



A Review of the Contribution of Mast Cells in Wound Healing: Involved Molecular and Cellular Mechanisms

Daniel Elieh Ali Komi^{1,2} · Kelly Khomtchouk³ · Peter Luke Santa Maria³

© Springer Science+Business Media, LLC, part of Springer Nature 2019

Abstract

Mast cells (MCs), apart from their classic role in allergy, contribute to a number of biologic processes including wound healing. In particular, two aspects of their histologic distribution within the skin have attracted the attention of researchers to study their wound healing role; they represent up to 8% of the total number of cells within the dermis and their cutaneous versions are localized adjacent to the epidermis and the subdermal vasculature and nerves. At the onset of a cutaneous injury, the accumulation of MCs and release of proinflammatory and immunomodulatory mediators have been well documented. The role of MC-derived mediators has been investigated through the stages of wound healing including inflammation, proliferation, and remodeling. They contribute to hemostasis and clot formation by enhancing the expression of factor XIIIa in dermal dendrocytes through release of TNF- α , and contribute to clot stabilization. Keratinocytes, by secreting stem cell factor (SCF), recruit MCs to the site. MCs in return release inflammatory mediators, including predominantly histamine, VEGF, interleukin (IL)-6, and IL-8, that contribute to increase of endothelial permeability and vasodilation, and facilitate migration of inflammatory cells, mainly monocytes and neutrophils to the site of injury. MCs are capable of activating the fibroblasts and keratinocytes, the predominant cells involved in wound healing. MCs stimulate fibroblast proliferation during the proliferative phase via IL-4, vascular endothelial growth factor (VEGF), and basic fibroblast growth factor (bFGF) to produce a new extracellular matrix (ECM). MC-derived mediators including fibroblast growth factor-2, VEGF, platelet-derived growth factor (PDGF), TGF- β , nerve growth factor (NGF), IL-4, and IL-8 contribute to neoangiogenesis, fibrinogenesis, or reepithelialization during the repair process. MC activation inhibition and targeting the MC-derived mediators are potential therapeutic strategies to improve wound healing through reduced inflammatory responses and scar formation.

Keywords Angiogenesis · Mast cells · Mediators · Scar formation · Wound healing

Abbreviations

bFGF	Basic fibroblast growth factor
ECM	Extracellular matrix
FGF	Fibroblast growth factor
IGF	Insulin-like growth factor
KGF	Keratinocyte growth factor
MC	Mast cell

MCP-1	Monocyte chemoattractant protein-1
MIP-2	Macrophage inflammatory protein-2
MMP	Matrix metalloproteinases
PAI-1	Plasminogen activator inhibitor 1
PDGF	Platelet-derived growth factor
SCF	Stem cell factor
TGF- β	Transforming growth factor β
t-PA	Tissue-type plasminogen activator
VEGF	Vascular endothelial growth factor

✉ Peter Luke Santa Maria
petersantamaria@stanford.edu

¹ Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

² Department of Immunology, Tabriz University of Medical Sciences, Tabriz, Iran

³ Department of Otolaryngology, Head and Neck Surgery, School of Medicine, Stanford University, 801 Welch Rd, Stanford, CA 94305, USA

Introduction

Wound healing is characterized by a well-orchestrated, progressive series of events aimed to ultimately restore the barrier function and mechanical integrity of the skin [1]. Impaired wound healing is associated with a variety of common diseases including diabetes, chronic inflammatory disorders, and

vascular and autoimmune diseases [2]. Inflammation, proliferation, and remodeling are the three cardinal phases of wound healing which require complicated intercellular interactions, contribution of released soluble factors, and extracellular matrix components [3, 4]. The inflammatory phase is characterized by neutrophil and macrophage infiltration, an important process for formation of new tissue in wound healing [5]. NADPH oxidase-dependent “oxidative burst” by these cells represents a main mechanism of pathogen removal at the wound site [6]. Impaired wound healing, characterized by excessive inflammation, is associated with increases in TNF- α level. TGF- β induces chemotaxis of inflammatory and immune cells to the wound site and mediates the replacement of collagen type III with collagen type I [7]. Wounds, due to any number of etiologies, may persist into chronicity; elevated levels of proinflammatory cytokines effect the resolution of healing [8]. Fibroblasts are the most prevalent cell type in the human dermis and encompass any stromal cell without expression of markers for a more specific mesenchymal lineage. Based on the location, fibroblasts are able to alter their function and physiology or even transform into a new cell type [9]. In the proliferative phase, fibroblasts proliferate and migrate to the wound area and secrete a new extracellular matrix (ECM); granulation tissue then fills the wound prior to reepithelialization. In this phase, keratinocytes proliferate and migrate from the edges of the wound toward the center. Neoangiogenesis contributes to establishing blood supply to the newly formed tissue during this proliferative phase [10]. The remodeling phase, characterized by maturation of the ECM, involves collagen bundles that increase in diameter and become organized to achieve greater tensile strength. Fibroblasts also undergo transformation into myofibroblasts to facilitate wound contraction. The wound healing process is complete after removal of the excess ECM [10]. A variety of cells undertake quite different duties during wound healing phases. For example, neutrophils, during the inflammatory phase, infiltrate into the injured tissue for the purpose of removing microbes, debris, and necrotic tissue [11, 12]. M1 macrophages are characterized by their proinflammatory function in parallel with M2 macrophages that induce angiogenesis and tissue remodeling in the early phases of healing and reducing inflammation in the late phase of wound healing [13]. Fibroblasts contribute to the wound healing process by initially migrating into the wound site and subsequently synthesizing various components of the new connective tissue matrix [14, 15]. MCs are specialized secretory cells of the innate immune system that originate from CD34+/CD117+ bone marrow progenitor cells [16–18]. MCs express the high-affinity IgE receptor (Fc ϵ RI). Cross-linking of IgE/Fc ϵ RI complex with the allergen-based epitope results in MC activation and release of three categories of effector molecules [19]. These effectors are preformed mediators stored in granules (e.g., serotonin, histamine, heparin, tryptase, and

chymase); mediators synthesized de novo upon cell stimulation (e.g., lipid mediators PAF, PDG2, and LTB4 and LTD4); and a variety of cytokines (e.g., IL-1, IL-3, IL-5, IL-8, IL-10, GM-CSF, TNF- α , TGF- β , and VEGF) [16, 20]. At the time of injury, MCs become activated, and through degranulation, they release a wide variety of these mediators. The process can be traced by observing the reduction of fully granulated MCs and the presence of extracellular granules in stained sections prepared from injured tissue [21]. In normal skin, MC_{TC} cells (containing tryptase, chymase, and carboxypeptidase) are the predominant cell type but together with MC_C cells (containing chymase and carboxypeptidase, but not tryptase) they have a role in fibrosis, inflammation, and biological regenerative processes [22]. Insights into the role of MCs in wound healing open up new therapeutic strategies that act via fine-tuning the MC degranulation mechanism during the repair stages at the precise location of injury. This article summarizes the MC-orchestrated cellular and molecular mechanisms involved in every stage of wound healing to provide potential therapeutic MC or MC-mediator targeting strategies.

Methods

To identify articles for inclusion in this review, an online search, from January 1986 to January 2018, was performed using the scientific reference search engine of the National Library of Medicine, PubMed, and Elsevier's Embase. The keywords used were “mast and cell and wound healing.” The search was performed in all languages. Using this search method, 547 articles were identified of which 75 were directly cited. Additionally, the basic and general information regarding mast cell biology were provided by citing the most recently published papers in the field.

Mast Cells in Hemostasis

MCs initiate their role in wound healing during this stage as they are recruited by anaphylatoxins C3a and C5a which are produced under influence of Hageman factor (XII) [14]. Moreover, MC-derived β -tryptase has been reported to generate C3a and C5a at sites of inflammation [23, 24]. It should be noted that only the MC_{TC} type of MCs (including skin MCs) has receptors for C3a and C5a [23, 25]. MC-derived tryptase inactivates the thrombin-induced clotting activity of fibrinogen. MCs, likely through secretion of TNF- α , enhance expression of XIIIa, a potent fibrin-stabilizing factor which catalyzes covalent cross-binding of fibrin fibrils in dermal dendrocytes [14, 26]. MCs, via producing tissue-type plasminogen activator (t-PA) in a resting state and through expressing plasminogen activator inhibitor 1 (PAI-1) upon activation by C5a [27], are hypothesized to participate in balancing the proteases and protease inhibitors in regulation

of tissue injury and repair [28] (Fig. 1). The results of Oschatz et al. reported that heparin isolated from either peritoneal or human lung MCs (HLMCs) when added to plasma activates contact system and promotes bradykinin (BK) generation [29]. Additionally, the coagulation system activity has been studied in MC-related diseases. In chronic spontaneous urticaria, tissue factor expressed on endothelial cells and eosinophils has been reported to activate the extrinsic coagulation pathway and is followed by leakage of plasma from blood vessels concurrent with MC activation [30]. For instance, plasma levels of activated factor VII were found higher in patients with acute urticaria, while levels of factor IX and tissue factor were lower in patients with chronic urticaria. Moreover, plasma levels of tissue factor pathway inhibitor/activated factor X (TFPI/Xa) were higher in chronic urticaria patients when compared with healthy controls [31]. Prolonged bleeding, mainly in gastrointestinal track and skin, has been documented in pediatric mastocytosis [32]. Menstrual-like bleeding in association with the anaphylactic episodes in some women has also been documented [33]. Considering the mMCP-6, mMCP-7, and hTryptase- β anticoagulant activity, tryptase inhibitors might be used to hinder the bleeding abnormalities that occur in patients with MC-related diseases [34].

Mast Cells in the Inflammatory Stage

Mast Cell Recruitment to the Site of Injury

MC recruitment to the site of wound has been investigated by Trautmann and colleagues [35]. They reported that monocyte chemoattractant protein-1 (MCP-1) released by keratinocytes and macrophages is correlated with recruitment of MC in situ. MCP-1-mediated recruitment of MCs to the site of injury places MCs and fibroblasts in close proximity, which provides the opportunity for fibroblast proliferation triggered by MC-released IL-4 [35]. The fivefold increase in MC numbers at the site of a wound during the wound healing process is a result of chemokine-dependent recruitment rather than proliferation [35, 36].

Mast Cell Mediated Monocyte Recruitment in the Inflammatory Stage

Monocytes are recruited from blood to sites of inflammation, utilizing integrin $\alpha 4 \beta 1$ and CD62L to arrest on the endothelium and chemokine receptors CCR2 and CCR5 to migrate toward gradients of inflammatory cytokines, mainly MCP-1 released by keratinocytes [36–38]. Monocytes, after being recruited to the wound site and under the influence of MC-released MCP-1, transform into efficient phagocytic cells; these macrophages are now capable of secreting PDGF,

fibroblast growth factor, vascular endothelial growth factor, TGF- β , and TGF- α [39].

Mast Cell Mediated Neutrophil Recruitment in the Inflammatory Stage

MC-derived mediators including TNF- α , MIP-2, and IL-8 have been shown to be potent recruiters of neutrophils during wound healing [40]. Rapid mobilization of neutrophils from the circulation depends on $\beta 2$ -integrin interactions with endothelial adhesion molecules such as intercellular adhesion molecule-1 [41]. Recruited neutrophils produce proinflammatory cytokines, such as IL-1 α and TNF- α which act as signals to activate local fibroblasts and keratinocytes by inducing the expression of FGF7 [42]. The role of MCs in neutrophil recruitment during the inflammatory stage has been already investigated using MC-deficient W/W^v mice and +/+ mice. This study reported that neutrophil infiltration was significantly decreased in W/W^v mice compared with wild-type +/+ control [43].

Mast Cells Released Mediators in the Inflammatory Stage

Once an injury occurs, MCs degranulate and release a wide spectrum of vasoactive and proinflammatory mediators including preformed molecules such as histamine, serotonin, TNF, kinins, and proteases stored in secretory granules [35, 44]. MC-derived histamine induces capillary permeability, in addition to vasodilation, resulting in a significantly induced influx of neutrophils to the site. Other potent vasoactive agents include vascular endothelial growth factor (VEGF), IL-6, and IL-8 [45]. Tryptase binds to protease-activated receptor-2 (PAR2) on endothelial cells and causes vasodilatation. Tryptase-induced vasodilation paves the way for infiltration of neutrophils and other inflammatory mediators into the injury site [46]. The exact mechanisms by which the MCs become activated during injury are still unknown. However, it is reported that thymic stromal lymphopoietin (TSLP), released in response to trauma in the presence of cytokines such as IL-1 and TNF, is a potent activating mediator [44] (Fig. 2). MCs are capable of promoting human β -defensin-3 (hBD-3) release from epidermal keratinocytes through H1 receptors by activating STAT3 and AP-1 via JAK2 and MEK/ERK [47]. The latter pathway is activated in early stages of inflammation during an infection [48]. hBD-3 acts among the first lines of defense after tissue bacterial invasion [49]. hBD-3 induces histamine release from MCs [47]. The other biological effects of the cationic antimicrobial peptide hBD-3 include promoting wound closure [50], promoting defense against gram-positive and gram-negative bacteria as well as fungi, chemo-attracting CCR6+ cells, and inducing the production of IL-18, IL-8, and IL-20 in epidermal keratinocytes. Histamine

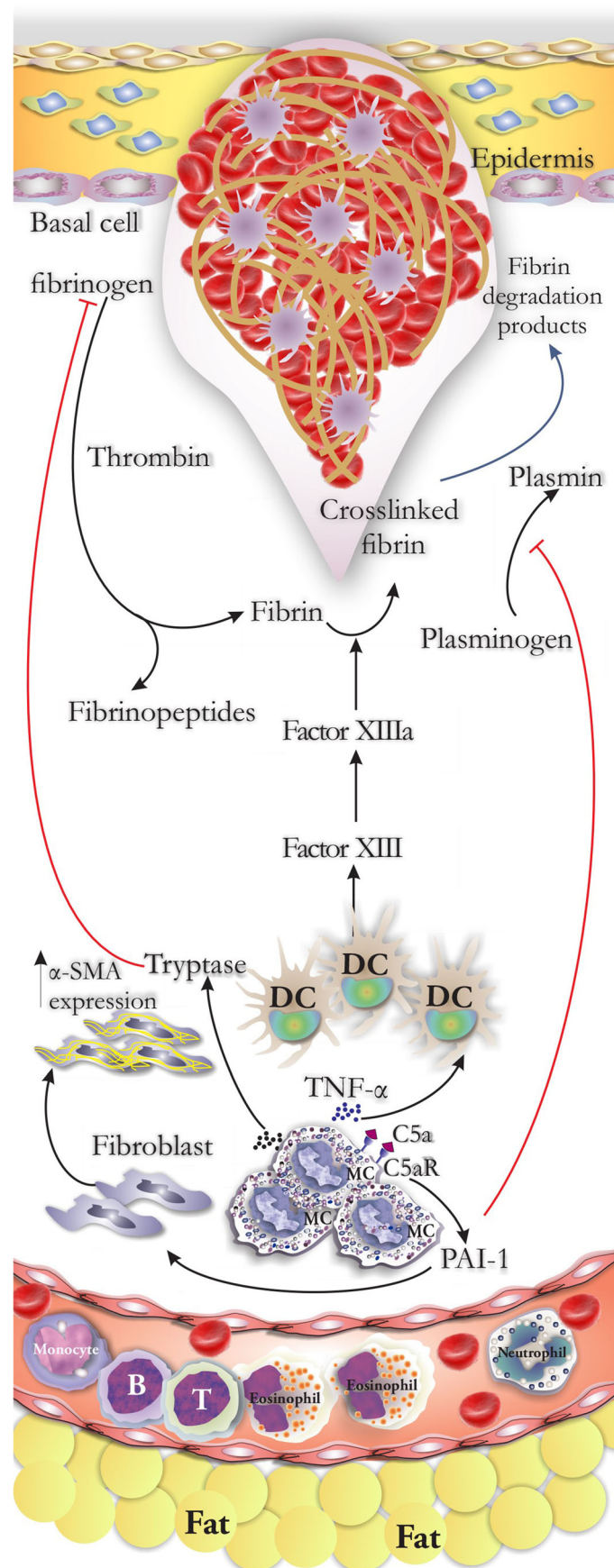


Fig. 1 Role of mast cells in hemostasis. MCs induce expression of factor XIII in dermal dendrocytes by releasing TNF- α . Factor XIII is converted to factor XIIIa in the presence of Ca²⁺, fibrin, and thrombin. Factor XIIIa then supports the fibrin cross-link and stabilizes the clot. Upon activation by C5a, MCs expressed PAI-1 inducing the α -SMA expression in fibroblasts. MCs also play a regulatory role in hemostasis; PAI-1 is capable of blocking the conversion of plasminogen to plasmin. MC-derived tryptase is able to inactivate the thrombin-induced clotting activity of fibrinogen

promotes antibacterial activity of keratinocytes via enhancing the expression of pathogen recognition receptors such as TLR-2 and Dectin-1 or increasing the production of GM-CSF to enhance opsonophagocytosis of macrophages and IL-8, a potent cytokine in neutrophil chemotaxis [47]. MC or other immune cell-mediated infiltration of inflammatory cells into the site of injury orchestrates the responses that contribute to removal of debris and pathogens and activation of residing cells to produce ECM proteins and induce the process of angiogenesis links the inflammatory and proliferation phases [51].

Mast Cells in the Proliferative Stage

Interaction of Mast Cells and Keratinocytes in the Proliferative Stage

This stage of wound healing is mostly predominated by cellular activity and includes reepithelialization, angiogenesis, and fibroplasia. Histologically, reepithelialization includes proliferation and migration of adjacent epidermal keratinocytes into the injury site, differentiation of the neoepithelium into a stratified epidermis, and finally the restoration of an intact basement membrane zone (BMZ) for anchoring the epidermis to the underlying dermis [39]. The implicated elements in keratinocyte migration include extracellular matrix, integrin receptors, a number of growth factors, fibronectin, type V collagen, and matrix metalloproteinases (MMPs) such as MMP-9. MMP-9 specifically degrades molecules in the basement membrane, including type IV collagen and laminins, to enable keratinocytes to elevate from the basement membrane and migrate into the wound [39]. MC-derived tryptase plays a key role in the interaction between MCs and keratinocytes owing to its capability of cleaving fibronectin and activating PAR-2 (a G protein-coupled receptor on keratinocytes). Keratinocytes, in return, may attract MCs toward the epidermis by releasing stem cell factor (SCF) [22]. Indeed, MCs as dermal cells have limited contact with keratinocytes in normal skin. In conditions, mainly chronic ulcers, epidermal MCs can be found within keratinocytes to interact with them [22]. In vitro studies of the interaction between MCs and keratinocytes revealed that MC-produced histamine inhibits keratinocyte mitosis. This heparin also acts as

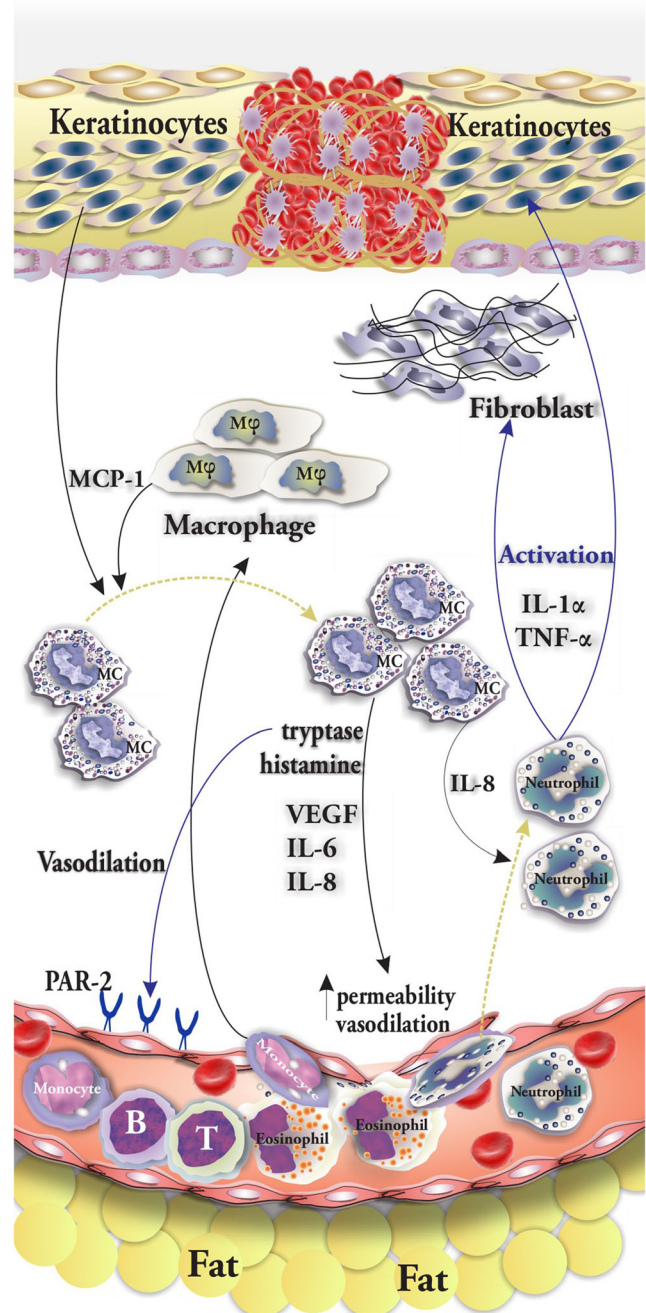


Fig. 2 Mast cells are recruited to the site of injury by macrophage and keratinocyte-derived MCP-1. MCs in return release mediators mainly histamine, VEGF, IL-6, and IL-8 that contribute to the increase of endothelial permeability and vasodilation and facilitate migration of inflammatory cells such as monocyte and neutrophil to the site of injury. Monocytes transform into macrophages and play a key role in wound healing. Neutrophils in humans are recruited by IL-8 to injury sites where they release IL-1 α and TNF- α and activate fibroblasts and keratinocytes to facilitate wound healing

an inhibitor of keratinocyte proliferation, without inducing cell differentiation. In contrast, metabolic products of arachidonic acid produced by MCs, including LTB₄, LTC₄, and LTD₄, stimulate keratinocyte proliferation [52]. The

contrasting and disparate functions of MCs have contributed to the complexity of unraveling their roles *in vivo* and in various pathogenic states.

Interaction of Mast Cells and Fibroblasts in the Proliferative Stage

After entering the wound, fibroblasts, under influence of PDGF and TGF- β , epidermal growth factor, and fibroblast growth factor, form the early extracellular matrix by secreting collagen, proteoglycans, and elastin [45]. To better understand the effect of MCs on fibroblast behavior, fibroblasts have been exposed to MC products *in vitro*, including histamine, heparin, and proteases; human MCs have also been co-cultured with fibroblasts [53]. Histamine and tryptase are able to stimulate dermal fibroblasts to release growth factors including FGF-2 or FGF-7, respectively [22]. Surprisingly, interactions between fibroblasts and their ECM control their morphology, growth, motility, differentiation, and even gene expression [54]. Fibroblasts also release SCF which has been reported to upregulate monocyte chemoattractant protein-1 (MCP-1) expression and synthesis in MCs. MCP-1 acts on fibroblasts in favor of enhancing the $\alpha 1(I)$ collagen mRNA expression [55]. A study comparing scald injury in MC-deficient W/W^V mice with controls showed there was no significant difference in the process of wound closure and reepithelialization, but less fibrous proliferation at the wound edge comparing higher neovascularization and less accumulation of chymase, FGF2, TGF-1, and VEGF producing MCs observed at the wound edge [56] (Fig. 3). Another study in cutaneous excisional wounds that were splinted open with rubber washers concluded that excisional wounds in MC-deficient mice close at rates and produce scars that are similar in terms of size, collagen content, collagen maturity, and collagen density to those of control mice. This supported that MCs are not essential for controlling the rate of reepithelialization, the ultimate collagen deposition, and scar maturation [57] (Fig. 4). MCs have been shown to stimulate fibroblast proliferation via IL-4, VEGF, and basic fibroblast growth factor (bFGF) [35, 58]. Fibroblasts generate a new ECM that replaces the fibrin clot which ultimately forms the mature scar [59, 60].

Role of Mast Cells in Promoting Angiogenesis in the Site of Injury

Angiogenesis begins with degradation of the basement membrane by cellular proteases including MC-derived tryptase [61]. This allows endothelial cells to penetrate and migrate into the ECM and then proliferate. Topographic distribution of connective tissue MCs within the vicinity of small vessels guarantees provisions of the essential mediators needed for normal angiogenesis [61]. During injury, not only are resident MCs contributing to angiogenesis but also further MCs

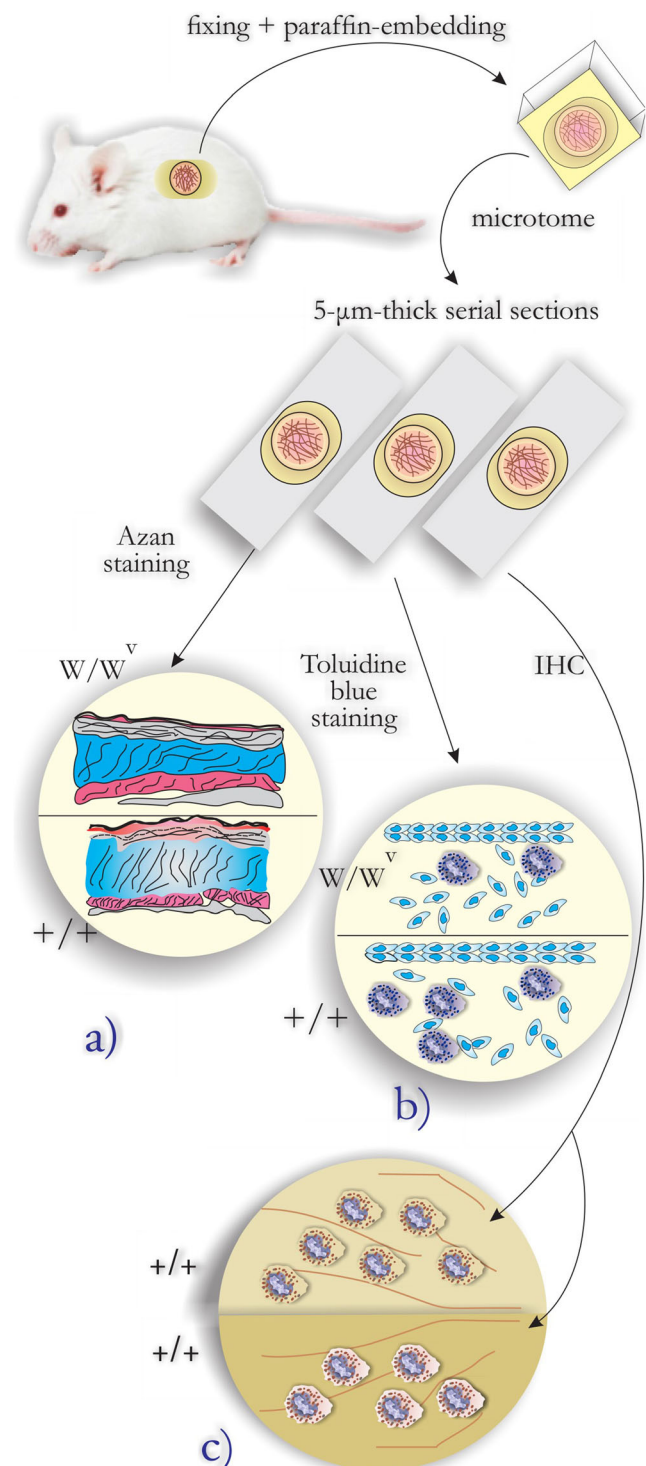


Fig. 3 Azan-stained cross sections of $+/+$ and W/W^V mouse skin showed an impaired fibrous proliferation and thinner dermis (blue) at the wound edge in W/W^V mice at 21 days after scald injury. Toluidine blue staining was used for observing mast cell distribution. Many mast cells were present at the wound edge in $+/+$ mice, while few were observed at the wound edge in W/W^V mice. Immunohistochemical localization of chymase, FGF2, TGF-1, and VEGF in $+/+$ mice was done using antibodies followed by a visualizing step with diaminobenzidine substrate

attracted to sites of neovascularization due to chemoattractive

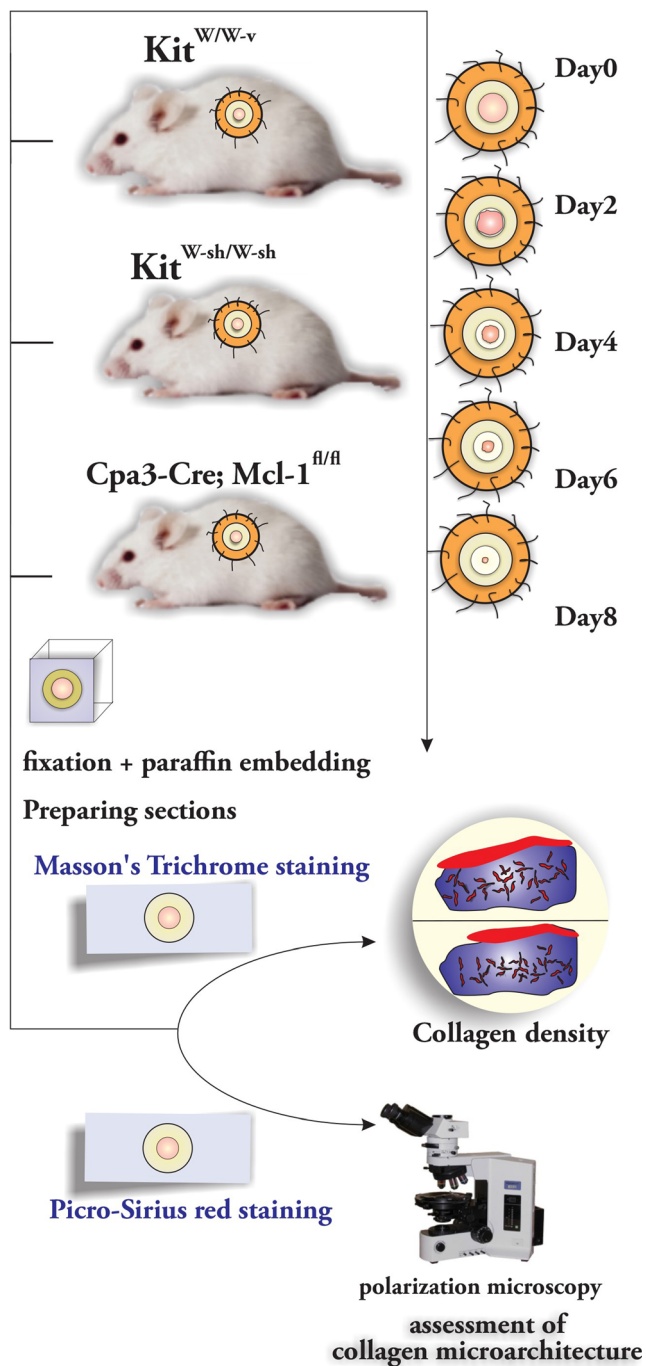


Fig. 4 Nauta et al., using three MC-deficient mice and comparing them with normal littermates, studied the role of MCs in a splinted model of excisional wound. Excisional wounds in MC-deficient mice of each group close at similar rates to control. They reported then that collagen density and its microarchitecture were similar between MC-deficient mice of each group and their wild-type counterparts

factors such as VEGF, bFGF, and platelet-derived growth factor (PDGF) [61]. An increasing number of studies have revealed a possible role for MCs in the modulation of wound angiogenesis. In this regard, MC-derived mediators including FGF-2, VEGF, PDGF, TGF- β , NGF, IL-4, and IL-8 contribute to neoangiogenesis, fibrinogenesis, or reepithelialization

during the repair process [14, 62]. Moreover, tryptase and chymase (MC-derived serine proteases) [40], bFGF, and angiopoietin-1-(ANG-1) possess proangiogenic activity [63]. During this stage, interaction between MCs recruited mainly from the inflammatory stage with fibroblasts guarantees MC survival within the site of injury and, in return, MCs activate fibroblasts to migrate to the site of injury to produce ECM proteins. Additionally, under the influence of MC-released mediators, the process of angiogenesis accelerates and neo-vascularization supplies the new tissue with cells and nutrients. The outcome of mutual MC fibroblast interactions promotes the formation of granulation tissue [45, 64].

Mast Cells in the Remodeling Stage

Vascular regression in the remodeling phase becomes predominant over angiogenesis leading to a transition from granulation tissue to collagen-rich avascular scar tissue [65]. MC-released tryptase via PAR-2 activation may promote fibroblast migration and proliferation, and support ECM synthesis [66]. Other MC mediators which directly support the growth and differentiation of fibroblasts include TGF- β , SCF, NGF, GM-CSF, PDGF, VEGF, and FGF-2, histamine, and proteases [67]. Additionally, other recruited cell types are able to participate in the remodeling stage. For instance, macrophages play a role in reepithelialization, fibroblast proliferation, and remodeling by releasing FGFs and TGF- β [68]. During wound healing, a portion of the wound fibroblasts differentiate into myofibroblasts, which are responsible for wound contraction [59]. Myofibroblasts are known for depositing high amounts of collagen and expressing α -smooth muscle actin (α -SMA), which later facilitates wound strength [69]. Th2-type cytokines (mainly IL-13 and IL-4) activate myofibroblasts through IL-4Ra/Sta6 signaling [64]. Activated myofibroblasts become the main producers and organizers of ECM and are required for restoring tissue integrity when injury occurs [64] (Fig. 5). Gap-junctional intercellular communication (GJIC) plays an important role in mast cell fibroblasts and/or myofibroblast interactions within granulation tissue in directing excessive fibrosis [70, 71]. The role of gap-junctional intercellular communication in MC-fibroblast interaction was studied by observations that the fluorescent dye, Calcein AM green, passes from the rat mast cell-1 (RMC-1) to the fibroblasts via gap-junction channels in MC-fibroblast co-culture [71].

Mast Cells and Scar Formation

The myofibroblasts undergo apoptosis when scarring is complete, while in pathological fibroses, myofibroblasts persist in the tissue and support fibrosis via increased matrix synthesis [72]. The results of experimental research aimed to reveal the

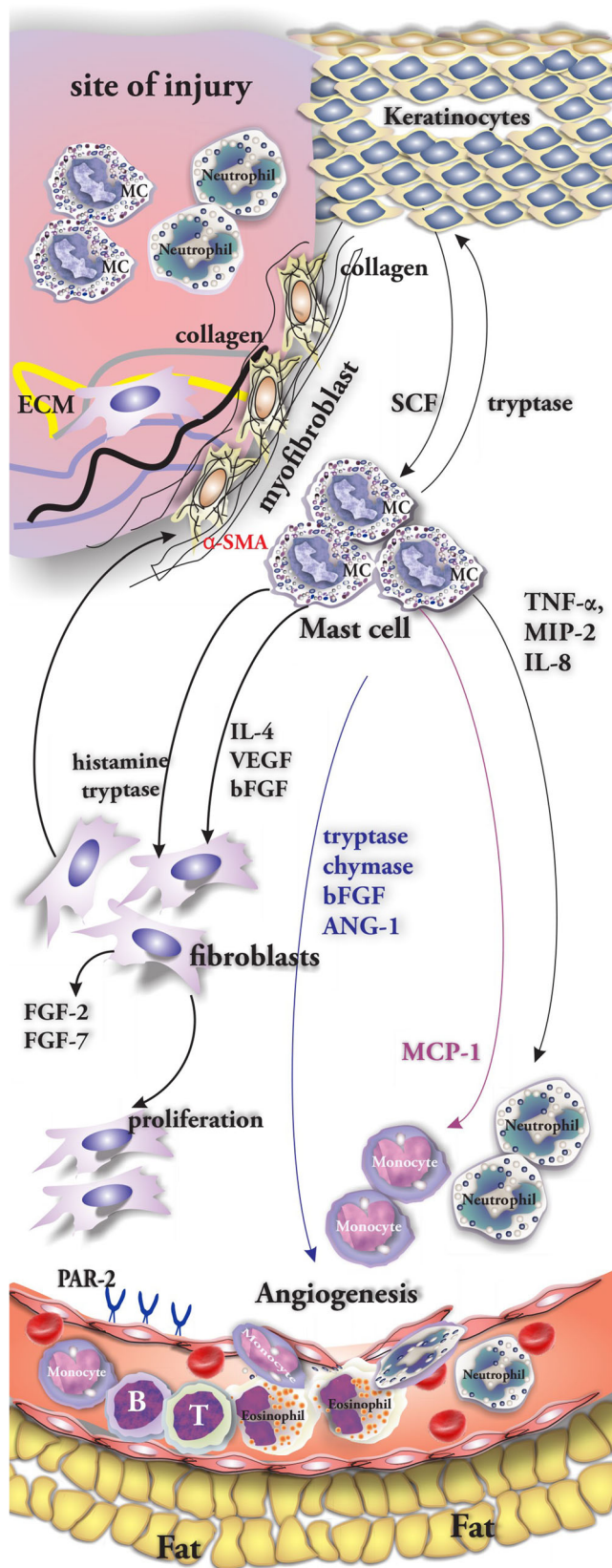


Fig. 5 Cellular and molecular interactions in wound site. Keratinocytes by secreting SCF recruit MCs to the site. MCs then recruit other inflammatory cells including neutrophils and monocytes. Proliferation of fibroblast and differentiation to myofibroblast are essential to produce ECM, collagen, and α -SMA to restore damaged barriers. MCs by releasing mediators facilitate angiogenesis for the purpose of nourishing infiltrated and recently proliferated cells in the wound site

that heal with minimal scarring, such as oral mucosal wounds, compared with other tissues that heal with more fibrotic scars [3, 73–76]. Cutaneous wound healing is accompanied by no inflammation or scarring at early stages of fetal development quite unlike the wounds of mature skin. There is a possible contribution of MCs in fetal wound as MCs of E15 (embryonic day 15) mice are smaller in size, less granular, and less abundant (by nearly half) when compared with MCs of E18 mice [73]. Interestingly, injection of lysate obtained from cultured skin MCs of E18 to dorsal wounds of E15 mice healed with scarring, while injection of lysate from E15 mice to E15 littermates healed without leaving a scar. Another approach to assess the role of MCs in wound healing is to compare the process of healing in terms of scar formation between wild-type mice and those with deficient MCs ($\text{Kit}^{\text{W/W-v}}$ mice). Wulff et al. [73] managed to wound E18 ($\text{Kit}^{\text{W/W-v}}$) and wild-type E18 ($\text{Kit}^{+/+}$) fetuses and harvested samples of skin at 7 or 10 days post-wounding to study the presence/absence of MCs in scar formation. Interestingly, the production of scar tissue was found significantly reduced in $\text{Kit}^{\text{W/W-v}}$ mice, suggesting that MCs play a role in enhancing scar formation [73] (Fig. 6).

Role of Mast Cell Mediators in Wound Healing

MCs participate in different phases of wound healing, largely through releasing mediators that induce the activity of the main cells involved in wound healing (including fibroblasts, endothelial cells, and keratinocytes) (reviewed in Table 1). Using transgenic (Tg) and knockdown mice with deficiency in producing MC mediators, the role of a majority of these mediators has been revealed in animal models. For instance, Succar et al., using Tg mice lacking mMCP-4, mMCP-5, and mMCP-6, revealed that these enzymes play a role in the proliferative phase of healing [79]. In some cases, to determine the role of a MC mediator, researchers benefit from co-culturing the MCs and cell lines that actively participate in wound healing and investigating the effects of a defined MC mediator or inhibitor on the process. For example, co-cultured HLMCs and human lung fibroblasts (HLFs) from patients with idiopathic pulmonary fibrosis (IPF) underwent MC degranulation induced using C48/80. The effect of tryptase on promoting the fibroblast proliferation was investigated by

role of MCs in scar formation are conflicting. Some studies report that MC numbers or their activation is lower in tissues

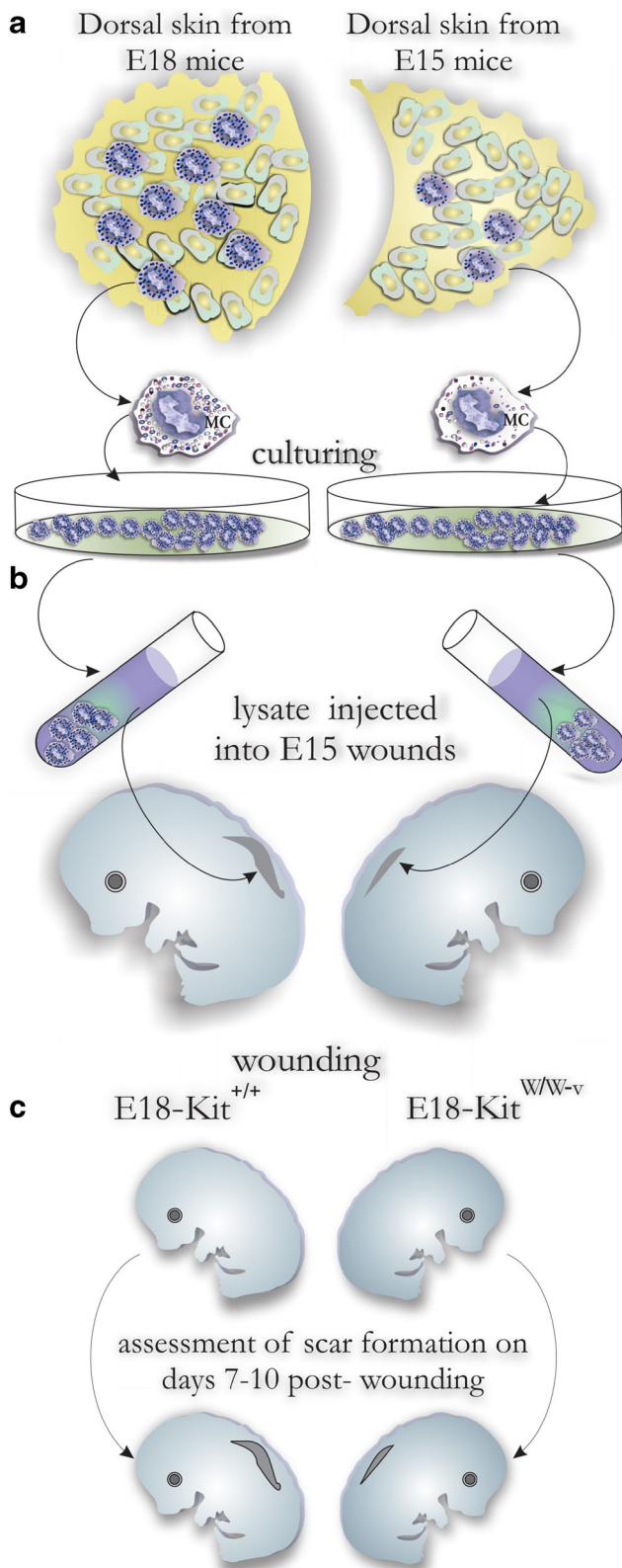


Fig. 6 **a** Morphologic comparison of MCs of E15 and E18 mice showed that MCs of E18 were larger in size and more granular. **b** Injection of lysate obtained from cultured skin MCs of E18 to dorsal wounds of E15 mice healed with scarring, while injection of lysate from E15 mice to E15 littermates healed without leaving a scar. **c** Comparison of wound healing in wounded E18 (Kit^{W/W-v}) and E18 (Kit^{+/+}) fetuses showed that MC-deficient Kit^{W/W-v} wound healed with significantly less production of scar

Mast Cells in Chronic Wounds

Various pathophysiologies of wounds that do not follow the normal healing process and have a significant delay in healing receive the clinical designation of a chronic wound. The incidence of chronic wounds is estimated to be about 1–2% in developed countries [88]. In normal tissue, MCs vary depending on source and are slightly more abundant in distal sites but proximal biopsies from healthy adult individuals are distributed at around 79.5 mast cells/mm² [89]. MCs may be more of a heterogeneous cell population than previously thought and evidence exists that they may readily change phenotype during the course of infection based on distance to wound margin and local inflammatory milieu [3]. In chronic wounds, MCs have a significantly different functional repertoire and location than in acute healing. It has been observed that MCs can migrate to the epithelium margin where they express tryptase, and to the perilesional skin and wound bed where they express chymase. Many MCs in the perilesional skin are double positive for TNF α and tryptase as measured by immunohistochemistry [22]. Tryptase-positive MCs go on to strongly express extracellular markers that appear tightly regulated in MCs of acute healing wounds, including SCF and the receptor Kit [90]. Signaling through Kit induces degranulation, promotes migration, and prevents apoptosis thereby potentially contributing to the chronic inflammatory microenvironment [91]. Functionally, in vitro assays show a role for MCs in inhibiting wound healing through the MC-keratinocyte axis mediated by histamine. This dose-dependent response on the inhibition of proliferation could be biologically relevant in areas where MCs and keratinocytes are in close proximity to one another [52]. An interesting epidermal source of MCs where chronic wounds regularly occur is the tympanic membrane—in particular, MCs are found abundantly (298.8 mast cells/mm²) in the pars flaccida of both rats and humans, and these cells are also prominent in the mucosa of the middle ear [92–94]. In chronic and secretory otitis media, MCs increase in number and degranulate histamine, heparin, and tryptase [95, 96]. The authors postulated that this degranulation is a key contributor to generating a state with chronic effusion, yet antihistamines were regularly used in treating this condition in the past and are now advised against as no substantial benefit has been observed [97]. Further work will undoubtedly be necessary to clarify these pathological disease states and potential mechanisms to aid in their resolution.

application of tryptase inhibitor APC366. They concluded that HLF proliferation induced by degranulating MCs could be attenuated through the application of APC366 [87].

Table 1 General properties and effects of mast cell-derived mediators in wound healing process

Mediator	General properties and effects in wound healing process	Ref
TGF- β 1	• Profibrotic multifunctional growth factor • Stimulates fibroblast proliferation and matrix formation • Some mast cell mediators including protease and chymase through activation of latent TGF-1 contribute in the regulation of extracellular matrix formation	[56]
TGF- β 2	• Is involved in: 1. Marked collagen deposition 2. Increasing mechanical strength of regenerated tissue 3. Accelerating the rate of wound healing	[56]
Histamine	• Capable of inducing proliferation and collagen synthesis of fibroblasts • Causes the dilatation of arterials and increases permeability of venules	[22] [39]
Chymase	• Possesses mitogenic effect on fibroblasts • Promotes myocardial and skin fibroblast proliferation • Regulates angiotensin (Ang) II generation • Regulates degradation of procollagen protein which participates in tissue remodeling and inflammation	[22, 77] [77]
IL-4	• Increases collagen production • Stimulates accumulation of extracellular matrix macromolecules • Stimulates synthesis of the extracellular matrix proteins including type I and III collagen and fibronectin in fibroblast culture	[22] [78] [22]
mMCP-6	• Stimulates dermal fibroblasts, inducing DNA synthesis in quiescent cells • Increases α-1 collagen production • Stimulates fibroblast and smooth muscle proliferation	[63] [79]
MMP-9	• Facilitates tissue remodeling by degradation of extracellular matrix (ECM)	[80]
mMCP-4	• Human homolog: chymase • Increases fibroblast proliferation • Directly proangiogenic by angiotensin I conversion • Indirectly proangiogenic by modulating ECM, releasing VEGF, and activating progelatinase B • Participates in neutrophil recruitment during the inflammatory phase	[79] [79] [81]
mMCP-5	• Human homolog: elastase • Induces the release of TGF-β1	[79]
Tryptase	• Contributes to angiogenesis through directly degrading the ECM components • Releases matrix-bound growth factors owing to its proteolytic activity • Activates latent matrix metalloproteases • Induces endothelial cell proliferation in a dose-dependent manner • Participates in inflammation by leukocyte recruitment • Induces α-SMA expression • Mitogenic for fibroblasts, smooth muscle cells • Possesses chemotactic and mitogenic effects on fibroblasts • Stimulates collagen synthesis • Involved in contraction, and fibroblast differentiation into myofibroblasts	[82] [82] [82] [82] [83] [84] [85] [21] [21] [21]
Carboxypeptidase A	• Increases fibroblast proliferation, and type I collagen production	[53]
bFGF	• Mitogenic factor for fibroblasts, endothelial cells	[86]

Defective Signaling and Molecular Interaction in Wound Healing Involved Cell Lines

In addition to the role of MCs in supporting the cells involved in wound healing in different phases, there are signaling or molecular deficiencies reported in cell lines that hamper the process of normal wound healing.

Fibroblasts

α 11 β 1 integrin is required for the conversion of dermal fibroblasts to myofibroblasts. The absence of α 11 β 1 results in formation of scar tissue but with compromised strength.

Schulz et al. studied the process of wound healing in α 11 β 1-deficient mice and used normal littermate mice as controls. They concluded that α 11 β 1 $^{-/-}$ mice have abnormal collagen remodeling due to TGF- β -dependent JNK signaling [98]. Discoidin domain receptor 2 (DDR2) acts as a tyrosine kinase receptor for fibrillar collagen. DDR2 $^{-/-}$ mice have been reported to have reduced α SMA expression, defective collagen type I synthesis, reduced MMP-2 activity, and defective recruitment of skin fibroblasts. Consequently, DDR2 $^{-/-}$ mice show defective remodeling of the extracellular matrix [99]. CCN1/CYR61 which is a matricellular protein expressed at sites of wound repair has been reported to induce fibroblast senescence through binding to integrin α 6 β 1 and heparan

sulfate proteoglycans and damaging DNA [100]. Additionally, deletion of type II TGF β receptor (T β RII) in fibroblasts results in defective wound contraction [101]. c-Myb oncogene induces IGF-I-independent growth and controls the proliferation of fibroblasts. c-Myb^{-/-} embryos have thinner skin when compared to wild type and their skins show complete absence of collagen type I. In vivo experiments showed that c-Myb deficiency hampers dermal cell proliferation during wound healing [102]. Additionally, CD18 (a common β 2 subunit) binding to variable α subunit (such as CD11) forms integrins that are expressed on a variety of cells. It has been reported that CD18^{-/-} mice have a severely delayed wound healing during granulation tissue contraction. These studied mice were found with reduced TGF- β 1 levels and defective myofibroblast differentiation [103].

Endothelial Cells

Nuclear receptor co-activator AIB1/SRC-3 defective mice were reported to have a defective neoangiogenic response. AIB1/SRC-3^{-/-} endothelial cells were found to have reduced proliferation in response to growth factors. Moreover, AIB1/SRC-3 knockout mice had delayed healing of excisional skin [104].

Keratinocyte

Interestingly, deficiency in FVII results in defective skin wound healing. The mechanism involved is associated with delayed keratinocyte migration. Keratinocytes of low-expressing FVII mice failed to actively participate in mechanical support of the injured site and reepithelialization phase [105]. Mice lacking CD151 have impaired wound healing. Lack of CD151 in mouse keratinocytes effected laminin-332-mediated biological functions during epithelialization—mainly adhesion, migration, and proliferation [106].

Mast Cell Stabilizers and TKIs in Wound Healing

Negative impacts of H1 blocker antihistamines, including mepyramine and promethazine, on wound healing include decreasing the strength of incision wounds and the strength of granulation tissue and collagen content which have previously been reported in rats [107]. Ketotifen, a MC stabilizer, has been reported to have positive effects in reducing excessive wound contraction and fibrosis when applied on post-excisional wounding in animal models [108]. The effects of disodium cromoglycate (DSCG), a widely used MC stabilizer on wound healing, were investigated by Chen et al. in Balb/c mice. They reported that application of DSCG could reduce the levels of inflammatory cytokines IL-1 α , IL-1 β , and

CXCL1, decrease scar width, and accelerate collagen reorganization [109]. Furthermore, the effects of cromolyn on mesh-induced host tissue reactions in subcutaneously mesh-implanted C57BL/6 J mice show that cromolyn treatment could decrease fibrotic responses and inflammation in response to the implemented meshes [110]. Several angiogenesis-targeting tyrosine kinase inhibitors (TKIs) have been suggested to be discontinued in patients with impaired wound healing. For instance, bevacizumab, which specifically binds to VEGF, prevents the mediator from interacting with VEGFR and may result in hemorrhage and the possibility of wound healing complications [111]. Furthermore, the capability of lenvatinib, a VEGFR tyrosine kinase inhibitor, to induce wound healing complications has been discussed [112].

Conclusion

Literature related to the role of MCs in wound healing process is increasingly full of research papers with inconsistent results. Some studies provide evidence of the positive or active contribution of MCs, for instance, by comparing the wound healing process in MC-deficient Kit^W/Kit^{W-v} mice and normal Kit^{+/+} mice, and then restoring the wound healing capacity of wild-type mice in MC-reconstituted Kit^W/Kit^{W-v} mice [80]. Some studies deliberately apply MC mediators to investigate the role of these cells on the molecular level. For example, Younan et al. reported the reconstitution of rmMCP-5 by topical application in scald burn injury to the burn site of mMCP-5^{-/-} mice; human MC chymase was also applied to the burn site of mMCP-4^{-/-} mice [113]. In contrast, the results of some studies just imply a fluctuation in the number of these cells during wound healing [114]. In this regard, one should note that a majority of this research have benefitted from the method of a simple skin incision or small excision to induce a cutaneous injury (mainly in rodents) and have only analyzed MCs in the early proliferative phase. In rodents, contraction is considered as the main mechanism of wound closure upon occurrence of surgical injury. The consequence of the contraction is rapid defect closure of the injured site before allowing time for granular tissue formation and MC proliferation [56]; therefore, the cellular and molecular mechanisms of wound closure may differ from mechanisms observed in humans and may not be applied to humans. One reason that studying the role of MCs in wound healing is problematic is the fact that recruitment of CD117+/CD34+ mast cell takes a few weeks to the site of the wound (plus the additional time needed for their proliferation); therefore, a small cutaneous injury may not be suitable for analyzing the role of MCs in the wound healing process in this model. One possible source of controversy in these results could be the type of studied wounds ranging the gamut from scald, surgical, and diabetes to burn wounds. For instance, Dong et al. used the end of a 50-ml syringe with no

plunger to expose the bared dorsal skin to 75 °C water for creating a wound by burning [77], while Chen et al. made a 4-cm full-thickness skin incision closed with surgical clips until the fifth post-operative day [109]. To the best of our knowledge, still there is no standard procedure of creating experimental wounds especially in rodents (perhaps, but some attempts have been made—see Dorsett-Martin 2004 [115]). It should also be noted that the biological mechanisms of healing differ among animals, for instance, while skin wound healing in Yorkshire pigs closely approximates human wound healing, the prominent mechanism in red Duroc pigs is forming fibroproliferative, hypercontractile scars [108]. Moreover, the precise role of MCs in negative-pressure wound therapy and involvement in wound healing through gap-junction communications [116] and the diabetic wound healing needs to be investigated. Finally, although direct interaction between MCs and fibroblasts through gap-junction protein has been documented, still its possible role in the development of fibrosis in vivo remains unknown. Targeting MCs by stabilizers including cromolyn (disodium cromoglycate) and ketotifen [117], in addition to application of c-Kit-blocking antibodies and small molecules such as tyrosine kinase inhibitors (to reduce MC accumulation during wound healing), has provided promising prospects in wound healing [21]. Mast cells in wounds are likely playing roles of yet undiscovered importance. Of great interest will be uncovering key mechanisms during wound healing to allow for future therapeutic successes directed at mast cells.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent No informed consent was required to prepare the manuscript.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

- Schultz GS, Davidson JM, Kirsner RS, Bornstein P, Herman IM (2011) Dynamic reciprocity in the wound microenvironment. *Wound Repair Regen* 19(2):134–148. <https://doi.org/10.1111/j.1524-475X.2011.00673.x>
- Brugues A, Anon E, Conte V, Veldhuis JH, Gupta M, Colombelli J, Munoz JJ, Brodland GW, Ladoux B, Treppe X (2014) Forces driving epithelial wound healing. *Nat Phys* 10(9):683–690. <https://doi.org/10.1038/nphys3040>
- Wulff BC, Wilgus TA (2013) Mast cell activity in the healing wound: more than meets the eye? *Exp Dermatol* 22(8):507–510. <https://doi.org/10.1111/exd.12169>
- Noli C, Miolo A (2001) The mast cell in wound healing. *Vet Dermatol* 12(6):303–313
- Chen L, DiPietro LA (2017) Toll-like receptor function in acute wounds. *Adv Wound Care* 6(10):344–355. <https://doi.org/10.1089/wound.2017.0734>
- Das A, Dickerson R, Ghatak PD, Gordillo GM, Chaffee S, Saha A, Khanna S, Roy S (2018) May dietary supplementation augment respiratory burst in wound-site inflammatory cells? *Antioxid Redox Signal* 28(5):401–405. <https://doi.org/10.1089/ars.2017.7304>
- Berksoy Hayta S, Durmus K, Altuntas EE, Yildiz E, Hisarciklio M, Akyol M (2018) The reduction in inflammation and impairment in wound healing by using strontium chloride hexahydrate. *Cutan Ocul Toxicol* 37(1):24–28. <https://doi.org/10.1080/15569527.2017.1326497>
- Sun L, Gao W, Fu X, Shi M, Xie W, Zhang W, Zhao F, Chen X (2017) Enhanced wound healing in diabetic rats by nanofibrous scaffolds mimicking the basketweave pattern of collagen fibrils in native skin. *Biomater Sci* 6:340–349. <https://doi.org/10.1039/c7bm00545h>
- Tracy LE, Minasian RA, Caterson EJ (2016) Extracellular matrix and dermal fibroblast function in the healing wound. *Adv Wound Care* 5(3):119–136. <https://doi.org/10.1089/wound.2014.0561>
- Yeh CJ, Chen CC, Leu YL, Lin MW, Chiu MM, Wang SH (2017) The effects of artocarpin on wound healing: in vitro and in vivo studies. *Sci Rep* 7(1):15599. <https://doi.org/10.1038/s41598-017-15876-7>
- Adamson R (2009) Role of macrophages in normal wound healing: an overview. *J Wound Care* 18(8):349–351. <https://doi.org/10.12968/jowc.2009.18.8.43636>
- Martin P, D'Souza D, Martin J, Grose R, Cooper L, Maki R, McKercher SR (2003) Wound healing in the PU.1 null mouse—tissue repair is not dependent on inflammatory cells. *Curr Biol* 13(13):1122–1128
- Yan J, Tie G, Wang S, Tutto A, DeMarco N, Khair L, Fazzio TG, Messina LM (2018) Diabetes impairs wound healing by Dnmt1-dependent dysregulation of hematopoietic stem cells differentiation towards macrophages. *Nat Commun* 9(1):33. <https://doi.org/10.1038/s41467-017-02425-z>
- Artuc M, Hermes B, Steckelings UM, Grutzkau A, Henz BM (1999) Mast cells and their mediators in cutaneous wound healing—active participants or innocent bystanders? *Exp Dermatol* 8(1):1–16
- Au SR, Au K, Saggars GC, Kame N, Ehrlich HP (2007) Rat mast cells communicate with fibroblasts via gap junction intercellular communications. *J Cell Biochem* 100(5):1170–1177. <https://doi.org/10.1002/jcb.21107>
- Komi DEA, Rambasek T, Wohrl S (2017) Mastocytosis: from a molecular point of view. <https://doi.org/10.1007/s12016-017-8619-2>
- Elieh Ali Komi D, Grauwet K (2017) Role of mast cells in regulation of T cell responses in experimental and clinical settings. *Clin Rev Allergy Immunol* 54:432–445. <https://doi.org/10.1007/s12016-017-8646-z>
- Elieh-Ali-Komi D, Cao Y (2017) Role of mast cells in the pathogenesis of multiple sclerosis and experimental autoimmune encephalomyelitis. *Clin Rev Allergy Immunol* 52(3):436–445. <https://doi.org/10.1007/s12016-016-8595-y>
- Elieh Ali Komi D, Bjerner L (2018) Mast cell-mediated orchestration of the immune responses in human allergic asthma: current insights. *Clin Rev Allergy Immunol*. <https://doi.org/10.1007/s12016-018-8720-1>

20. Elieh Ali Komi D, Rambasek T, Bielory L (2017) Clinical implications of mast cell involvement in allergic conjunctivitis. *Allergy* 73:528–539. <https://doi.org/10.1111/all.13334>
21. Wilgus TA, Wulff BC (2014) The importance of mast cells in dermal scarring. *Adv Wound Care* 3(4):356–365. <https://doi.org/10.1089/wound.2013.0457>
22. Huttunen M, Aalto ML, Harvima RJ, Horsmanheimo M, Harvima IT (2000) Alterations in mast cells showing tryptase and chymase activity in epithelializing and chronic wounds. *Exp Dermatol* 9(4):258–265
23. Fukuoka Y, Hite MR, Dellinger AL, Schwartz LB (2013) Human skin mast cells express complement factors C3 and C5. *J Immunol* (Baltimore, Md: 1950) 191(4):1827–1834. <https://doi.org/10.4049/jimmunol.1202889>
24. Bowser C, Erstein DP, Silverberg JI, Nowakowski M, Joks R (2010) Correlation of plasma complement split product levels with allergic respiratory disease activity and relation to allergen immunotherapy. *Ann Allergy Asthma Immunol* 104(1):42–49. <https://doi.org/10.1016/j.anai.2009.11.021>
25. Oskeritzian CA, Zhao W, Min HK, Xia HZ, Pozez A, Kiev J, Schwartz LB (2005) Surface CD88 functionally distinguishes the MCTC from the MCT type of human lung mast cell. *J Allergy Clin Immunol* 115(6):1162–1168. <https://doi.org/10.1016/j.jaci.2005.02.022>
26. Weisel JW (2005) Fibrinogen and fibrin. *Adv Protein Chem* 70: 247–299. [https://doi.org/10.1016/s0065-3233\(05\)70008-5](https://doi.org/10.1016/s0065-3233(05)70008-5)
27. Hartmann K, Henz BM, Kruger-Krasagakes S, Kohl J, Burger R, Guhl S, Haase I, Lippert U, Zuberbier T (1997) C3a and C5a stimulate chemotaxis of human mast cells. *Blood* 89(8):2863–2870
28. Wojta J, Kaun C, Zorn G, Ghannadan M, Hauswirth AW, Sperr WR, Fritsch G, Printz D, Binder BR, Schatzl G, Zwirner J, Maurer G, Huber K, Valent P (2002) C5a stimulates production of plasminogen activator inhibitor-1 in human mast cells and basophils. *Blood* 100(2):517–523
29. Oschatz C, Maas C, Lecher B, Jansen T, Bjorkqvist J, Tradler T, Sedlmeier R, Burfeind P, Cichon S, Hammerschmidt S, Muller-Esterl W, Willemin WA, Nilsson G, Renne T (2011) Mast cells increase vascular permeability by heparin-initiated bradykinin formation in vivo. *Immunity* 34(2):258–268. <https://doi.org/10.1016/j.immuni.2011.02.008>
30. Yanase Y, Takahagi S, Hide M (2017) Chronic spontaneous urticaria and the extrinsic coagulation system. *Allergol Int* 67:191–194. <https://doi.org/10.1016/j.alit.2017.09.003>
31. Zhu H, Liang B, Li R, Li J, Lin L, Ma S, Wang J (2013) Activation of coagulation, anti-coagulation, fibrinolysis and the complement system in patients with urticaria. *Asian Pac J Allergy Immunol* 31(1):43–50
32. Kettelhut BV, Metcalfe DD (1991) Pediatric mastocytosis. *J Invest Dermatol* 96(3 Suppl):15S–18S; discussion 18S, 60S–65S. <https://doi.org/10.1111/1523-1747.ep12468942>
33. Gonzalo-Garijo MA, Perez-Rangel I, Alvarado-Izquierdo MI, Perez-Calderon R, Sanchez-Vega S, Zambonino MA (2010) Metrorrhagia as an uncommon symptom of anaphylaxis. *J Invest Allergol Clin Immunol* 20(6):540–541
34. Prieto-Garcia A, Castells MC, Hansbro PM, Stevens RL (2014) Mast cell-restricted tetramer-forming tryptases and their beneficial roles in hemostasis and blood coagulation. *Immunol Allergy Clin N Am* 34(2):263–281. <https://doi.org/10.1016/j.iac.2014.01.001>
35. Trautmann A, Toksoy A, Engelhardt E, Brocker EB, Gillitzer R (2000) Mast cell involvement in normal human skin wound healing: expression of monocyte chemoattractant protein-1 is correlated with recruitment of mast cells which synthesize interleukin-4 in vivo. *J Pathol* 190(1):100–106. [https://doi.org/10.1002/\(sici\)1096-9896\(200001\)190:1<100::aid-path496>3.0.co;2-q](https://doi.org/10.1002/(sici)1096-9896(200001)190:1<100::aid-path496>3.0.co;2-q)
36. Gillitzer R, Goebeler M (2001) Chemokines in cutaneous wound healing. *J Leukoc Biol* 69(4):513–521
37. Ogle ME, Segar CE, Sridhar S, Botchwey EA (2016) Monocytes and macrophages in tissue repair: implications for immunoregenerative biomaterial design. *Exp Biol Med* (Maywood, NJ) 241(10):1084–1097. <https://doi.org/10.1177/1535370216650293>
38. Gordon JR (2000) Monocyte chemoattractant peptide-1 expression during cutaneous allergic reactions in mice is mast cell dependent and largely mediates the monocyte recruitment response. *J Allergy Clin Immunol* 106(1 Pt 1):110–116. <https://doi.org/10.1067/mai.2000.107036>
39. Li J, Chen J, Kirsner R (2007) Pathophysiology of acute wound healing. *Clin Dermatol* 25(1):9–18. <https://doi.org/10.1016/j.clindermatol.2006.09.007>
40. Egozi EI, Ferreira AM, Burns AL, Gamelli RL, Dipietro LA (2003) Mast cells modulate the inflammatory but not the proliferative response in healing wounds. *Wound Repair Regen* 11(1):46–54
41. Leoni G, Neumann PA, Sumagin R, Denning TL, Nusrat A (2015) Wound repair: role of immune-epithelial interactions. *Mucosal Immunol* 8(5):959–968. <https://doi.org/10.1038/mi.2015.63>
42. van der Veer WM, Bloemen MC, Ulrich MM, Molema G, van Zuijlen PP, Middelkoop E, Niessen FB (2009) Potential cellular and molecular causes of hypertrophic scar formation. *Burns* 35(1): 15–29. <https://doi.org/10.1016/j.burns.2008.06.020>
43. Iba Y, Shibata A, Kato M, Masukawa T (2004) Possible involvement of mast cells in collagen remodeling in the late phase of cutaneous wound healing in mice. *Int Immunopharmacol* 4(14): 1873–1880. <https://doi.org/10.1016/j.intimp.2004.08.009>
44. Theoharides TC, Alysandratos KD, Angelidou A, Delivanis DA, Sismanopoulos N, Zhang B, Asadi S, Vasiadi M, Weng Z, Miniati A, Kalogeromitros D (2012) Mast cells and inflammation. *Biochim Biophys Acta* 1822(1):21–33. <https://doi.org/10.1016/j.bbadis.2010.12.014>
45. Kennelly R, Conneely JB, Bouchier-Hayes D, Winter DC (2011) Mast cells in tissue healing: from skin to the gastrointestinal tract. *Curr Pharm Des* 17(34):3772–3775
46. Ng MF (2010) The role of mast cells in wound healing. *Int Wound J* 7(1):55–61. <https://doi.org/10.1111/j.1742-481X.2009.00651.x>
47. Ishikawa T, Kanda N, Hau CS, Tada Y, Watanabe S (2009) Histamine induces human beta-defensin-3 production in human keratinocytes. *J Dermatol Sci* 56(2):121–127. <https://doi.org/10.1016/j.jdermsci.2009.07.012>
48. Soliman M, Kim DS, Park JG, Kim JY, Alfajaro MM, Baek YB, Cho EH, Park CH, Kang MI, Park SI, Cho KO (2018) PI3K/Akt and MEK/ERK signaling pathways facilitate sapovirus trafficking and late endosomal acidification for viral uncoating in LLC-PK cells. *J Virol* 92. <https://doi.org/10.1128/jvi.01674-18>
49. Zhu C, Bao NR, Chen S, Zhao JN (2016) HBD-3 regulation of the immune response and the LPS/TLR4-mediated signaling pathway. *Exp Ther Med* 12(4):2150–2154. <https://doi.org/10.3892/etm.2016.3579>
50. Rohrl J, Yang D, Oppenheim JJ, Hehlhans T (2008) Identification and biological characterization of mouse beta-defensin 14, the orthologue of human beta-defensin 3. *J Biol Chem* 283(9):5414–5419. <https://doi.org/10.1074/jbc.M709103200>
51. Kasuya A, Tokura Y (2014) Attempts to accelerate wound healing. *J Dermatol Sci* 76(3):169–172. <https://doi.org/10.1016/j.jdermsci.2014.11.001>
52. Huttunen M, Hyttinen M, Nilsson G, Butterfield JH, Horsmanheimo M, Harvima IT (2001) Inhibition of keratinocyte growth in cell culture and whole skin culture by mast cell mediators. *Exp Dermatol* 10(3):184–192
53. Gruber BL (2003) Mast cells in the pathogenesis of fibrosis. *Curr Rheumatol Rep* 5(2):147–153

54. Yamamoto T, Hartmann K, Eckes B, Krieg T (2000) Mast cells enhance contraction of three-dimensional collagen lattices by fibroblasts by cell-cell interaction: role of stem cell factor/c-kit. *Immunology* 99(3):435–439
55. Yamamoto T, Hartmann K, Eckes B, Krieg T (2001) Role of stem cell factor and monocyte chemoattractant protein-1 in the interaction between fibroblasts and mast cells in fibrosis. *J Dermatol Sci* 26(2):106–111
56. Shiota N, Nishikori Y, Kakizoe E, Shimoura K, Niibayashi T, Shimbori C, Tanaka T, Okunishi H (2010) Pathophysiological role of skin mast cells in wound healing after scald injury: study with mast cell-deficient W/W^V mice. *Int Arch Allergy Immunol* 151(1):80–88. <https://doi.org/10.1159/000232573>
57. Nauta AC, Grova M, Montoro DT, Zimmermann A, Tsai M, Gurtner GC, Galli SJ, Longaker MT (2013) Evidence that mast cells are not required for healing of splinted cutaneous excisional wounds in mice. *PLoS One* 8(3):e59167. <https://doi.org/10.1371/journal.pone.0059167>
58. Tellechea A, Leal EC, Kafanas A, Auster ME, Kuchibhotla S, Ostrovsky Y, Tecilazich F, Baltzis D, Zheng Y, Carvalho E, Zabolotny JM, Weng Z, Petra A, Patel A, Panagiotidou S, Pradhan-Nabzdyk L, Theoharides TC, Veves A (2016) Mast cells regulate wound healing in diabetes. *Diabetes* 65(7):2006–2019. <https://doi.org/10.2337/db15-0340>
59. Oskeritzian CA (2012) Mast cells and wound healing. *Adv Wound Care* 1(1):23–28. <https://doi.org/10.1089/wound.2011.0357>
60. Wilgus TA (2008) Immune cells in the healing skin wound: influential players at each stage of repair. *Pharmacol Res* 58(2):112–116. <https://doi.org/10.1016/j.phrs.2008.07.009>
61. Hiromatsu Y, Toda S (2003) Mast cells and angiogenesis. *Microsc Res Tech* 60(1):64–69. <https://doi.org/10.1002/jemt.10244>
62. Rao KN, Brown MA (2008) Mast cells: multifaceted immune cells with diverse roles in health and disease. *Ann N Y Acad Sci* 1143:83–104. <https://doi.org/10.1196/annals.1443.023>
63. Maltby S, Khazaie K, McNagny KM (2009) Mast cells in tumor growth: angiogenesis, tissue remodelling and immune-modulation. *Biochim Biophys Acta* 1796(1):19–26. <https://doi.org/10.1016/j.bbcan.2009.02.001>
64. Hinz B (2016) The role of myofibroblasts in wound healing. *Curr Res Transl Med* 64(4):171–177. <https://doi.org/10.1016/j.retram.2016.09.003>
65. Nishikori Y, Shiota N, Okunishi H (2014) The role of mast cells in cutaneous wound healing in streptozotocin-induced diabetic mice. *Arch Dermatol Res* 306(9):823–835. <https://doi.org/10.1007/s00403-014-1496-0>
66. Bagher M, Larsson-Callert AK, Rosmark O, Hallgren O, Bjermer L, Westergren-Thorsson G (2018) Mast cells and mast cell tryptase enhance migration of human lung fibroblasts through protease-activated receptor 2. *Cell Commun Signal* 16(1):59. <https://doi.org/10.1186/s12964-018-0269-3>
67. Landolina N, Gangwar RS, Levi-Schaffer F (2015) Mast cells' integrated actions with eosinophils and fibroblasts in allergic inflammation: implications for therapy. *Adv Immunol* 125:41–85. <https://doi.org/10.1016/bs.ai.2014.09.002>
68. Takeo M, Lee W, Ito M (2015) Wound healing and skin regeneration. *Cold Spring Harbor Perspect Med* 5(1):a023267. <https://doi.org/10.1101/cshperspect.a023267>
69. Glim JE, van Egmond M, Niessen FB, Everts V, Beelen RH (2013) Detrimental dermal wound healing: what can we learn from the oral mucosa? *Wound Repair Regen* 21(5):648–660. <https://doi.org/10.1111/wrr.12072>
70. Moyer KE, Saggars GC, Ehrlich HP (2004) Mast cells promote fibroblast populated collagen lattice contraction through gap junction intercellular communication. *Wound Repair Regen* 12(3):269–275. <https://doi.org/10.1111/j.1067-1927.2004.012310.x>
71. Ehrlich HP (2013) A snapshot of direct cell-cell communications in wound healing and scarring. *Adv Wound Care* 2(4):113–121. <https://doi.org/10.1089/wound.2012.0414>
72. Darby IA, Hewitson TD (2007) Fibroblast differentiation in wound healing and fibrosis. *Int Rev Cytol* 257:143–179. [https://doi.org/10.1016/s0074-7696\(07\)57004-x](https://doi.org/10.1016/s0074-7696(07)57004-x)
73. Wulff BC, Parent AE, Meleski MA, DiPietro LA, Schrementi ME, Wilgus TA (2012) Mast cells contribute to scar formation during fetal wound healing. *J Invest Dermatol* 132(2):458–465. <https://doi.org/10.1038/jid.2011.324>
74. Whitby DJ, Ferguson MW (1991) The extracellular matrix of lip wounds in fetal, neonatal and adult mice. *Development* 112(2):651–668
75. Glim JE, Beelen RH, Niessen FB, Everts V, Ulrich MM (2015) The number of immune cells is lower in healthy oral mucosa compared to skin and does not increase after scarring. *Arch Oral Biol* 60(2):272–281. <https://doi.org/10.1016/j.archoralbio.2014.10.008>
76. Natah SS, Hayrinen-Immonen R, Hietanen J, Malmstrom M, Kontinen YT (1998) Quantitative assessment of mast cells in recurrent aphthous ulcers (RAU). *J Oral Pathol Med* 27(3):124–129
77. Dong X, Xu T, Ma S, Wen H (2015) Expression and activity levels of chymase in mast cells of burn wound tissues increase during the healing process in a hamster model. *Exp Ther Med* 9(6):2190–2194. <https://doi.org/10.3892/etm.2015.2424>
78. Salmon-Ehr V, Ramont L, Godeau G, Birembaut P, Guenounou M, Bernard P, Maquart FX (2000) Implication of interleukin-4 in wound healing. *Lab Invest* 80(8):1337–1343
79. Succar J, Douaiher J, Lancerotto L, Li Q, Yamaguchi R, Younan G, Pejler G, Orgill DP (2014) The role of mouse mast cell proteases in the proliferative phase of wound healing in microdeformational wound therapy. *Plast Reconstr Surg* 134(3):459–467. <https://doi.org/10.1097/prs.0000000000000432>
80. Weller K, Foitzik K, Paus R, Syska W, Maurer M (2006) Mast cells are required for normal healing of skin wounds in mice. *FASEB J* 20(13):2366–2368. <https://doi.org/10.1096/fj.06-5837je>
81. Lin L, Bankaitis E, Heimbach L, Li N, Abrink M, Pejler G, An L, Diaz LA, Werb Z, Liu Z (2011) Dual targets for mouse mast cell protease-4 in mediating tissue damage in experimental bullous pemphigoid. *J Biol Chem* 286(43):37358–37367. <https://doi.org/10.1074/jbc.M111.272401>
82. Honnégowda TM, Kumar P, Padmanabha Udupa EG, Kumar S, Kumar U, Rao P (2015) Role of angiogenesis and angiogenic factors in acute and chronic wound healing. *Plast Aesthet Res* 2:243–249. <https://doi.org/10.4103/2347-9264.165438>
83. Caughey GH (2007) Mast cell tryptases and chymases in inflammation and host defense. *Immunol Rev* 217:141–154. <https://doi.org/10.1111/j.1600-065X.2007.00509.x>
84. Gailit J, Marchese MJ, Kew RR, Gruber BL (2001) The differentiation and function of myofibroblasts is regulated by mast cell mediators. *J Invest Dermatol* 117(5):1113–1119. <https://doi.org/10.1046/j.1523-1747.2001.15211.x>
85. Kondo S, Kagami S, Kido H, Strutz F, Muller GA, Kuroda Y (2001) Role of mast cell tryptase in renal interstitial fibrosis. *J Am Soc Nephrol* 12(8):1668–1676
86. Li CY, Baek JY (2002) Mastocytosis and fibrosis: role of cytokines. *Int Arch Allergy Immunol* 127(2):123–126
87. Wygrecka M, Dahal BK, Kosanovic D, Petersen F, Taborski B, von Gerlach S, Didiasova M, Zakrzewicz D, Preissner KT, Schermuly RT, Markart P (2013) Mast cells and fibroblasts work in concert to aggravate pulmonary fibrosis: role of transmembrane SCF and the PAR-2/PKC- α /Raf-1/p44/42 signaling pathway. *Am J Pathol* 182(6):2094–2108. <https://doi.org/10.1016/j.ajpath.2013.02.013>

88. Jarbrink K, Ni G, Sonnergren H, Schmidtchen A, Pang C, Bajpai R, Car J (2016) Prevalence and incidence of chronic wounds and related complications: a protocol for a systematic review. 5(1): 152. <https://doi.org/10.1186/s13643-016-0329-y>
89. Janssens AS, Heide R, den Hollander JC, Mulder PG, Tank B, Oranje AP (2005) Mast cell distribution in normal adult skin. *J Clin Pathol* 58(3):285–289. <https://doi.org/10.1136/jcp.2004.017210>
90. Huttunen M, Naukkarinen A, Horsmanheimo M, Harvima IT (2002) Transient production of stem cell factor in dermal cells but increasing expression of Kit receptor in mast cells during normal wound healing. *Arch Dermatol Res* 294(7):324–330. <https://doi.org/10.1007/s00403-002-0331-1>
91. Iemura A, Tsai M, Ando A, Wershil BK, Galli SJ (1994) The c-kit ligand, stem cell factor, promotes mast cell survival by suppressing apoptosis. *Am J Pathol* 144(2):321–328
92. Alm PE, Bloom GD, Hellstrom S, Stenfors LE, Widemar L (1983) Mast cells in the pars flaccida of the tympanic membrane. A quantitative morphological and biochemical study in the rat. *Experientia* 39(3):287–289
93. Widemar L, Hellstrom S, Stenfors LE, Bloom GD (1986) An overlooked site of tissue mast cells—the human tympanic membrane. Implications for middle ear affections. *Acta Otolaryngol* 102(5–6):391–395
94. Ichimiya I, Kawauchi H, Mogi G (1990) Analysis of immunocompetent cells in the middle ear mucosa. *Arch Otolaryngol Head Neck Surg* 116(3):324–330
95. Sankovic S, Dergenc R, Bojic P (2005) Mast cells in chronic inflammation of the middle ear mucosa. *Rev Laryngol Otol Rhinol* 126(1):15–18
96. Hurst DS, Amin K, Seveus L, Venge P (1999) Evidence of mast cell activity in the middle ears of children with otitis media with effusion. *Laryngoscope* 109(3):471–477
97. Griffin G, Flynn CA (2011) Antihistamines and/or decongestants for otitis media with effusion (OME) in children. *Cochrane Database Syst Rev* 9:CD003423. <https://doi.org/10.1002/14651858.CD003423.pub3>
98. Schulz JN, Zeltz C, Sorensen IW, Barczyk M, Carracedo S, Hallinger R, Niehoff A, Eckes B, Gullberg D (2015) Reduced granulation tissue and wound strength in the absence of α 11 β 1 integrin. *J Invest Dermatol* 135(5):1435–1444. <https://doi.org/10.1038/jid.2015.24>
99. Olaso E, Lin HC, Wang LH, Friedman SL (2011) Impaired dermal wound healing in discoidin domain receptor 2-deficient mice associated with defective extracellular matrix remodeling. *Fibrogenesis Tissue Repair* 4(1):5. <https://doi.org/10.1186/1755-1536-4-5>
100. Jun JI, Lau LF (2010) The matricellular protein CCN1 induces fibroblast senescence and restricts fibrosis in cutaneous wound healing. *Nat Cell Biol* 12(7):676–685. <https://doi.org/10.1038/ncb2070>
101. Denton CP, Khan K, Hoyles RK, Shiwen X, Leoni P, Chen Y, Eastwood M, Abraham DJ (2009) Inducible lineage-specific deletion of TbetRII in fibroblasts defines a pivotal regulatory role during adult skin wound healing. *J Invest Dermatol* 129(1):194–204. <https://doi.org/10.1038/jid.2008.171>
102. Kopecki Z, Luchetti MM, Adams DH, Strudwick X, Mantamadiotis T, Stoppacciaro A, Gabrielli A, Ramsay RG, Cowin AJ (2007) Collagen loss and impaired wound healing is associated with c-Myb deficiency. *J Pathol* 211(3):351–361. <https://doi.org/10.1002/path.2113>
103. Peters T, Sindrilaru A, Hinz B, Hinrichs R, Menke A, Al-Azzeh EA, Holzwarth K, Oreshkova T, Wang H, Kess D, Walzog B, Sulyok S, Sunderkotter C, Friedrich W, Wlaschek M, Krieg T, Scharffetter-Kochanek K (2005) Wound-healing defect of CD18(–/–) mice due to a decrease in TGF- β 1 and myofibroblast differentiation. *EMBO J* 24(19):3400–3410. <https://doi.org/10.1038/sj.emboj.7600809>
104. Al-Otaiby M, Tassi E, Schmidt MO, Chien CD, Baker T, Salas AG, Xu J, Furlong M, Schlegel R, Riegel AT, Wellstein A (2012) Role of the nuclear receptor coactivator AIB1/SRC-3 in angiogenesis and wound healing. *Am J Pathol* 180(4):1474–1484. <https://doi.org/10.1016/j.ajpath.2011.12.032>
105. Xu Z, Xu H, Ploplis VA, Castellino FJ (2010) Factor VII deficiency impairs cutaneous wound healing in mice. *Mol Med* 16(5–6): 167–176. <https://doi.org/10.2119/molmed.2009.00171>
106. Geary SM, Cowin AJ, Copeland B, Baleato RM, Miyazaki K, Ashman LK (2008) The role of the tetraspanin CD151 in primary keratinocyte and fibroblast functions: implications for wound healing. *Exp Cell Res* 314(11–12):2165–2175. <https://doi.org/10.1016/j.yexcr.2008.04.011>
107. Bairy KL, Rao CM, Ramesh KV, Kulkarni DR (1991) Effects of antihistamines on wound healing. *Indian J Exp Biol* 29(4):398–399
108. Gallant-Behm CL, Hildebrand KA, Hart DA (2008) The mast cell stabilizer ketotifen prevents development of excessive skin wound contraction and fibrosis in red Duroc pigs. *Wound Repair Regen* 16(2):226–233. <https://doi.org/10.1111/j.1524-475X.2008.00363.x>
109. Chen L, Schrementi ME, Ranzer MJ, Wilgus TA, DiPietro LA (2014) Blockade of mast cell activation reduces cutaneous scar formation. *PLoS One* 9(1):e85226. <https://doi.org/10.1371/journal.pone.0085226>
110. Orenstein SB, Saberski ER, Klueh U, Kreutzer DL, Novitsky YW (2010) Effects of mast cell modulation on early host response to implanted synthetic meshes. *Hernia* 14(5):511–516. <https://doi.org/10.1007/s10029-010-0680-1>
111. Shah DR, Dholakia S, Shah RR (2014) Effect of tyrosine kinase inhibitors on wound healing and tissue repair: implications for surgery in cancer patients. *Drug Saf* 37(3):135–149. <https://doi.org/10.1007/s40264-014-0139-x>
112. Cheng C, Nayemama A, Christopher Jones S, Casey D, Waldron PE (2018) Wound healing complications with lenvatinib identified in a pharmacovigilance database. *J Oncol Pharm Pract*. <https://doi.org/10.1177/1078155218817109>
113. Younan G, Suber F, Xing W, Shi T, Kunori Y, Abrink M, Pejler G, Schlenner SM, Rodewald HR, Moore FD Jr, Stevens RL, Adachi R, Austen KF, Gurish MF (2010) The inflammatory response after an epidermal burn depends on the activities of mouse mast cell proteases 4 and 5. *J Immunol* 185(12):7681–7690. <https://doi.org/10.4049/jimmunol.1002803>
114. el Sayed SO, Dyson M (1993) Responses of dermal mast cells to injury. *J Anat* 182(Pt 3):369–376
115. Dorsett-Martin WA (2004) Rat models of skin wound healing: a review. *Wound Repair Regen* 12(6):591–599. <https://doi.org/10.1111/j.1067-1927.2004.12601.x>
116. Garner WL, Ehrlich HP (2011) Discussion: mast cells are required in the proliferation and remodeling phases of microdeformational wound therapy. *Plast Reconstr Surg* 128(6):659e–660e. <https://doi.org/10.1097/PRS.0b013e318230bec1>
117. Dong X, Geng Z, Zhao Y, Chen J, Cen Y (2013) Involvement of mast cell chymase in burn wound healing in hamsters. *Exp Ther Medicine* 5(2):643–647. <https://doi.org/10.3892/etm.2012.836>