Review

Exploring the Role of Dermal Sheath Cells in Wound Healing and Fibrosis

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Review

Exploring the Role of Dermal Sheath Cells in Wound Healing and Fibrosis

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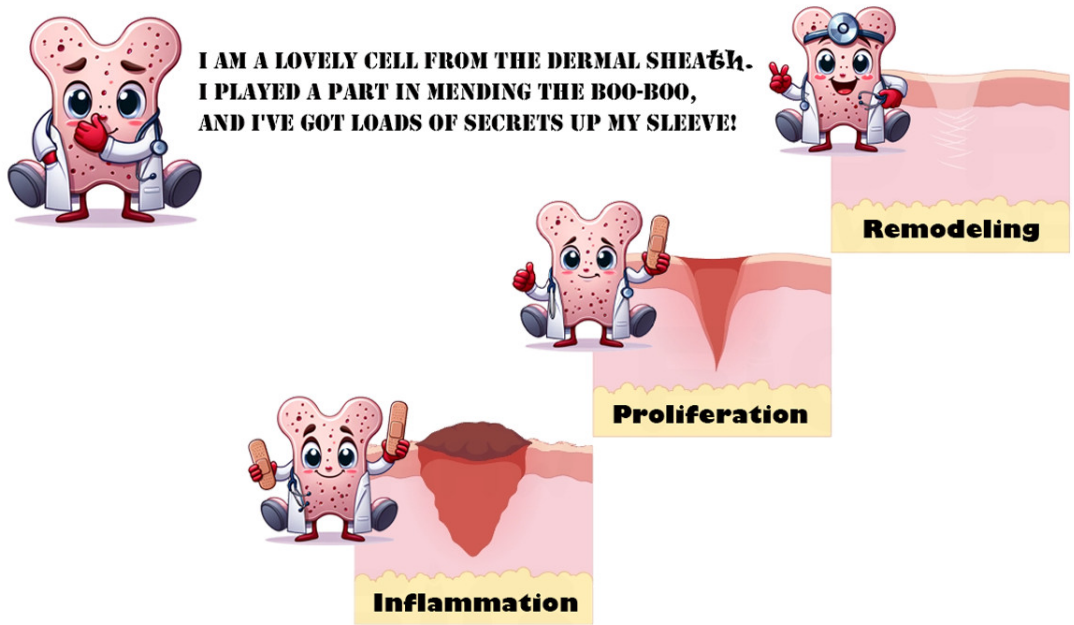
Abstract: Wound healing is a complex, dynamic process involving the coordinated interaction of diverse cell types, growth factors, cytokines, and extracellular matrix components. Despite emerging evidence highlighting their importance, dermal sheath cells remain a largely overlooked aspect of wound healing research. This review explores the multifunctional roles of dermal sheath cells in various phases of wound healing, including modulating inflammation, aiding in proliferation, and contributing to extracellular matrix remodeling. Special attention is devoted to the paracrine effects of dermal sheath cells and their role in fibrosis, highlighting their potential in improving healing outcomes, especially in differentiating between hairy and non-hairy skin sites. By drawing connections between dermal sheath cells activity and wound healing outcomes, this work proposes new insights into the mechanisms of tissue regeneration and repair, marking a step forward in our understanding of wound healing processes.

Keywords: Dermal sheath cell; Wound healing; Extracellular matrix; Fibrosis; Paracrine



This review brings to light the significant yet often overlooked role of dermal sheath cells (DSCs) in wound healing. It emphasizes their multifunctional roles in inflammation modulation, proliferation aid, and ECM remodeling, and illuminates DSCs' paracrine effects and their involvement in fibrosis, offering a new perspective on skin repair processes.

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Introduction

Wound healing in medical practice is challenging, requiring a multifaceted approach that includes both the body's natural healing processes and advanced therapies like cell transplantation and bioengineered skin. Overcoming complications such as infection, inadequate blood supply, and excessive inflammation is critical to prevent delayed healing, chronic wounds, or excessive scarring. The orchestration of key biological processes, such as cell migration, proliferation, and the secretion of the extracellular matrix (ECM) by skin cells, is essential for successful wound healing.

Dermal fibroblasts (DFs) have been widely studied in the mechanism of wound repair. They contain multiple subtypes stored mainly in the papillary and reticular dermis, exhibiting functional heterogeneity and undergoing significant dynamic changes during skin wound healing [1–3]. Currently, Dermal sheath cells (DSCs) are considered to be one of the subgroups of DFs [4–7]. The dermal sheath (DS) is a vital component of the hair follicle mesenchyme, situated at the outermost layer of the hair follicle and separated from epidermal cells by a basement membrane [8,9]. DSCs are known for their roles in hair follicle formation and cycling, acting as resident stem cells within the skin [5,10,11]. These cells have shown potential in modulating key processes in wound healing, such as inflammation, cell proliferation, and ECM remodeling.

Exploring the intricate roles of DSCs and their potential to modulate wound healing processes promises to advance strategies in wound care and regenerative medicine. By understanding how DSCs contribute to these processes, we can develop new therapeutic approaches to improve healing outcomes, particularly in distinguishing between hairy and non-hairy skin sites.

Background

Wound healing is typically divided into three overlapping phases: inflammation, proliferation, and remodeling [12,13]. During the inflammation phase, hemostasis is achieved through the activation of platelets, which form a clot to prevent excessive bleeding [14]. This clot provides a provisional matrix for subsequent cell migration and tissue repair [15,16]. Meanwhile, immune cells are recruited to the wound site to clear debris, neutralize pathogens, and release cytokines and growth factors that initiate the healing process [17–20]. The proliferation stage is marked by the migration and proliferation of diverse cell types, notably fibroblasts, endothelial cells, and keratinocytes [21]. Fibroblasts play a crucial role in producing and remodeling the ECM, which provides structural support to the healing tissue [22]. Endothelial cells contribute to angiogenesis to ensure sufficient blood supply to the affected area [23]. Keratinocytes migrate and proliferate to reestablish the epidermal barrier [24,25]. This stage also involves the breakdown of the provisional ECM formed during hemostasis, mediated by matrix metalloproteinases (MMPs) and proteinases secreted by fibroblasts, while concurrently, fibroblasts synthesize ECM proteins, contributing to the formation of granulation tissue [26]. In the final stage, remodeling, the newly formed ECM undergoes maturation and reorganization, enhancing the tensile strength of the healing tissue. Collagen fibers align along tension lines, and excessive scar tissue undergoes gradual remodeling, transitioning into a more organized and functional structure [27].

Epithelial-mesenchymal interactions are pivotal in the wound healing process. As depicted in Figure 1, skin epithelial and mesenchymal cells exhibit notable heterogeneity and plasticity. Post injury, an inflammatory response dominates the very beginning of the healing process. Under the sustained influence of Wnt/β-catenin signaling, reticular fibroblasts undergo significant activation, characterized by proliferation, migration, and synthesis of thick and well-organized collagen fibers [1,28]. Simultaneously, adipose precursor cells proliferate and evolve into mature adipocytes, collaboratively contributing to wound filling and granulation tissue formation [29]. Dermal papillary fibroblasts, in contrast, migrate into the wound site at a slightly later stage (during epithelialization) and produce poorly organized ECM [28]. Epidermal cells move into the healing wound by polymerizing cytoskeletal actin fibers in the outgrowth and forming new adhesion complexes, culminating in wound closure [30].

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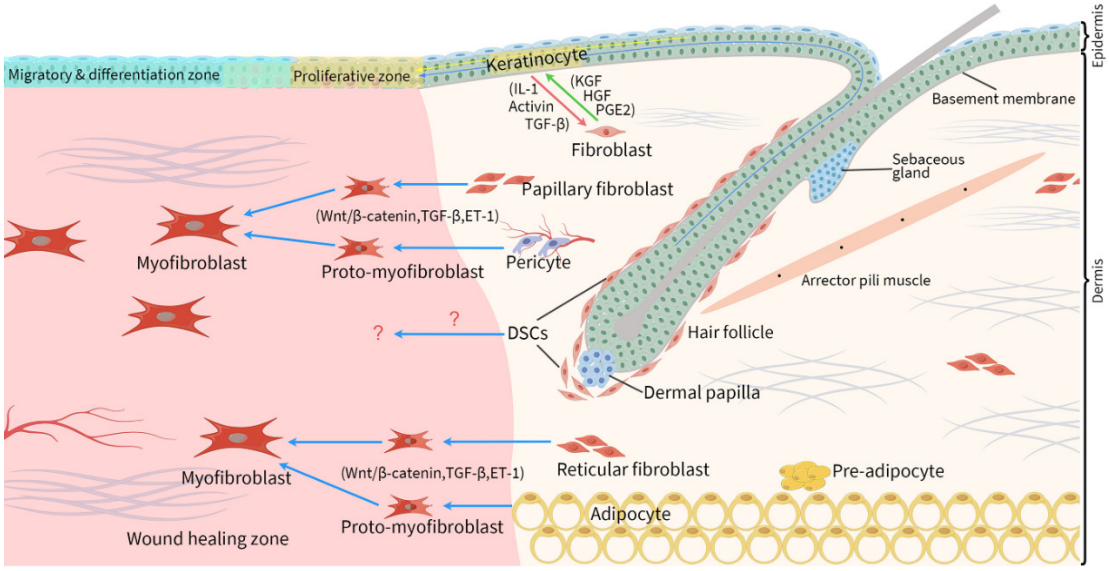

Figure 1. Heterogeneity and plasticity of skin epithelial and mesenchymal cells during wound healing. After an injury, mesenchymal cells and keratinocytes are recruited to the site. These cells begin proliferating, migrating, and either differentiating or dedifferentiating. The thick blue arrows highlight the migration and differentiation of mesenchymal cells, which include reticular fibroblasts, papillary fibroblasts, and adipocytes. The narrow arrows show the proliferation and migration of epithelial cells from adjacent epidermis and hair follicles. Reticular fibroblasts are among the first and most numerous fibroblasts to migrate to the wound area and serve as a primary source for the differentiation of myofibroblasts. Mesenchymal cells transform into myofibroblasts under the influence of TGF-β signaling. Keratinocytes, through the paracrine action of IL-1 and other cytokines, stimulate fibroblasts to produce many kinds of soluble factors, including KGF, HGF, and PGE2. These cytokines, in turn, can promote the proliferation, migration, and differentiation of keratinocytes, creating a synergistic interaction that aids in wound healing. Following the wound, some DSCs migrate into the granulation tissue. As wound healing concludes, some myofibroblasts may transform back into adipocytes and fibroblasts (not shown). DSCs, dermal sheath cells. (this diagram was made with Figdraw).

Initially, cytokines such as interleukin-1 (IL-1) from epidermal cells prompt adjacent DFs to synthesize and secrete a spectrum of growth factors (Figure 1), including keratinocyte growth factor (KGF), granulocyte macrophage colony-stimulating factor (GM-CSF), IL-6, IL-8, IL-1, hepatocyte growth factor (HGF), transforming growth factor-beta (TGF-β), and heparin-binding epidermal growth factor-like growth factor (HB-EGF), and to express cyclooxygenase 2 (COX2) and prostaglandin E2 (PGE2) [31,32]. In its turn, these soluble factors, mainly KGF, HGF, and PGE2 (Figure 1), enhance the proliferation, physiological differentiation and basement membrane deposition of epidermal cells [32]. In later stages, an increase in TGF-β-dependent gene expression and a decrease in nuclear factor kappa B (NF-κB) activation occur. Concurrently, under the combined influence of TGF-β and endothelin-1 (ET-1), a subset of DFs begins to express significant levels of alpha-smooth muscle actin (αSMA), transitioning into myofibroblasts [33]. This transition is crucial for establishing mechanical tension within the wound area [28]. At the same time, a delicate balance between pro-fibrotic and anti-fibrotic signals, cellular activities, and mechanical cues mitigates excessive fibrosis in the wound repair area.

Another crucial process is ECM remodeling, involving the synthesis and arrangement of collagen, elastin, and other constituents. In the early stages of wound healing, fibrin is deposited to create a temporary matrix clot. This clot helps to stop bleeding and provides a provisional scaffold for cell migration and tissue repair [34,35]. As the wound healing process progresses, fibroblasts migrate into the wound area and secrete new collagen, primarily Type III collagen, to replace the temporary fibrin clot. Type III collagen provides initial strength to the healing tissue. Over time, the Type III collagen is gradually replaced by Type I collagen, which is stronger and more durable [36,37].

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Disruptions in collagen synthesis, degradation, or organization can lead to impaired wound healing and the formation of abnormal scars.

Dermal Sheath Cells: Characteristics and Heterogeneity

DSCs are derived from the neural crest during embryonic development and are characterized by their distinct molecular markers and phenotypic features. Key markers used to sort DSCs include αSMA, SOX2 and platelet-derived growth factor receptor alpha (PDGFRα) [8,38,39]. Recent single-cell transcriptome sequencing (scRNA-seq) analyses have identified new markers for DSCs, such as *COL11A1* in humans,[2,6]. and *ACAN* and *ITGA8 in mice* [38,40]. DSCs are now recognized as resident stem cells in the skin [5,10,11], and their functions vary according to their specific locations. A key characteristic of DSCs is their ability to induce the formation of new hair follicles [5,41,42]. A particular subset of DSCs that remain after catagen and tightly wrap the telogen dermal papilla (DP) are a stem cell population that self-renews and replenishes the entire DS and contributes to the DP during the following anagen hair growth, and have been termed hair follicle dermal stem cells [8,40,43]. Additionally, in humans, DSCs derived from the lower part of the DS have been considered as hair follicle-derived mesenchymal stromal cells [44]. These cells express neural markers and possess stem cell properties, and are viewed as a promising source for immunomodulation [44]. There are also DSCs in humans that express CD36, undergo changes in location in accordance with the hair cycle, and appear to be involved in the process of angiogenesis [45]. Furthermore, DSCs are also linked to the production of ECM proteins.

In this review, we examined every subset and all name variations of DSCs across different species; however, the differences and similarities among them have not yet been fully explained. These include hair follicle dermal stem cells in mice [43,46]; hair follicle-derived mesenchymal stromal cells [44], CD36-expressing DSCs [45], and hair follicle dermal sheath mesenchymal stromal cells in humans [47]; and upper and lower follicle DSCs in rats [48].

Modulatory Potential of Dermal Sheath Cells in Inflammatory Responses

The inflammatory phase of wound healing is characterized by the activation of immune cells and the release of various inflammatory mediators. Recent research suggests that DSCs may play a crucial role in this phase, potentially influencing immune cell behavior and cytokine secretion, thereby modulating the inflammatory response and promoting tissue repair.

Studies have indicated that DSCs may influence the inflammatory response by secreting several cytokines and chemokines, like platelet-derived growth factor C (PDGF-C), PDGF-D, IL-6 and IL-8. [38,47]PDGFs drive cell recruitment to damaged tissue [49]. They initiate chemotaxis of neutrophils, macrophages, fibroblasts, and smooth muscle cells, facilitating the healing process to the inflammation stage [50,51]. A conditioned medium from human hair follicle dermal sheath mesenchymal stromal cells demonstrated enhanced wound-healing effects on human skin keratinocytes, fibroblasts and endothelial cells *in vitro*, and shorter wound-healing time in diabetic mice *in vivo* [47]. Later these cells were found to secrete paracrine factors such as IL-6 and IL-8 [47]. IL-6 is a pro-inflammatory cytokine that helps in recruiting immune cells, such as neutrophils and macrophages, to the wound site [52,53]. In IL-6 knockout mice, the reduction of wound area was delayed with attenuated leukocyte infiltration, re-epithelialization, angiogenesis, and collagen accumulation [53]. IL-8 primarily functions as a chemoattractant for neutrophils, involved in balancing pro- and anti-inflammatory responses, as well as the polarization and depolarization of neutrophils at the wound site [54,55]. Under the influence of these cytokines, DSCs may modulate neutrophil infiltration to the wound site, promoting their recruitment or limiting excessive infiltration. Additionally,

Moreover, human hair follicle-derived mesenchymal stromal cells from the lower DS have been found to promote the polarization of macrophages towards the M2 phenotype, which is associated with a more regenerative environment [44]. M1 macrophages promote inflammation. This M1–M2 transition is critical for the resolution of inflammation and tipping the balance to tissue repair [56].

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Additionally, an unbiased reassessment of unpublished single-cell RNA-Seq data by Jeff Biernaskie et al. revealed significant interactions between $CD200^{+}Stmn2^{+}$ DSCs (including dermal stem cells of the dermal cup) and $CD68^{+}F480^{+}$ peri-follicular macrophages [57]. These cells were co-isolated from 28-day-old anagen backskin of mice using the 10× Chromium platform. By employing the Cell-Cell Interactions (CCInx) R package to analyze intercellular communication interactomes, a variety of macrophage-derived ligands (e.g., C1qa, C1qb, C1qc, ApoE, and Gelsolin) were identified with corresponding receptors on dermal sheath cells [57]. Multiple macrophage receptors were found predicted to be activated by DS-derived ligands, primarily components of the ECM [57]. These interactions suggest a complex communication network between DSCs and macrophages, which may have significant implications for wound healing and fibrosis.

In summary, while DSCs show promise in modulating inflammatory responses through the secretion of various cytokines and chemokines, the complex interactions between DSCs and macrophages, as suggested by recent findings, indicate significant potential for influencing wound healing and fibrosis. Further research is needed to directly link DSCs to specific immune cell infiltration and to fully elucidate their role in these processes.

Dermal Sheath Cells Are Activated in the Proliferation Phase

In the proliferation phase, there is an increase in the activity of various cell types. These cells proliferate and migrate to the wound site, contributing to the rebuilding of the dermal and epidermal tissues.

Jahoda et al. first proposed the hypothesis that DSCs might have significant value in skin wound healing [5]. Experiments using fluorescent dye to trace cells revealed that hair follicle DSCs appear in the newly formed granulation tissue during the proliferation phase [48]. Certain research has suggested that hair follicle–associated fibroblasts, including DSCs and DP cells, are vital for the regeneration of hair follicles following injury [40]. Jahoda et al. argued that DSCs from different parts of the hair follicle have distinct roles during the healing process: DSCs from the upper part (upper follicle dermal sheath, UDS) only participate in wound healing, whereas those from the lower part (lower follicle dermal sheath, LDS) not only contribute to wound healing but also engage in the growth cycle of adjacent follicles [48]. This discovery aligns with the findings of Abbasi et al. in 2019 [46]. They conducted cell lineage tracing studies using αSMACreER^{T}[2]:Rosa26^{YFP} mice and found that the DS-derived dermal stem cells are activated after a wound occurs [46]. A portion of these cells actively migrate into the wound area to participate in skin wound healing. Another portion integrates into the DP of peri-wound hair follicles; this bias toward a DP fate only occurred when a wound was induced during certain stages of the hair cycle. This finding was also supported by Rahmani's research [43]. In addition, a lineage tracing study has shown that in wound-induced hair follicle neogenesis (WIHN) – a phenomenon involving the reemergence of hair follicles at the center of large-size wounds in adult mouse back skin – the fibroblasts of the DS and DP within hair follicles contribute minorly to the formation of new hair follicles. Instead, a separate extrafollicular lineage of fibroblasts distinguished by Hic1 expression gave rise to 90% of the DP cells within the newly formed follicles [58]. For a long time, DSCs have been considered as the cellular pool that replenishes the DP during the hair follicle regeneration cycle [48,59,60], with the control of thrombin signaling through PI3K being a mechanism that underlies this process [61]. Incredibly, it now appears that this concept is not applicable to WIHN. In the field of tissue-engineered skin, Higgins et al. reported that four subtypes of DFs (papillary fibroblasts, reticular fibroblasts, DSCs, and DP cells) all support the growth of overlying epidermal cells, both in vivo and *in vitro* [62]. Surprisingly, DSCs were found to be more conducive to the formation of the basement membrane [48,62]. This may be related to the characteristic of DSCs in abundantly expressing type IV collagen and laminin, both critical components of the basement membrane [38,63]. Additionally, it is worth mentioning that DP cells do not participate in skin wound healing [64,65].

Dermal Sheath Cells may Contribute to Angiogenesis and Vascularization

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Angiogenesis and vascularization are crucial for delivering oxygen and nutrients to the healing tissue and typically occur during the proliferative phase of wound healing. Studies have suggested that DSCs may contribute to these processes.

Yoshida et al. reported a subset of DSCs exhibits high levels of CD36 expression, a characteristic not observed in DP cells or DFs [45]. CD36 expression was observed at the perivascular region in the entire LDS at anagen III-IV, and in the UDS at anagen V-VI. Co-culture experiment confirmed that CD36-enriched DSCs promoted proliferation of blood endothelial cells in vitro in a cell-cell contact-dependent manner [45]. These interactions may contribute to the stabilization and maturation of newly formed blood vessels, facilitating their integration into the surrounding tissue. Besides, CD36-enriched DSCs, compared to CD36-negative DSCs, demonstrated increased expression of HGF, which is a known pro-angiogenic factor [45]. However, it should be noted that Yoshida *et al.*'s study did not specifically separate DSCs from other cell types within the connective tissue sheath (CTS), which consists of multiple cell types such as DSCs, blood vessels, immune cells, fat cells, and sparsely intermingled αSMA- fibroblasts distinct from the DSCs [66]. Further, they have not provided any evidence that CD36 is expressed by DSCs by performing any dual stains.

The secretion of cytokines such as PDGF-C and PDGF-D by DSCs [38]. can influence endothelial cell behavior and promote vascularization. PDGF-C [38], for instance, is known to be involved in angiogenesis [67–69]. While endothelial cells are directly responsible for the formation of these new blood vessels, PDGF-C influences this process primarily acting on pericytes and smooth muscle cells [70]. There are other studies confirmed that PDGF-C can not only promote the angiogenic effect of vascular endothelial growth factor (VEGF) but also independently promote the formation of new blood vessels [71]. PDGF-D [38], another member of the PDGF family that involved in angiogenesis, exerts its effects by binding to specific cell surface receptors, primarily PDGF receptor β (PDGFR-β) [72]. This binding activates signaling pathways that lead to cell proliferation, migration, and survival [73–75], all of which are essential for angiogenesis and vascularization.

Overall, while DSCs are likely to contribute to angiogenesis and vascularization through direct cell-cell contact and the secretion of cytokines that promote the formation and maturation of new blood vessels, it remains unclear whether DSCs enhance vascularization during follicle regeneration and cannot be extrapolated to the wound healing scenario without more evidence.

Extracellular Matrix Remodeling and Dermal Sheath Cells

ECM remodeling is a sophisticated and intricate process that plays a crucial role in the final phase of healing. Currently, there is limited research available on the direct involvement of DSCs in ECM remodeling. However, the findings presented here suggest that DSCs possess the capability to regulate ECM remodeling.

Collagen is the main structural component and the most abundant protein in the ECM, with 85% of the dermis being collagen. Heitman et al. characterized 483 enriched genes reflecting specialized functions of DS compared to DP and DF in the anagen skin of mice [76]. From Table 1, we can see collagen types 1, 3, 4, 5, 6, 8, 11, 12, 16, and 27 were expressed in DSCs. In the study, DSCs expressed more types of collagen compared to other cell types. Specifically, collagen type I was expressed in both DSCs and DFs, while collagen type III could only be seen in DSCs. During remodeling, the wound area experiences a significant transition from type III to type I collagen, enhancing tissue strength and resilience [36,37]. DSCs have been shown to migrate into granulation tissue during wound healing [46,48], suggesting they are involved in collagen remodeling.

Table 1. Collagen expression signatures in DSCs, DFs, and DP cells *.

DSC	DP cell	DF	DSC, DP cell	DSC, DF	DSC, DF, DP cell
Col3a1	Col9a2	Col4a5	Col5a3	Col1a1	Col6a3
Col4a1	Col15a1	Col7a1		Col1a2	Col16a1
Col4a2	Col23a1	Col25a1		Col5a1	

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Col6a6	Col26a1	Col5a2
Col8a1		Col6a1
Col8a2		Col6a2
Col11a1		
Col12a1		
Col27a1		

*Data extracted from Heitman *et al.*'s study [76]. DSC, dermal sheath cell; DF, dermal fibroblast; DP, dermal papilla.

MMPs are key enzymes in this phase, responsible for degrading old or damaged ECM components, and are balanced by tissue inhibitors to prevent excessive breakdown. DFs and myofibroblasts are central to this process, whereas DSCs have not been reported in this respect. In spite of this, we still attempted to explore the MMPs that can be expressed by DSCs (Table 2) and to delve into their roles in wound healing. One in situ study found that the expression of MMP1 in human scalp hair follicle DSCs elevated with age [77]. In a rat model, MMP-1 improved the wound-healing process of skin with higher epithelial hyperplasia and reduced scar formation [78]. Heitman *et al.*'s study [38], which used P5 mice for transcriptome analysis, revealed that MMP11 and tissue inhibitor of metalloproteinase 1 (TIMP1) were expressed in anagen hair follicle DSCs, in contrast to MMP2, MMP19, and MMP27, which were expressed in DFs; MMP23 was found to be expressed in both cell types (Table 2). Recent research has shown that MMP23 is involved in inflammatory bowel disease [79] and wound healing after traumatic injury [80]. MMP11 was also found to be expressed in DSCs of human scalp [81]. Unlike most MMPs, which are secreted as inactive proenzymes and activated extracellularly, MMP11 is secreted in its active form yet cannot degrade any major ECM components [82,83]. Active MMP11 released by fibroblasts promotes epithelial cell apoptosis and growth of connective tissues [84]. MMP11 may thus play a unique role in tissue remodeling processes. A study using the tissue-engineered human cornea investigated MMPs during corneal wound healing. It found that MMP11 expression significantly increased in the central area of wounded, reconstructed corneas within 24 hours and then progressively decreased as the wounds were closing, with MMP11 appearing to be dose-dependently upregulated by IL-1β and TNF-α [85]. Research in the field of tumor has reported that MMP11 can promote cell proliferation, migration, and invasion, while microRNA-125a/b can inhibit its effects by directly targeting MMP11 [83,86]. MMPs can be inhibited in 1:1 stoichiometry by TIMP family (TIMP1~4). TIMP1 inhibits most MMPs but weak for MMP14, MMP16, MMP19 and MMP24 (Table 2) [87], binding particularly strongly to MMP9 and pro-MMP9 [88]. Besides, TIMP1 can interact with the proforms of MMPs in a non-inhibitory manner, and also have functions independent of MMP inhibition by directly binding to CD63 [89]. This suggests that DSCs may establish contact with endothelial cells and platelets by paracrine signaling. In psoriasis, TIMP1 and TIMP3 are present in the inflammatory infiltrate and in the endothelial cells of the papillary dermis [89]. In the treatment of diabetic foot ulcers, an increased ratio of serum MMP9 against TIMP1 predicts poor wound healing [90]. We also determined that TIMP1, TIMP2, and TIMP3 were differentially expressed between human healthy skin DFs and those from patients with systemic sclerosis (SSc) [2], indicating potential therapeutic targets in SSc. Additionally, TIMP1 is a negative regulator of adipogenesis [91,92], suggesting that it may play a role in the recovery of the subcutaneous adipose layer after wound. Above all, although we have identified specific MMPs and their inhibitors expressed by DSCs, the comprehensive mapping of their expression profiles within DSCs throughout the different phases of wound healing remains a largely unexplored territory, presenting a significant opportunity for future studies to unravel these complex interactions and their implications in tissue repair and regeneration.

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Table 2. Expression and substrate specificities of Mammalian MMPs (parts) and human tissue inhibitors of MMPs (TIMPs).

MMPs	DSCs[76]	DFs[2,76]	DP cells[76]	Substrates[84,93,94]
Gelatinases				
MMP2 (gelatinase A)		+ (human healthy DFs; human SSc DFs)		Gelatin; collagen I, IV, V, VII and X; laminin; aggrecan; fibronectin; tenascin
Stromelysins				
MMP11 (stromelysin-3)	+ (mouse; human scalp)	+ (human SSc DFs)		Serine protease inhibitors; α1-proteinase inhibitor
Membrane-type MMPs				
MMP14 (MT1-MMP)		+ (human SSc DFs)		Collagen I, II and III; gelatin; fibronectin; laminin; vitronectin; aggrecan; tenascin; nidogen; perlecan; fibrillin; α1-proteinase inhibitor; alpha2-macroglobulin; fibrin
MMP17 (MT4-MMP)			+ (mouse)	Fibrin; fibrinogen; tumor necrosis factor
Other MMPs				
MMP19		+ (mouse)	+ (mouse)	Gelatin; aggrecan; cartilage oligomeric matrix protein; collagen IV; laminin; nidogen; tenascin
MMP23	+ (mouse)	+ (mouse; human healthy DFs; human SSc DFs)		Unknown
MMP27		+ (mouse)		Unknown
TIMPs				MMP inhibition[87]
TIMP1	+ (mouse)	+ (human healthy DFs; human SSc DFs)		weak for MMP14, MMP16, MMP19 and MMP24
TIMP2		+ (human healthy DFs; human SSc DFs)		All
TIMP3		+ (human healthy DFs; human SSc DFs)		All
TIMP4				Most

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Abbreviations: MMP, matrix metalloproteinase; TIMP, tissue inhibitors of metalloproteinase; DSC, dermal sheath cell; DP, dermal papilla; DF, dermal fibroblast; SSc, systemic sclerosis.

Paracrine Effects of Dermal Sheath Cells

As mentioned earlier, DSCs exhibit the ability to produce IL-6 and IL-8 during the inflammatory phase of wound healing. Moreover, the discussion extends to the secreted protein CD36, HGF, PDGF-C, and PDGF-D concerning the proliferation stage. The remodeling phase further delves into the role of MMPs and their inhibitors. Ahlers et al. demonstrated that DS-secreted proteins exerted paracrine effects on primary keratinocytes and DFs, promoting proliferation, epidermal thickness and procollagen production [6]. Specifically, all tested proteins, including midkine, activin A and retinol binding protein 4, increased the proliferation of primary keratinocytes [6]. In particular, activin A significantly increased epidermal thickness and fibroblast procollagen type I c-peptide production in a 3D skin model [6]. Importantly, the paracrine effects of DSCs unveil a spectrum of functions that may surpass initial expectations in terms of breadth and complexity.

Potential Role of Dermal Sheath Cells in Fibrosis

The remodeling phase also involves scar maturation, where the scar tissue undergoes changes to become more like the original tissue in terms of appearance and functionality. Research indicates that DSCs are not just passive bystanders but may participate in the fibrotic process. When triggered by certain stimuli, such as injury or inflammatory signals, these cells can differentiate or transform into a myofibroblast or wound healing phenotype [5,27,95]. ScRNA-seq analysis revealed multiple genes shared between DSCs and myofibroblasts [2], including high *ACTA2* expression, the gene encoding smooth muscle actin (SMA). DSCs express other markers common to myofibroblasts, including *COL11A1*, and cluster proximal to myofibroblasts in Uniform Manifold Approximation and Projection (UMAP) plots [2], suggesting that DSCs have similar characteristics or expression profiles to myofibroblasts. In wound healing, myofibroblasts are instrumental in fibrosis by producing ECM, which is essential for tissue repair but can also lead to excessive scarring if dysregulated. As mentioned earlier, anagen hair follicle DSCs were found to express TIMP1. TIMP1 is the best predictor of TGF-β1, which has been demonstrated to promote myofibroblast formation and is a hallmark of fibrosis [89,96], indicating that DSCs may impact fibrosis. However, it is important to note that the contribution of DSCs to wound healing by differentiating into myofibroblasts is minimal, as suggested by Abbasi et al. (2020) [58].

Another study also indicated that DSCs might be involved in the process of fibrosis, particularly through the Wnt/β-catenin signaling pathway [8]. TGF-β, Notch, Hedgehog, and Wnt/β-catenin pathways are widely recognized as major players for regenerative wound healing. The Wnt/β-catenin pathway is a well-known key player for enhancement of the overall healing process involving tissue regeneration via crosstalk with other signaling pathways [97]. The activation of the Wnt/β-catenin pathway elicits a range of healing responses, including the promotion of angiogenesis [98,99], enhancement of fibroblast migration, proliferation, and differentiation [1,97], stimulation of keratinocyte proliferation and differentiation [100,101], facilitation of re-epithelialization [102,103], and the induction of wound-induced hair folliculogenesis [104,105]. A cell tracing study reported that constitutive activation of β-catenin in DS not only generated ectopic hair follicle outgrowth, endowing DSCs with hair-inducing ability, but also induced cell-autonomous progressive skin fibrosis in the dermis, where the excessive fibroblasts largely originated from the DS [8]. Gene expression analysis of purified DSCs with activated β-catenin revealed a significant increase in the expression of Bmp, Fgf, and Notch ligands [8], potentially serving as precursors to the signaling cascade crucial for skin regeneration. However, it should be noted that there is no direct evidence of DSCs contributing to skin fibrosis during wound healing under physiological conditions. The study by Tao et al. (2019) [8] represents a scenario where β-catenin is overexpressed in αSMA+ cells, which is not typically seen under normal physiological conditions.

En1-lineage-past fibroblasts (EPFs) of fascia were shown to be responsible for most connective tissue deposition in skin fibrosis during wound healing [106]. Notably, in all the GEO datasets

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(GSE215133[107], GSE136996[76], GSE81615[8]) from the transcriptome sequencing studies we have examined, DSCs consistently expresses En1, at levels that are consistent with or even higher than those of DFs (GSE215133 & GSE136996). However, this information alone is not sufficient to definitively determine that DSCs belong to the En1-lineage-past fibroblasts (EPFs), as the identification of EPFs typically involves lineage tracing experiments and additional validation techniques such as dual staining and comparative gene expression profiling.

Abnormal contractions of myofibroblasts result in tissue contractures and stiffness. Myofibroblast contractions are separately regulated by Ca^{2+} dependent myosin light chain kinase (MLCK) and Rho-associated protein kinase (ROCK) within the same cell [108–110]. ROCK maintains stress fibers in the center of cells whereas MLCK drives stress fiber assembly in the periphery [109]. A recent study demonstrates that ET-1, derived from epithelial cells, can trigger DS contraction through both ET_A and ET_B receptors by activating the Ca^{2+} dependent MLCK pathway [107]. This finding suggests that DSCs share a similar contraction mechanism with myofibroblasts. Consequently, when a wound occurs and DSCs migrate to the wound site, abnormal contractions in DSCs could potentially contribute to tissue fibrosis.

Dermal Sheath Cells Work Differently in Healing Hairy and Non-Hairy Sites

Clinicians have long reported that hair-bearing areas (hairy sites) tend to heal more rapidly than those lacking hair follicles (non-hairy sites) [111]. A study notes that in animals with high densities of hair follicles, differences in wound healing are observed alongside changes in the hair growth cycle [112]. This observation extends to humans, where apparent differences in wound healing responses are seen between hairy sites and non-hairy sites [5]. This suggests a link between hair follicle density, DSCs activity, and wound healing efficacy. Furthermore, the study indicates that the involvement of DSCs may lead to qualitatively improved dermal repair. This opens up therapeutic possibilities, such as using DSCs to create dermal or full skin equivalents to improve wound healing and reduce scarring. Additionally, the inductive properties of these cells make them promising for tissue engineering applications. This potential could lead to the development of skin equivalents capable of growing hair follicles when grafted.

Conclusions and Future Challenges

This review has extensively discussed the multifaceted role of DSCs in the context of wound healing. DSCs are pivotal in modulating cell activity, orchestrating collagen synthesis, and influencing ECM remodeling through intricate networks of paracrine signaling and cell-cell interactions. Their contribution is vital for the maintenance and restructuring of the dermal ECM, which is a cornerstone in achieving effective wound healing and tissue homeostasis.

Despite the advancements in understanding the role of DSCs, numerous aspects remain underexplored or unclear, presenting considerable challenges and opportunities for future research. One significant area is the specific molecular pathways and mechanisms by which DSCs regulate these critical processes. Although their influence on cell activity and collagen synthesis is recognized, the detailed molecular interactions and signaling pathways involved are not fully delineated.

Another critical gap lies in our understanding of the heterogeneity and plasticity of DSCs. The diverse subpopulations of DSCs and their respective roles in different phases of wound healing are not comprehensively understood. This diversity hints at a complex regulatory network, where different DSC subsets may have unique functions or interactions with other cell types in the wound microenvironment.

In summary, while DSCs have emerged as crucial players in wound healing, their full potential and the breadth of their roles are yet to be fully uncovered. Future studies should aim to unravel these aspects, potentially leading to novel therapeutic approaches for wound care and tissue regeneration.

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List of Abbreviations

αSMA, alpha-smooth muscle actin; COX2, cyclooxygenase 2; CTS, connective tissue sheath; DFs, dermal fibroblasts; DP, dermal papilla; DSCs, dermal sheath cells; ET-1, endothelin-1; GM-CSF, granulocyte macrophage colony-stimulating factor; HB-EGF, heparin-binding epidermal growth factor-like growth factor; HGF, hepatocyte growth factor; IL-1, interleukin-1; KGF, keratinocyte growth factor; LDS, lower follicle dermal sheath; MLCK, myosin light chain kinase; MMPs, matrix metalloproteinases; NF-κB, nuclear factor kappa B; PDGF-C, platelet-derived growth factor C; PDGFRα, platelet-derived growth factor receptor alpha; PGE2, prostaglandin E2; ROCK, rho-associated protein kinase; scRNA-seq, single-cell transcriptome sequencing; SSc, systemic sclerosis; TIMP1, tissue inhibitor of metalloproteinase 1; TGF-β, transforming growth factor-beta; UMAP, uniform manifold approximation and projection; UDS, upper follicle dermal sheath; VEGF, vascular endothelial growth factor; WIHN, wound-induced hair follicle neogenesis.

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