D.E.A., K. Khomtchouk, and P.L. Santa Maria, *A review of the contribution of mast cells in wound healing: involved molecular and cellular mechanisms.* Clinical reviews in allergy & immunology, 2020. **58**(3): p. 298-312.
104. Su, Y. and A. Richmond, *Chemokine regulation of neutrophil infiltration of skin wounds.* Advances in wound care, 2015. **4**(11): p. 631-640.
105. Bonavita, O., M. Massara, and R. Bonecchi, *Chemokine regulation of neutrophil function in tumors.* Cytokine & growth factor reviews, 2016. **30**: p. 81-86.
106. Gillitzer, R. and M. Goebeler, *Chemokines in cutaneous wound healing.* Journal of leukocyte biology, 2001. **69**(4): p. 513-521.
107. Oskeritzian, C.A., *Mast cells and wound healing.* Advances in wound care, 2012. **1**(1): p. 23-28.
108. Singer, A.J. and R.A. Clark, *Cutaneous wound healing.* New England journal of medicine, 1999. **341**(10): p. 738-746.
109. Wang, J., *Neutrophils in tissue injury and repair.* Cell and tissue research, 2018. **371**(3): p. 531-539.

 doi:10.20944/preprints202211.0170.v1

110. Larouche, J., et al., *Immune regulation of skin wound healing: mechanisms and novel therapeutic targets.* Advances in wound care, 2018. **7**(7): p. 209-231.
111. Reeves, E., *Lu H, Jacobs HL, Messina CG, Bolsover S, Gabella G, Potma EO, Warley A, Roes J, Segal AW.* Killing activity of neutrophils is mediated through activation of proteases by K+ flux. Nature, 2002. **416**: p. 291-297.
112. Belaaouaj, A., et al., *Mice lacking neutrophil elastase reveal impaired host defense against gram negative bacterial sepsis.* Nature medicine, 1998. **4**(5): p. 615-618.
113. Segel, G.B., M.W. Halterman, and M.A. Lichtman, *The paradox of the neutrophil's role in tissue injury.* Journal of leukocyte biology, 2011. **89**(3): p. 359-372.
114. Jorch, S.K. and P. Kubes, *An emerging role for neutrophil extracellular traps in noninfectious disease.* Nature medicine, 2017. **23**(3): p. 279-287.
115. Nordenfelt, P. and H. Tapper, *Phagosome dynamics during phagocytosis by neutrophils.* Journal of leukocyte biology, 2011. **90**(2): p. 271-284.
116. Levin, R., S. Grinstein, and J. Canton, *The life cycle of phagosomes: formation, maturation, and resolution.* Immunological reviews, 2016. **273**(1): p. 156-179.
117. Lee, W.L., R.E. Harrison, and S. Grinstein, *Phagocytosis by neutrophils.* Microbes and infection, 2003. **5**(14): p. 1299-1306.
118. Faurschou, M. and N. Borregaard, *Neutrophil granules and secretory vesicles in inflammation.* Microbes and infection, 2003. **5**(14): p. 1317-1327.
119. Burg, N.D. and M.H. Pillinger, *The neutrophil: function and regulation in innate and humoral immunity.* Clinical immunology, 2001. **99**(1): p. 7-17.
120. Bratton, D.L. and P.M. Henson, *Neutrophil clearance: when the party is over, clean-up begins.* Trends in immunology, 2011. **32**(8): p. 350-357.
121. Witko-Sarsat, V., et al., *Regulating neutrophil apoptosis: new players enter the game.* Trends in immunology, 2011. **32**(3): p. 117-124.
122. De Oliveira, S., E.E. Rosowski, and A. Huttenlocher, *Neutrophil migration in infection and wound repair: going forward in reverse.* Nature Reviews Immunology, 2016. **16**(6): p. 378.
123. Wang, J., et al., *Visualizing the function and fate of neutrophils in sterile injury and repair.* Science, 2017. **358**(6359): p. 111-116.
124. Jablonski, K.A., et al., *Novel markers to delineate murine M1 and M2 macrophages.* PloS one, 2015. **10**(12): p. e0145342.
125. Ambarus, C.A., et al., *Systematic validation of specific phenotypic markers for in vitro polarized human macrophages.* Journal of immunological methods, 2012. **375**(1-2): p. 196-206.
126. He, L. and A.G. Marneros, *Macrophages are essential for the early wound healing response and the formation of a fibrovascular scar.* The American journal of pathology, 2013. **182**(6): p. 2407-2417.
127. Dipietro, L.A., et al., *Modulation of macrophage recruitment into wounds by monocyte chemoattractant protein-1.* Wound Repair and Regeneration, 2001. **9**(1): p. 28-33.
128. Olingy, C.E., et al., *Non-classical monocytes are biased progenitors of wound healing macrophages during soft tissue injury.* Scientific reports, 2017. **7**(1): p. 1-16.
129. Das, A., et al., *Monocyte and macrophage plasticity in tissue repair and regeneration.* The American journal of pathology, 2015. **185**(10): p. 2596-2606.
130. Wynn, T.A. and K.M. Vannella, *Macrophages in tissue repair, regeneration, and fibrosis.* Immunity, 2016. **44**(3): p. 450-462.
131. Gentek, R., K. Molawi, and M.H. Sieweke, *Tissue macrophage identity and self-renewal.* Immunological reviews, 2014. **262**(1): p. 56-73.
132. Singla, D.K., J. Wang, and R. Singla, *Primary human monocytes differentiate into M2 macrophages and involve Notch-1 pathway.* Canadian journal of physiology and pharmacology, 2017. **95**(3): p. 288-294.
133. Sordet, O., et al., *Specific involvement of caspases in the differentiation of monocytes into macrophages.* Blood, The Journal of the American Society of Hematology, 2002. **100**(13): p. 4446-4453.
134. Chatterjee, M., et al., *Platelet-derived CXCL12 regulates monocyte function, survival, differentiation into macrophages and foam cells through differential involvement of CXCR4–CXCR7.* Cell death & disease, 2015. **6**(11): p. e1989-e1989.
135. DiPietro, L.A., et al., *Modulation of JE/MCP-1 expression in dermal wound repair.* The American journal of pathology, 1995. **146**(4): p. 868.
136. Lucas, T., et al., *Differential roles of macrophages in diverse phases of skin repair.* The Journal of Immunology, 2010. **184**(7): p. 3964-3977.
137. Zhu, Z., et al., *Systemic depletion of macrophages in the subacute phase of wound healing reduces hypertrophic scar formation.* Wound Repair and Regeneration, 2016. **24**(4): p. 644-656.
138. Hu, M.S., et al., *Delivery of monocyte lineage cells in a biomimetic scaffold enhances tissue repair.* JCI insight, 2017. **2**(19).
139. Navarrete, M., et al., *Interferon-γ, interleukins-6 and-4, and factor XIII-A as indirect markers of the classical and alternative macrophage activation pathways in chronic periodontitis.* Journal of periodontology, 2014. **85**(5): p. 751-760.
140. Garaicoa-Pazmino, C., et al., *Characterization of macrophage polarization in periodontal disease.* Journal of clinical periodontology, 2019. **46**(8): p. 830-839.
141. Slauch, J.M., *How does the oxidative burst of macrophages kill bacteria? Still an open question.* Molecular microbiology, 2011. **80**(3): p. 580-583.
142. Fang, F.C., *Antimicrobial actions of reactive oxygen species.* MBio, 2011. **2**(5).
143. Sorokin, L., *The impact of the extracellular matrix on inflammation.* Nature Reviews Immunology, 2010. **10**(10): p. 712-723.
144. Takeuchi, O. and S. Akira, *Pattern recognition receptors and inflammation.* Cell, 2010. **140**(6): p. 805-820.

 doi:10.20944/preprints202211.0170.v1

145. Mills, C., *M1 and M2 macrophages: oracles of health and disease.* Critical Reviews™ in Immunology, 2012. **32**(6).
146. Fantin, A., et al., *Tissue macrophages act as cellular chaperones for vascular anastomosis downstream of VEGF-mediated endothelial tip cell induction.* Blood, The Journal of the American Society of Hematology, 2010. **116**(5): p. 829-840.
147. Jetten, N., et al., *Anti-inflammatory M2, but not pro-inflammatory M1 macrophages promote angiogenesis in vivo.* Angiogenesis, 2014. **17**(1): p. 109-118.
148. Outtz, H.H., et al., *Notch1 controls macrophage recruitment and Notch signaling is activated at sites of endothelial cell anastomosis during retinal angiogenesis in mice.* Blood, The Journal of the American Society of Hematology, 2011. **118**(12): p. 3436-3439.
149. Willenborg, S., et al., *CCR2 recruits an inflammatory macrophage subpopulation critical for angiogenesis in tissue repair.* Blood, 2012. **120**(3): p. 613-625.
150. Martin, J., et al., *The dialogue between endothelial cells and monocytes/macrophages in vascular syndromes.* Current pharmaceutical design, 2007. **13**(17): p. 1751-1759.
151. Zhu, Z., et al., *Alternatively activated macrophages derived from THP-1 cells promote the fibrogenic activities of human dermal fibroblasts.* Wound Repair and Regeneration, 2017. **25**(3): p. 377-388.
152. Pakshir, P. and B. Hinz, *The big five in fibrosis: Macrophages, myofibroblasts, matrix, mechanics, and miscommunication.* Matrix Biology, 2018. **68**: p. 81-93.
153. Meng, X.M., et al., *Inflammatory macrophages can transdifferentiate into myofibroblasts during renal fibrosis.* Cell Death Dis, 2016. **7**(12): p. e2495.
154. Tang, P.M., D.J. Nikolic-Paterson, and H.Y. Lan, *Macrophages: versatile players in renal inflammation and fibrosis.* Nat Rev Nephrol, 2019. **15**(3): p. 144-158.
155. Wong, V.W., et al., *Mechanical force prolongs acute inflammation via T-cell-dependent pathways during scar formation.* Faseb j, 2011. **25**(12): p. 4498-510.
156. Suga, H., et al., *Tracking the elusive fibrocyte: identification and characterization of collagen-producing hematopoietic lineage cells during murine wound healing.* Stem Cells, 2014. **32**(5): p. 1347-60.
157. Shapouri-Moghaddam, A., et al., *Macrophage plasticity, polarization, and function in health and disease.* J Cell Physiol, 2018. **233**(9): p. 6425-6440.
158. Thorsson, V., et al., *The Immune Landscape of Cancer.* Immunity, 2018. **48**(4): p. 812-830.e14.
159. Lech, M. and H.J. Anders, *Macrophages and fibrosis: How resident and infiltrating mononuclear phagocytes orchestrate all phases of tissue injury and repair.* Biochim Biophys Acta, 2013. **1832**(7): p. 989-97.
160. Weidenbusch, M. and H.J. Anders, *Tissue microenvironments define and get reinforced by macrophage phenotypes in homeostasis or during inflammation, repair and fibrosis.* J Innate Immun, 2012. **4**(5-6): p. 463-77.
161. Weiskirchen, R., S. Weiskirchen, and F. Tacke, *Organ and tissue fibrosis: Molecular signals, cellular mechanisms and translational implications.* Mol Aspects Med, 2019. **65**: p. 2-15.
162. Boyce, D.E., et al., *Inflammatory-cell subpopulations in keloid scars.* Br J Plast Surg, 2001. **54**(6): p. 511-6.
163. Wernig, G., et al., *Unifying mechanism for different fibrotic diseases.* Proc Natl Acad Sci U S A, 2017. **114**(18): p. 4757-4762.
164. Wynn, T.A. and L. Barron, *Macrophages: master regulators of inflammation and fibrosis.* Semin Liver Dis, 2010. **30**(3): p. 245-57.
165. Wetzler, C., et al., *Large and sustained induction of chemokines during impaired wound healing in the genetically diabetic mouse: prolonged persistence of neutrophils and macrophages during the late phase of repair.* Journal of Investigative Dermatology, 2000. **115**(2): p. 245-253.
166. Maruyama, K., et al., *Decreased macrophage number and activation lead to reduced lymphatic vessel formation and contribute to impaired diabetic wound healing.* Am J Pathol, 2007. **170**(4): p. 1178-91.
167. Khanna, S., et al., *Macrophage dysfunction impairs resolution of inflammation in the wounds of diabetic mice.* PloS one, 2010. **5**(3): p. e9539.
168. Buchwalow, I., W. Boecker, and M. Tiemann, *The contribution of Paul Ehrlich to histochemistry: a tribute on the occasion of the centenary of his death.* Virchows Archiv, 2015. **466**(1): p. 111-116.
169. Ehrlich, P., *Beitrage zur Theorie und Praxis der Histologischen Farbung [doctoral thesis].* Germany: University of Leipzig, 1878.
170. Metcalfe, D.D., D. Baram, and Y.A. Mekori, *Mast cells.* Physiological reviews, 1997.
171. Siebenhaar, F., et al., *Control of Pseudomonas aeruginosa skin infections in mice is mast cell-dependent.* Am J Pathol, 2007. **170**(6): p. 1910-6.
172. Wang, Z., et al., *Skin mast cells protect mice against vaccinia virus by triggering mast cell receptor S1PR2 and releasing antimicrobial peptides.* J Immunol, 2012. **188**(1): p. 345-57.
173. Younan, G., et al., *The inflammatory response after an epidermal burn depends on the activities of mouse mast cell proteases 4 and 5.* J Immunol, 2010. **185**(12): p. 7681-90.
174. Weller, K., et al., *Mast cells are required for normal healing of skin wounds in mice.* Faseb j, 2006. **20**(13): p. 2366-8.
175. Wilgus, T.A. and B.C. Wulff, *The Importance of Mast Cells in Dermal Scarring.* Adv Wound Care (New Rochelle), 2014. **3**(4): p. 356-365.
176. Wulff, B.C., et al., *Mast cells contribute to scar formation during fetal wound healing.* J Invest Dermatol, 2012. **132**(2): p. 458-65.
177. Rao, K.N. and M.A. Brown, *Mast cells: multifaceted immune cells with diverse roles in health and disease.* Ann N Y Acad Sci, 2008. **1143**: p. 83-104.
178. Nakano, T., et al., *Fate of bone marrow-derived cultured mast cells after intracutaneous, intraperitoneal, and intravenous transfer into genetically mast cell-deficient W/Wv mice. Evidence that cultured mast cells can give rise to both connective tissue type and mucosal mast cells.* J Exp Med, 1985. **162**(3): p. 1025-43.

 doi:10.20944/preprints202211.0170.v1

179. Wulff, B.C. and T.A. Wilgus, *Mast cell activity in the healing wound: more than meets the eye?* Exp Dermatol, 2013. **22**(8): p. 507-10.
180. Douaiher, J., et al., *Development of mast cells and importance of their tryptase and chymase serine proteases in inflammation and wound healing.* Adv Immunol, 2014. **122**: p. 211-52.
181. Sasaki, A., et al., *Mast cells: an unexpected finding in the modulation of cutaneous wound repair by charged beads.* Plast Reconstr Surg, 2003. **111**(4): p. 1446-53.
182. Komi, D.E.A., K. Khomtchouk, and P.L. Santa Maria, *A Review of the Contribution of Mast Cells in Wound Healing: Involved Molecular and Cellular Mechanisms.* Clin Rev Allergy Immunol, 2020. **58**(3): p. 298-312.
183. Romani, N., et al., *Langerhans cells - dendritic cells of the epidermis.* Apmis, 2003. **111**(7-8): p. 725-40.
184. Steinman, R.M. and Z.A. Cohn, *Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution.* J Exp Med, 1973. **137**(5): p. 1142-62.
185. Steinman, R.M., et al., *Dendritic cells are the principal stimulators of the primary mixed leukocyte reaction in mice.* J Exp Med, 1983. **157**(2): p. 613-27.
186. Hume, D.A., *Macrophages as APC and the dendritic cell myth.* J Immunol, 2008. **181**(9): p. 5829-35.
187. Tamoutounour, S., et al., *Origins and functional specialization of macrophages and of conventional and monocyte-derived dendritic cells in mouse skin.* Immunity, 2013. **39**(5): p. 925-38.
188. Harman, A.N., et al., *Identification of lineage relationships and novel markers of blood and skin human dendritic cells.* J Immunol, 2013. **190**(1): p. 66-79.
189. Kissenpfennig, A., et al., *Dynamics and function of Langerhans cells in vivo: dermal dendritic cells colonize lymph node areas distinct from slower migrating Langerhans cells.* Immunity, 2005. **22**(5): p. 643-54.
190. Ahrens, S., et al., *F-actin is an evolutionarily conserved damage-associated molecular pattern recognized by DNGR-1, a receptor for dead cells.* Immunity, 2012. **36**(4): p. 635-45.
191. Zhang, J.G., et al., *The dendritic cell receptor Clec9A binds damaged cells via exposed actin filaments.* Immunity, 2012. **36**(4): p. 646-57.
192. Heath, W.R. and F.R. Carbone, *The skin-resident and migratory immune system in steady state and memory: innate lymphocytes, dendritic cells and T cells.* Nat Immunol, 2013. **14**(10): p. 978-85.
193. Edwards, A.D., et al., *Toll-like receptor expression in murine DC subsets: lack of TLR7 expression by CD8 alpha+ DC correlates with unresponsiveness to imidazoquinolines.* Eur J Immunol, 2003. **33**(4): p. 827-33.
194. Merad, M., F. Ginhoux, and M. Collin, *Origin, homeostasis and function of Langerhans cells and other langerin-expressing dendritic cells.* Nat Rev Immunol, 2008. **8**(12): p. 935-47.
195. Mackenzie, I.C., *Relationship between mitosis and the ordered structure of the stratum corneum in mouse epidermis.* Nature, 1970. **226**(5246): p. 653-5.
196. Merad, M., et al., *Langerhans cells renew in the skin throughout life under steady-state conditions.* Nat Immunol, 2002. **3**(12): p. 1135-41.
197. Malissen, B., S. Tamoutounour, and S. Henri, *The origins and functions of dendritic cells and macrophages in the skin.* Nat Rev Immunol, 2014. **14**(6): p. 417-28.
198. Gregorio, J., et al., *Plasmacytoid dendritic cells sense skin injury and promote wound healing through type I interferons.* J Exp Med, 2010. **207**(13): p. 2921-30.
199. Wollenberg, A., et al., *Plasmacytoid dendritic cells: a new cutaneous dendritic cell subset with distinct role in inflammatory skin diseases.* J Invest Dermatol, 2002. **119**(5): p. 1096-102.
200. Conrad, C., S. Meller, and M. Gilliet, *Plasmacytoid dendritic cells in the skin: to sense or not to sense nucleic acids.* Semin Immunol, 2009. **21**(3): p. 101-9.
201. Chen, L., et al., *The dynamic changes of CD3e(-)CD11c(+) dendritic cells in spleens and bone marrow of mice infected with Schistosoma japonicum.* Parasitol Res, 2017. **116**(3): p. 1007-1011.
202. Ebrahimi-Nik, H., et al., *CD11c(+) MHCII(lo) GM-CSF-bone marrow-derived dendritic cells act as antigen donor cells and as antigen presenting cells in neoepitope-elicited tumor immunity against a mouse fibrosarcoma.* Cancer Immunol Immunother, 2018. **67**(9): p. 1449-1459.
203. Bourdely, P., et al., *Transcriptional and Functional Analysis of CD1c(+) Human Dendritic Cells Identifies a CD163(+) Subset Priming CD8(+)CD103(+) T Cells.* Immunity, 2020. **53**(2): p. 335-352.e8.
204. Roberts, E.W., et al., *Critical Role for CD103(+)/CD141(+) Dendritic Cells Bearing CCR7 for Tumor Antigen Trafficking and Priming of T Cell Immunity in Melanoma.* Cancer Cell, 2016. **30**(2): p. 324-336.
205. Dutertre, C.A., et al., *Single-Cell Analysis of Human Mononuclear Phagocytes Reveals Subset-Defining Markers and Identifies Circulating Inflammatory Dendritic Cells.* Immunity, 2019. **51**(3): p. 573-589.e8.
206. Papalexi, E. and R. Satija, *Single-cell RNA sequencing to explore immune cell heterogeneity.* Nat Rev Immunol, 2018. **18**(1): p. 35-45.
207. Villani, A.C., et al., *Single-cell RNA-seq reveals new types of human blood dendritic cells, monocytes, and progenitors.* Science, 2017. **356**(6335).
208. Bos, J.D., et al., *T-cell receptor gamma delta bearing cells in normal human skin.* J Invest Dermatol, 1990. **94**(1): p. 37-42.
209. Yamaguchi, Y., et al., *Enhanced angiogenic potency of monocytic endothelial progenitor cells in patients with systemic sclerosis.* Arthritis Res Ther, 2010. **12**(6): p. R205.
210. Gray, E.E., K. Suzuki, and J.G. Cyster, *Cutting edge: Identification of a motile IL-17-producing gammadelta T cell population in the dermis.* J Immunol, 2011. **186**(11): p. 6091-5.
211. Keyes, B.E., et al., *Impaired Epidermal to Dendritic T Cell Signaling Slows Wound Repair in Aged Skin.* Cell, 2016. **167**(5): p. 1323-1338.e14.

212. Jameson, J., et al., *A role for skin gammadelta T cells in wound repair.* Science, 2002. **296**(5568): p. 747-9.
213. Witherden, D.A., et al., *The CD100 receptor interacts with its plexin B2 ligand to regulate epidermal γδ T cell function.* Immunity, 2012. **37**(2): p. 314-25.
214. Havran, W.L. and J.M. Jameson, *Epidermal T cells and wound healing.* J Immunol, 2010. **184**(10): p. 5423-8.
215. Jameson, J.M., et al., *A keratinocyte-responsive gamma delta TCR is necessary for dendritic epidermal T cell activation by damaged keratinocytes and maintenance in the epidermis.* J Immunol, 2004. **172**(6): p. 3573-9.
216. Li, Y., et al., *Functions of Vγ4 T Cells and Dendritic Epidermal T Cells on Skin Wound Healing.* Front Immunol, 2018. **9**: p. 1099.
217. Sumaria, N., et al., *Cutaneous immunosurveillance by self-renewing dermal gammadelta T cells.* J Exp Med, 2011. **208**(3): p. 505-18.
218. Nakamizo, S., et al., *Dermal Vγ4(+) γδ T cells possess a migratory potency to the draining lymph nodes and modulate CD8(+) T-cell activity through TNF-α production.* J Invest Dermatol, 2015. **135**(4): p. 1007-1015.
219. Jameson, J. and W.L. Havran, *Skin gammadelta T-cell functions in homeostasis and wound healing.* Immunol Rev, 2007. **215**: p. 114-22.
220. Jiang, X., et al., *Dermal γδ T Cells Do Not Freely Re-Circulate Out of Skin and Produce IL-17 to Promote Neutrophil Infiltration during Primary Contact Hypersensitivity.* PLoS One, 2017. **12**(1): p. e0169397.
221. Gebhardt, T., et al., *Different patterns of peripheral migration by memory CD4+ and CD8+ T cells.* Nature, 2011. **477**(7363): p. 216-9.
222. Gilliam, A.C., *Scleroderma.* Curr Dir Autoimmun, 2008. **10**: p. 258-79.
223. Li, G., et al., *Skin-Resident Effector Memory CD8(+)CD28(-) T Cells Exhibit a Profibrotic Phenotype in Patients with Systemic Sclerosis.* J Invest Dermatol, 2017. **137**(5): p. 1042-1050.
224. Philip, M. and A. Schietinger, *Heterogeneity and fate choice: T cell exhaustion in cancer and chronic infections.* Curr Opin Immunol, 2019. **58**: p. 98-103.
225. Yu, X., et al., *Unravelling the heterogeneity and dynamic relationships of tumor-infiltrating T cells by single-cell RNA sequencing analysis.* J Leukoc Biol, 2020. **107**(6): p. 917-932.
226. Zhu, J., *T Helper Cell Differentiation, Heterogeneity, and Plasticity.* Cold Spring Harb Perspect Biol, 2018. **10**(10).
227. Tschachler, E., et al., *Dendritic epidermal T cells: activation requirements and phenotypic characterization of proliferating cells.* J Invest Dermatol, 1989. **92**(5): p. 763-8.
228. Steiner, G., et al., *Characterization of T cell receptors on resident murine dendritic epidermal T cells.* Eur J Immunol, 1988. **18**(9): p. 1323-8.
229. Broughton, G., 2nd, J.E. Janis, and C.E. Attinger, *Wound healing: an overview.* Plast Reconstr Surg, 2006. **117**(7 Suppl): p. 1e-S-32e-S.
230. Carlson, M.A. and J.S. Thompson, *Wound splinting modulates granulation tissue proliferation.* Matrix Biol, 2004. **23**(4): p. 243-50.
231. Gurtner, G.C., et al., *Wound repair and regeneration.* Nature, 2008. **453**(7193): p. 314-21.
232. Yokoyama, H., *Initiation of limb regeneration: the critical steps for regenerative capacity.* Dev Growth Differ, 2008. **50**(1): p. 13-22.
233. Eilken, H.M. and R.H. Adams, *Dynamics of endothelial cell behavior in sprouting angiogenesis.* Curr Opin Cell Biol, 2010. **22**(5): p. 617-25.
234. Fukuhara, S., *[Live Imaging of Angiogenesis during Wound Healing].* Yakugaku Zasshi, 2020. **140**(4): p. 513-519.
235. Sorg, H., et al., *Skin Wound Healing: An Update on the Current Knowledge and Concepts.* Eur Surg Res, 2017. **58**(1-2): p. 81-94.
236. Veith, A.P., et al., *Therapeutic strategies for enhancing angiogenesis in wound healing.* Adv Drug Deliv Rev, 2019. **146**: p. 97-125.
237. Ferguson, J.E., 3rd, R.W. Kelley, and C. Patterson, *Mechanisms of endothelial differentiation in embryonic vasculogenesis.* Arterioscler Thromb Vasc Biol, 2005. **25**(11): p. 2246-54.
238. Asahara, T., et al., *Isolation of putative progenitor endothelial cells for angiogenesis.* Science, 1997. **275**(5302): p. 964-7.
239. Tonnesen, M.G., X. Feng, and R.A. Clark, *Angiogenesis in wound healing.* J Investig Dermatol Symp Proc, 2000. **5**(1): p. 40-6.
240. Gerhardt, H., et al., *VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia.* J Cell Biol, 2003. **161**(6): p. 1163-77.
241. Herbert, S.P. and D.Y. Stainier, *Molecular control of endothelial cell behaviour during blood vessel morphogenesis.* Nat Rev Mol Cell Biol, 2011. **12**(9): p. 551-64.
242. Naito, H., T. Iba, and N. Takakura, *Mechanisms of new blood-vessel formation and proliferative heterogeneity of endothelial cells.* Int Immunol, 2020. **32**(5): p. 295-305.
243. Hellström, M., et al., *Dll4 signalling through Notch1 regulates formation of tip cells during angiogenesis.* Nature, 2007. **445**(7129): p. 776-80.
244. Suchting, S., et al., *The Notch ligand Delta-like 4 negatively regulates endothelial tip cell formation and vessel branching.* Proceedings of the National Academy of Sciences, 2007. **104**(9): p. 3225-3230.
245. Liu, Z.J., et al., *Regulation of Notch1 and Dll4 by vascular endothelial growth factor in arterial endothelial cells: implications for modulating arteriogenesis and angiogenesis.* Mol Cell Biol, 2003. **23**(1): p. 14-25.
246. Ruhrberg, C., et al., *Spatially restricted patterning cues provided by heparin-binding VEGF-A control blood vessel branching morphogenesis.* Genes Dev, 2002. **16**(20): p. 2684-98.
247. Siekmann, A.F. and N.D. Lawson, *Notch signalling and the regulation of angiogenesis.* Cell Adh Migr, 2007. **1**(2): p. 104-6.
248. Bodnar, R.J., *Chemokine Regulation of Angiogenesis During Wound Healing.* Adv Wound Care (New Rochelle), 2015. **4**(11): p. 641-650.
249. Satish, L., *Chemokines as Therapeutic Targets to Improve Healing Efficiency of Chronic Wounds.* Adv Wound Care (New Rochelle), 2015. **4**(11): p. 651-659.
250. Balaji, S., et al., *Chemokine Involvement in Fetal and Adult Wound Healing.* Adv Wound Care (New Rochelle), 2015. **4**(11): p. 660-672.

 doi:10.20944/preprints202211.0170.v1

251. Weller, A., S. Isenmann, and D. Vestweber, *Cloning of the mouse endothelial selectins. Expression of both E- and P-selectin is inducible by tumor necrosis factor alpha.* J Biol Chem, 1992. **267**(21): p. 15176-83.
252. Gotsch, U., et al., *Expression of P-selectin on endothelial cells is upregulated by LPS and TNF-alpha in vivo.* Cell Adhes Commun, 1994. **2**(1): p. 7-14.
253. Sugama, Y. and A.B. Malik, *Thrombin receptor 14-amino acid peptide mediates endothelial hyperadhesivity and neutrophil adhesion by P-selectin-dependent mechanism.* Circ Res, 1992. **71**(4): p. 1015-9.
254. Nagaoka, T., et al., *Delayed wound healing in the absence of intercellular adhesion molecule-1 or L-selectin expression.* Am J Pathol, 2000. **157**(1): p. 237-47.
255. Subramaniam, M., et al., *Role of endothelial selectins in wound repair.* Am J Pathol, 1997. **150**(5): p. 1701-9.
256. Folkman, J. and M. Klagsbrun, *Angiogenic factors.* Science, 1987. **235**(4787): p. 442-7.
257. Cheresh, D.A., et al., *Recognition of distinct adhesive sites on fibrinogen by related integrins on platelets and endothelial cells.* Cell, 1989. **58**(5): p. 945-53.
258. Attwell, D., et al., *What is a pericyte?* Journal of Cerebral Blood Flow & Metabolism, 2016. **36**(2): p. 451-455.
259. Ribatti, D., B. Nico, and E. Crivellato, *The role of pericytes in angiogenesis.* International Journal of Developmental Biology, 2011. **55**(3): p. 261-268.
260. Armulik, A., G. Genové, and C. Betsholtz, *Pericytes: developmental, physiological, and pathological perspectives, problems, and promises.* Dev Cell, 2011. **21**(2): p. 193-215.
261. Hall, C.N., et al., *Capillary pericytes regulate cerebral blood flow in health and disease.* Nature, 2014. **508**(7494): p. 55-60.
262. Crisan, M., et al., *A perivascular origin for mesenchymal stem cells in multiple human organs.* Cell Stem Cell, 2008. **3**(3): p. 301-13.
263. Caplan, A.I., *All MSCs are pericytes?* Cell Stem Cell, 2008. **3**(3): p. 229-30.
264. Crisan, M., et al., *Perivascular multipotent progenitor cells in human organs.* Ann N Y Acad Sci, 2009. **1176**: p. 118-23.
265. Trost, A., et al., *Brain and retinal pericytes: origin, function and role.* Frontiers in cellular neuroscience, 2016. **10**: p. 20.
266. Yamazaki, T. and Y.-s. Mukouyama, *Tissue specific origin, development, and pathological perspectives of pericytes.* Frontiers in Cardiovascular Medicine, 2018. **5**: p. 78.
267. Prazeres, P.H.D.M., et al., *Pericytes are heterogeneous in their origin within the same tissue.* Developmental biology, 2017. **427**(1): p. 6-11.
268. Nehls, V. and D. Drenckhahn, *Heterogeneity of microvascular pericytes for smooth muscle type alpha-actin.* J Cell Biol, 1991. **113**(1): p. 147-54.
269. Dore-Duffy, P. and K. Cleary, *Morphology and properties of pericytes.* The blood-brain and other neural barriers, 2011: p. 49-68.
270. Díaz-Flores, L., et al., *Microvascular pericytes, a review of their morphological and functional characteristics.* Histology and histopathology, 1991.
271. Birbrair, A., et al., *How plastic are pericytes?* Stem cells and development, 2017. **26**(14): p. 1013-1019.
272. Stopak, D. and A.K. Harris, *Connective tissue morphogenesis by fibroblast traction: I. Tissue culture observations.* Developmental biology, 1982. **90**(2): p. 383-398.
273. Alberts, B., et al., *Fibroblasts and their transformations: the connective-tissue cell family*, in *Molecular Biology of the Cell. 4th edition*. 2002, Garland Science.
274. Fries, K.M., et al., *Evidence of fibroblast heterogeneity and the role of fibroblast subpopulations in fibrosis.* Clin Immunol Immunopathol, 1994. **72**(3): p. 283-92.
275. Darby, I.A. and T.D. Hewitson, *Fibroblast differentiation in wound healing and fibrosis.* Int Rev Cytol, 2007. **257**: p. 143-79.
276. Darby, I.A., et al., *Fibroblasts and myofibroblasts in wound healing.* Clin Cosmet Investig Dermatol, 2014. **7**: p. 301-11.
277. Jinno, H., et al., *Convergent genesis of an adult neural crest-like dermal stem cell from distinct developmental origins.* Stem Cells, 2010. **28**(11): p. 2027-40.
278. Driskell, R.R., et al., *Distinct fibroblast lineages determine dermal architecture in skin development and repair.* Nature, 2013. **504**(7479): p. 277-281.
279. Lee, J. and T. Tumbar, *Hairy tale of signaling in hair follicle development and cycling.* Semin Cell Dev Biol, 2012. **23**(8): p. 906-16.
280. Plikus, M.V., et al., *Regeneration of fat cells from myofibroblasts during wound healing.* Science, 2017. **355**(6326): p. 748-752.
281. Senthebane, D.A., et al., *The Role of Tumor Microenvironment in Chemoresistance: To Survive, Keep Your Enemies Closer.* Int J Mol Sci, 2017. **18**(7).
282. Mishra, P.J. and D. Banerjee, *Activation and Differentiation of Mesenchymal Stem Cells.* Methods Mol Biol, 2017. **1554**: p. 201-209.
283. Tomasek, J.J., et al., *Myofibroblasts and mechano-regulation of connective tissue remodelling.* Nat Rev Mol Cell Biol, 2002. **3**(5): p. 349-63.
284. Schultz, G.S., et al., *Dynamic reciprocity in the wound microenvironment.* Wound Repair Regen, 2011. **19**(2): p. 134-48.
285. Simpson, R.M., et al., *Age-related changes in pericellular hyaluronan organization leads to impaired dermal fibroblast to myofibroblast differentiation.* Am J Pathol, 2009. **175**(5): p. 1915-28.
286. Leask, A., *Potential therapeutic targets for cardiac fibrosis: TGFbeta, angiotensin, endothelin, CCN2, and PDGF, partners in fibroblast activation.* Circ Res, 2010. **106**(11): p. 1675-80.
287. Grinnell, F., *Fibroblasts, myofibroblasts, and wound contraction.* J Cell Biol, 1994. **124**(4): p. 401-4.
288. Sappino, A.P., W. Schürch, and G. Gabbiani, *Differentiation repertoire of fibroblastic cells: expression of cytoskeletal proteins as marker of phenotypic modulations.* Lab Invest, 1990. **63**(2): p. 144-61.
289. Rinkevich, Y., et al., *Skin fibrosis. Identification and isolation of a dermal lineage with intrinsic fibrogenic potential.* Science, 2015. **348**(6232): p. aaa2151.

290. Hinz, B., et al., *Recent developments in myofibroblast biology: paradigms for connective tissue remodeling.* Am J Pathol, 2012. **180**(4): p. 1340-55.
291. Serini, G., et al., *The fibronectin domain ED-A is crucial for myofibroblastic phenotype induction by transforming growth factor-beta1.* J Cell Biol, 1998. **142**(3): p. 873-81.
292. Obland, G.F., *The fine structure of the interrelationship of cells in the human epidermis.* The Journal of Cell Biology, 1958. **4**(5): p. 529-538.
293. Lindemann, B. and C. Voute, *Structure and function of the epidermis*, in *Frog neurobiology*. 1976, Springer. p. 169-210.
294. Mascré, G., et al., *Distinct contribution of stem and progenitor cells to epidermal maintenance.* Nature, 2012. **489**(7415): p. 257-62.
295. Hobbs, R.M., et al., *Expression of activated MEK1 in differentiating epidermal cells is sufficient to generate hyperproliferative and inflammatory skin lesions.* J Invest Dermatol, 2004. **123**(3): p. 503-15.
296. Reynolds, L.E., et al., *alpha3beta1 integrin-controlled Smad7 regulates reepithelialization during wound healing in mice.* J Clin Invest, 2008. **118**(3): p. 965-74.
297. Savagner, P., et al., *Developmental transcription factor slug is required for effective re-epithelialization by adult keratinocytes.* J Cell Physiol, 2005. **202**(3): p. 858-66.
298. Pastar, I., et al., *Epithelialization in Wound Healing: A Comprehensive Review.* Adv Wound Care (New Rochelle), 2014. **3**(7): p. 445-464.
299. Nakamura, K., I.R. Williams, and T.S. Kupper, *Keratinocyte-derived monocyte chemoattractant protein 1 (MCP-1): analysis in a transgenic model demonstrates MCP-1 can recruit dendritic and Langerhans cells to skin.* J Invest Dermatol, 1995. **105**(5): p. 635-43.
300. Brown, L.F., et al., *Expression of vascular permeability factor (vascular endothelial growth factor) by epidermal keratinocytes during wound healing.* J Exp Med, 1992. **176**(5): p. 1375-9.
301. Costin, G.E. and V.J. Hearing, *Human skin pigmentation: melanocytes modulate skin color in response to stress.* Faseb j, 2007. **21**(4): p. 976-94.
302. Cichorek, M., et al., *Skin melanocytes: biology and development.* Advances in Dermatology and Allergology/Postępy Dermatologii I Alergologii, 2013. **30**(1): p. 30.
303. Solanas, G. and S.A. Benitah, *Regenerating the skin: a task for the heterogeneous stem cell pool and surrounding niche.* Nat Rev Mol Cell Biol, 2013. **14**(11): p. 737-48.
304. Tanimura, S., et al., *Hair follicle stem cells provide a functional niche for melanocyte stem cells.* Cell Stem Cell, 2011. **8**(2): p. 177-87.
305. Matsumura, H., et al., *Hair follicle aging is driven by transepidermal elimination of stem cells via COL17A1 proteolysis.* Science, 2016. **351**(6273): p. aad4395.
306. Snell, R.S., *A study of the melanocytes and melanin in a healing deep wound.* J Anat, 1963. **97**(Pt 2): p. 243-53.
307. Chou, W.C., et al., *Direct migration of follicular melanocyte stem cells to the epidermis after wounding or UVB irradiation is dependent on Mc1r signaling.* Nat Med, 2013. **19**(7): p. 924-9.
308. Hirobe, T., *Proliferation of epidermal melanocytes during the healing of skin wounds in newborn mice.* J Exp Zool, 1983. **227**(3): p. 423-31.
309. Gao, F.L., et al., *The contribution of melanocytes to pathological scar formation during wound healing.* Int J Clin Exp Med, 2013. **6**(7): p. 609-13.
310. Sirimahachaiyakul, P., et al., *Race Does Not Predict Melanocyte Heterogeneous Responses to Dermal Fibroblast-Derived Mediators.* PLoS One, 2015. **10**(9): p. e0139135.
311. Haukipuro, K., et al., *Synthesis of type I collagen in healing wounds in humans.* Annals of surgery, 1991. **213**(1): p. 75.
312. Caley, M.P., V.L. Martins, and E.A. O'Toole, *Metalloproteinases and Wound Healing.* Adv Wound Care (New Rochelle), 2015. **4**(4): p. 225-234.
313. Visse, R. and H. Nagase, *Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry.* Circ Res, 2003. **92**(8): p. 827-39.
314. Gill, S.E., et al., *A null mutation for tissue inhibitor of metalloproteinases-3 (Timp-3) impairs murine bronchiole branching morphogenesis.* Dev Biol, 2003. **261**(2): p. 313-23.
315. Telgenhoff, D. and B. Shroot, *Cellular senescence mechanisms in chronic wound healing.* Cell Death Differ, 2005. **12**(7): p. 695-8.
316. Hinz, B. and G. Gabbiani, *Cell-matrix and cell-cell contacts of myofibroblasts: role in connective tissue remodeling.* Thromb Haemost, 2003. **90**(6): p. 993-1002.
317. Azevedo, P.O., et al., *Endothelial cells maintain neural stem cells quiescent in their niche.* Neuroscience, 2017. **363**: p. 62-65.
318. Robles, D.T. and D. Berg, *Abnormal wound healing: keloids.* Clin Dermatol, 2007. **25**(1): p. 26-32.
319. Tuan, T.L. and L.S. Nichter, *The molecular basis of keloid and hypertrophic scar formation.* Mol Med Today, 1998. **4**(1): p. 19-24.
320. Slemp, A.E. and R.E. Kirschner, *Keloids and scars: a review of keloids and scars, their pathogenesis, risk factors, and management.* Curr Opin Pediatr, 2006. **18**(4): p. 396-402.
321. Xue, M. and C.J. Jackson, *Extracellular matrix reorganization during wound healing and its impact on abnormal scarring.* Advances in wound care, 2015. **4**(3): p. 119-136.
322. Aarabi, S., M.T. Longaker, and G.C. Gurtner, *Hypertrophic scar formation following burns and trauma: new approaches to treatment.* PLoS Med, 2007. **4**(9): p. e234.
323. Wang, M.L., et al., *Peripheral nerve injury, scarring, and recovery.* Connective tissue research, 2019. **60**(1): p. 3-9.
324. Alster, T.S. and E.L. Tanzi, *Hypertrophic scars and keloids.* American journal of clinical dermatology, 2003. **4**(4): p. 235-243.
325. Deitch, E.A., et al., *Hypertrophic burn scars: analysis of variables.* The Journal of trauma, 1983. **23**(10): p. 895-898.

 doi:10.20944/preprints202211.0170.v1

326. Wolfram, D., et al., *Hypertrophic scars and keloids—a review of their pathophysiology, risk factors, and therapeutic management.* Dermatologic surgery, 2009. **35**(2): p. 171-181.
327. Ud-Din, S. and A. Bayat, *New insights on keloids, hypertrophic scars, and striae.* Dermatologic clinics, 2014. **32**(2): p. 193-209.
328. Michael, K.E., et al., *Focal adhesion kinase modulates cell adhesion strengthening via integrin activation.* Molecular biology of the cell, 2009. **20**(9): p. 2508-2519.
329. Wen, H., P.A. Blume, and B.E. Sumpio, *Role of integrins and focal adhesion kinase in the orientation of dermal fibroblasts exposed to cyclic strain.* International wound journal, 2009. **6**(2): p. 149-158.
330. Duscher, D., et al., *Mechanotransduction and fibrosis.* J Biomech, 2014. **47**(9): p. 1997-2005.
331. Ding, Q., et al., *Focal adhesion kinase (FAK)-related non-kinase inhibits myofibroblast differentiation through differential MAPK activation in a FAK-dependent manner.* J Biol Chem, 2008. **283**(40): p. 26839-49.
332. Chin, Y.R. and A. Toker, *Function of Akt/PKB signaling to cell motility, invasion and the tumor stroma in cancer.* Cell Signal, 2009. **21**(4): p. 470-6.
333. Duscher, D., et al., *Transdermal deferoxamine prevents pressure-induced diabetic ulcers.* Proc Natl Acad Sci U S A, 2015. **112**(1): p. 94-9.
334. Januszyk, M., et al., *The role of focal adhesion kinase in keratinocyte fibrogenic gene expression.* International journal of molecular sciences, 2017. **18**(9): p. 1915.
335. Liu, W., et al., *The Abnormal Architecture of Healed Diabetic Ulcers Is the Result of FAK Degradation by Calpain 1.* J Invest Dermatol, 2017. **137**(5): p. 1155-1165.
336. Wong, V.W., et al., *Loss of keratinocyte focal adhesion kinase stimulates dermal proteolysis through upregulation of MMP9 in wound healing.* Ann Surg, 2014. **260**(6): p. 1138-46.
337. Florin, L., et al., *Increased keratinocyte proliferation by JUN-dependent expression of PTN and SDF-1 in fibroblasts.* J Cell Sci, 2005. **118**(Pt 9): p. 1981-9.
338. Frykberg, R.G. and J. Banks, *Challenges in the Treatment of Chronic Wounds.* Adv Wound Care (New Rochelle), 2015. **4**(9): p. 560-582.
339. *Management of chronic pressure ulcers: an evidence-based analysis.* Ont Health Technol Assess Ser, 2009. **9**(3): p. 1-203.
340. Kranke, P., et al., *Hyperbaric oxygen therapy for chronic wounds.* Cochrane Database Syst Rev, 2004(2): p. Cd004123.
341. Januszyk, M., et al., *Diabetes irreversibly depletes bone marrow-derived mesenchymal progenitor cell subpopulations.* Diabetes, 2014. **63**(9): p. 3047-56.
342. Rodrigues, M., et al., *Progenitor cell dysfunctions underlie some diabetic complications.* Am J Pathol, 2015. **185**(10): p. 2607-18.
343. Wetzler, C., et al., *Large and sustained induction of chemokines during impaired wound healing in the genetically diabetic mouse: prolonged persistence of neutrophils and macrophages during the late phase of repair.* J Invest Dermatol, 2000. **115**(2): p. 245-53.
344. Roy, S., et al., *Dermal wound healing is subject to redox control.* Molecular therapy, 2006. **13**(1): p. 211-220.
345. Sen, C.K., *The general case for redox control of wound repair.* Wound repair and regeneration, 2003. **11**(6): p. 431-438.
346. Lerman, O.Z., et al., *Cellular dysfunction in the diabetic fibroblast: impairment in migration, vascular endothelial growth factor production, and response to hypoxia.* Am J Pathol, 2003. **162**(1): p. 303-12.
347. Hu, S.C. and C.E. Lan, *High-glucose environment disturbs the physiologic functions of keratinocytes: Focusing on diabetic wound healing.* J Dermatol Sci, 2016. **84**(2): p. 121-127.
348. Grice, E.A. and J.A. Segre, *The skin microbiome.* Nat Rev Microbiol, 2011. **9**(4): p. 244-53.
349. Grice, E.A., et al., *A diversity profile of the human skin microbiota.* Genome Res, 2008. **18**(7): p. 1043-50.
350. Chiller, K., B.A. Selkin, and G.J. Murakawa, *Skin microflora and bacterial infections of the skin.* J Investig Dermatol Symp Proc, 2001. **6**(3): p. 170-4.
351. Kong, H.H. and J.A. Segre, *Skin microbiome: looking back to move forward.* J Invest Dermatol, 2012. **132**(3 Pt 2): p. 933-9.
352. Vijaya Chandra, S.H., et al., *Cutaneous Malassezia: Commensal, Pathogen, or Protector?* Front Cell Infect Microbiol, 2020. **10**: p. 614446.
353. Kalan, L.R. and M.B. Brennan, *The role of the microbiome in nonhealing diabetic wounds.* Annals of the New York Academy of Sciences, 2019. **1435**(1): p. 79-92.
354. Høiby, N., et al., *The clinical impact of bacterial biofilms.* Int J Oral Sci, 2011. **3**(2): p. 55-65.
355. Parsek, M.R. and P.K. Singh, *Bacterial biofilms: an emerging link to disease pathogenesis.* Annu Rev Microbiol, 2003. **57**: p. 677-701.
356. Demidova-Rice, T.N., J.T. Durham, and I.M. Herman, *Wound healing angiogenesis: innovations and challenges in acute and chronic wound healing.* Advances in wound care, 2012. **1**(1): p. 17-22.
357. Okonkwo, U.A. and L.A. DiPietro, *Diabetes and wound angiogenesis.* International journal of molecular sciences, 2017. **18**(7): p. 1419.
358. DiPietro, L.A., *Angiogenesis and wound repair: when enough is enough.* Journal of leukocyte biology, 2016. **100**(5): p. 979-984.
359. Plafki, C., et al., *Complications and side effects of hyperbaric oxygen therapy.* Aviat Space Environ Med, 2000. **71**(2): p. 119-24.
360. Rennert, R.C., et al., *Biological therapies for the treatment of cutaneous wounds: phase III and launched therapies.* Expert Opin Biol Ther, 2013. **13**(11): p. 1523-41.
361. Romanelli, M., V. Dini, and M.S. Bertone, *Randomized comparison of OASIS wound matrix versus moist wound dressing in the treatment of difficult-to-heal wounds of mixed arterial/venous etiology.* Adv Skin Wound Care, 2010. **23**(1): p. 34-8.
362. Veves, A., P. Sheehan, and H.T. Pham, *A randomized, controlled trial of Promogran (a collagen/oxidized regenerated cellulose dressing) vs standard treatment in the management of diabetic foot ulcers.* Arch Surg, 2002. **137**(7): p. 822-7.

363. Wieman, T.J., J.M. Smiell, and Y. Su, *Efficacy and safety of a topical gel formulation of recombinant human platelet-derived growth factor-BB (becaplermin) in patients with chronic neuropathic diabetic ulcers. A phase III randomized placebo-controlled double-blind study.* Diabetes Care, 1998. **21**(5): p. 822-7.
364. Marler, J.J., et al., *Transplantation of cells in matrices for tissue regeneration.* Adv Drug Deliv Rev, 1998. **33**(1-2): p. 165-182.
365. Dzobo, K., K. Motaung, and A. Adesida, *Recent Trends in Decellularized Extracellular Matrix Bioinks for 3D Printing: An Updated Review.* Int J Mol Sci, 2019. **20**(18).
366. Yukna, R.A., D.W. Turner, and L.J. Robinson, *Variable antigenicity of lyophilized allogeneic and lyophilized xenogeneic skin in guinea pigs.* J Periodontal Res, 1977. **12**(3): p. 197-203.
367. Khoshnood, N. and A. Zamanian, *Decellularized extracellular matrix bioinks and their application in skin tissue engineering.* Bioprinting, 2020: p. e00095.
368. Halim, A.S., T.L. Khoo, and S.J.M. Yussof, *Biologic and synthetic skin substitutes: an overview.* Indian journal of plastic surgery: official publication of the Association of Plastic Surgeons of India, 2010. **43**(Suppl): p. S23.
369. Kawai, K., et al., *Accelerated tissue regeneration through incorporation of basic fibroblast growth factor-impregnated gelatin microspheres into artificial dermis.* Biomaterials, 2000. **21**(5): p. 489-99.
370. Sun, G., et al., *Dextran hydrogel scaffolds enhance angiogenic responses and promote complete skin regeneration during burn wound healing.* Proc Natl Acad Sci U S A, 2011. **108**(52): p. 20976-81.
371. Huang, S., et al., *Wound dressings containing bFGF-impregnated microspheres.* J Microencapsul, 2006. **23**(3): p. 277-90.
372. Takemoto, S., et al., *Preparation of collagen/gelatin sponge scaffold for sustained release of bFGF.* Tissue Eng Part A, 2008. **14**(10): p. 1629-38.
373. Garcia, Y., et al., *Assessment of cell viability in a three-dimensional enzymatically cross-linked collagen scaffold.* Journal of Materials Science: Materials in Medicine, 2007. **18**(10): p. 1991-2001.
374. Davidenko, N., et al., *Control of crosslinking for tailoring collagen-based scaffolds stability and mechanics.* Acta Biomaterialia, 2015. **25**: p. 131-142.
375. Kubow, K.E., et al., *Crosslinking of cell-derived 3D scaffolds up-regulates the stretching and unfolding of new extracellular matrix assembled by reseeded cells.* Integrative Biology, 2009. **1**(11-12): p. 635-648.
376. Frame, J.D., et al., *Use of dermal regeneration template in contracture release procedures: a multicenter evaluation.* Plast Reconstr Surg, 2004. **113**(5): p. 1330-8.
377. Huss, F.R., et al., *Characterization of a new degradable polymer scaffold for regeneration of the dermis: In vitro and in vivo human studies.* Organogenesis, 2008. **4**(3): p. 195-200.
378. Boyce, S.T. and G.D. Warden, *Principles and practices for treatment of cutaneous wounds with cultured skin substitutes.* Am J Surg, 2002. **183**(4): p. 445-56.
379. Dantzer, E. and F.M. Braye, *Reconstructive surgery using an artificial dermis (Integra): results with 39 grafts.* Br J Plast Surg, 2001. **54**(8): p. 659-64.
380. Haertsch, P., *Reconstructive surgery using an artificial dermis (Integra).* Br J Plast Surg, 2002. **55**(4): p. 362-3.
381. Unglaub, F., D. Ulrich, and N. Pallua, *[Reconstructive surgery using an artificial dermis (Integra): results with 19 grafts].* Zentralbl Chir, 2005. **130**(2): p. 157-61.
382. Suchánek, I., et al., *Reconstructive surgeries after extensive burns in children.* Acta Chir Plast, 2003. **45**(4): p. 139-43.
383. Truong, A.T., et al., *Comparison of dermal substitutes in wound healing utilizing a nude mouse model.* J Burns Wounds, 2005. **4**: p. e4.
384. Koob, T.J., et al., *Properties of dehydrated human amnion/chorion composite grafts: Implications for wound repair and soft tissue regeneration.* J Biomed Mater Res B Appl Biomater, 2014. **102**(6): p. 1353-62.
385. Koob, T.J., et al., *Angiogenic properties of dehydrated human amnion/chorion allografts: therapeutic potential for soft tissue repair and regeneration.* Vasc Cell, 2014. **6**: p. 10.
386. Koob, T.J., et al., *Biological properties of dehydrated human amnion/chorion composite graft: implications for chronic wound healing.* Int Wound J, 2013. **10**(5): p. 493-500.
387. Rössner, E., et al., *Epiflex® a new decellularised human skin tissue transplant: manufacture and properties.* Cell and tissue banking, 2011. **12**(3): p. 209-217.
388. Roessner, E.D., M. Vitacolonna, and P. Hohenberger, *Confocal laser scanning microscopy evaluation of an acellular dermis tissue transplant (Epiflex®).* PloS one, 2012. **7**(10): p. e45991.
389. Forbes, J. and D.E. Fetterolf, *Dehydrated amniotic membrane allografts for the treatment of chronic wounds: a case series.* J Wound Care, 2012. **21**(6): p. 290, 292, 294-6.
390. Sheikh, E.S., E.S. Sheikh, and D.E. Fetterolf, *Use of dehydrated human amniotic membrane allografts to promote healing in patients with refractory non healing wounds.* Int Wound J, 2014. **11**(6): p. 711-7.
391. Zelen, C.M., et al., *Treatment of chronic diabetic lower extremity ulcers with advanced therapies: a prospective, randomised, controlled, multi-centre comparative study examining clinical efficacy and cost.* Int Wound J, 2016. **13**(2): p. 272-82.
392. Zelen, C.M., et al., *A prospective, randomised, controlled, multi-centre comparative effectiveness study of healing using dehydrated human amnion/chorion membrane allograft, bioengineered skin substitute or standard of care for treatment of chronic lower extremity diabetic ulcers.* Int Wound J, 2015. **12**(6): p. 724-32.
393. Bianchi, C., et al., *A multicentre randomised controlled trial evaluating the efficacy of dehydrated human amnion/chorion membrane (EpiFix(®)) allograft for the treatment of venous leg ulcers.* Int Wound J, 2018. **15**(1): p. 114-122.
394. Mostow, E.N., et al., *Effectiveness of an extracellular matrix graft (OASIS Wound Matrix) in the treatment of chronic leg ulcers: a randomized clinical trial.* J Vasc Surg, 2005. **41**(5): p. 837-43.

395. Hodde, J.P., D.M. Ernst, and M.C. Hiles, *An investigation of the long-term bioactivity of endogenous growth factor in OASIS Wound Matrix.* J Wound Care, 2005. **14**(1): p. 23-5.
396. Shores, J.T., A. Gabriel, and S. Gupta, *Skin substitutes and alternatives: a review.* Adv Skin Wound Care, 2007. **20**(9 Pt 1): p. 493-508; quiz 509-10.
397. Sclafani, A.P., T. Romo, 3rd, and A.A. Jacono, *Rejuvenation of the aging lip with an injectable acellular dermal graft (Cymetra).* Arch Facial Plast Surg, 2002. **4**(4): p. 252-7.
398. Wainwright, D.J., *Use of an acellular allograft dermal matrix (AlloDerm) in the management of full-thickness burns.* Burns, 1995. **21**(4): p. 243-8.
399. Athavale, S.M., et al., *AlloDerm and DermaMatrix implants for parotidectomy reconstruction: a histologic study in the rat model.* Head Neck, 2013. **35**(2): p. 242-9.
400. Xu, H., et al., *Host response to human acellular dermal matrix transplantation in a primate model of abdominal wall repair.* Tissue Eng Part A, 2008. **14**(12): p. 2009-19.
401. Park, J.W., S.R. Hwang, and I.-S. Yoon, *Advanced Growth Factor Delivery Systems in Wound Management and Skin Regeneration.* Molecules, 2017. **22**(8): p. 1259.
402. Dzobo, K., et al., *The Future of Tissue Engineering and Regenerative Medicine in Africa.* Tissue Eng Part A, 2017. **23**(19-20): p. 1023-1025.
403. Dzobo, K., et al., *Advances in Regenerative Medicine and Tissue Engineering: Innovation and Transformation of Medicine.* Stem Cells Int, 2018. **2018**: p. 2495848.
404. Yamakawa, S. and K. Hayashida, *Advances in surgical applications of growth factors for wound healing.* Burns & trauma, 2019. **7**.
405. Wong, C., et al., *Fibrin-based biomaterials to deliver human growth factors.* Thrombosis and haemostasis, 2003. **89**(03): p. 573-582.
406. Liu, M., et al., *Electrospun nanofibers for wound healing.* Materials Science and Engineering: C, 2017. **76**: p. 1413-1423.
407. El-Aassar, M., et al., *Wound healing of nanofiber comprising Polygalacturonic/Hyaluronic acid embedded silver nanoparticles: In-vitro and in-vivo studies.* Carbohydrate polymers, 2020. **238**: p. 116175.
408. Varkey, M., J. Ding, and E.E. Tredget, *Advances in Skin Substitutes-Potential of Tissue Engineered Skin for Facilitating Anti-Fibrotic Healing.* J Funct Biomater, 2015. **6**(3): p. 547-63.
409. Eaglstein, W.H., M. Iriondo, and K. Laszlo, *A composite skin substitute (graftskin) for surgical wounds. A clinical experience.* Dermatol Surg, 1995. **21**(10): p. 839-43.
410. Hayes, D.W., Jr., et al., *Full-thickness burn of the foot: successful treatment with Apligraf. A case report.* Clin Podiatr Med Surg, 2001. **18**(1): p. 179-88.
411. Cooper, M.L., et al., *In vivo optimization of a living dermal substitute employing cultured human fibroblasts on a biodegradable polyglycolic acid or polyglactin mesh.* Biomaterials, 1991. **12**(2): p. 243-8.
412. Hart, C.E., A. Loewen-Rodriguez, and J. Lessem, *Dermagraft: Use in the Treatment of Chronic Wounds.* Adv Wound Care (New Rochelle), 2012. **1**(3): p. 138-141.
413. Joncas, V., et al., *The closure of a large chronic abdominal wound in a neonate utilizing a biologic dressing.* Adv Skin Wound Care, 2010. **23**(9): p. 404-5.
414. Hartmann-Fritsch, F., D. Marino, and E. Reichmann, *About ATMPs, SOPs and GMP: the hurdles to produce novel skin grafts for clinical use.* Transfusion Medicine and Hemotherapy, 2016. **43**(5): p. 344-352.
415. Bergmeier, V., et al., *Identification of a myofibroblast-specific expression signature in skin wounds.* Matrix Biol, 2018. **65**: p. 59-74.
416. Brewer, D.B., *Max Schultze (1865), G. Bizzozero (1882) and the discovery of the platelet.* Br J Haematol, 2006. **133**(3): p. 251-8.
417. Gibbons, G.W., *Grafix(®), a Cryopreserved Placental Membrane, for the Treatment of Chronic/Stalled Wounds.* Adv Wound Care (New Rochelle), 2015. **4**(9): p. 534-544.
418. Kim, S.W., et al., *Delivery of a spheroids-incorporated human dermal fibroblast sheet increases angiogenesis and M2 polarization for wound healing.* Biomaterials, 2021. **275**: p. 120954.
419. Kim, T.H., et al., *Electricity auto-generating skin patch promotes wound healing process by activation of mechanosensitive ion channels.* Biomaterials, 2021. **275**: p. 120948.