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Review Article

Matrix Metalloproteinases And Their Tissue Inhibitors In Wound Healing Cascade: An Update

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ABSTRACT
Wound healing is a complex process involving various cellular and molecular events to restore tissue integrity. Matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) are crucial in regulating extracellular matrix (ECM) remodeling, a pivotal step in wound healing. MMPs are zinc-dependent endopeptidases that degrade components of the ECM, including collagens, elastin, and proteoglycans. They are produced by various cell types involved in wound healing, such as fibroblasts, keratinocytes, and inflammatory cells. Their expression is tightly regulated during different phases of wound healing. In the initial inflammatory phase, MMPs recruit and activate immune cells, facilitating the clearance of pathogens and debris. In the proliferative phase, MMPs promote cell migration, angiogenesis, and the formation of granulation tissue, serving as a provisional matrix for tissue regeneration. In the remodeling phase, MMPs are essential for the degradation of the provisional matrix and the synthesis of new ECM components, leading to wound closure and scar formation. Understanding the roles of MMPs and TIMPs is crucial for developing effective therapeutic interventions.

Keywords: Wound healing,TIMPs, MMPs, inflammatory, Remoulding

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1. INTRODUCTION

Skin, the largest organ of the human body, is responsible for protecting the body from outer infection or damage.Skin provides a waterproof barrier, sweat glands to maintain body temperature, and cushioning effect on the skeleton. Any damage to the epithelial cells which are present in the epidermal layer of the skin may infringe on the integrity of the skin [1][1]. Matrix metalloproteinases (MMPs) and endopeptidases(also called metallopeptidases or endoproteinases)are calcium-dependent zinc-containing enzymes that help in the regeneration of the skin[2] [2]. Apart from that, it is also responsible for sustaining fluid balance and thermoregulation [3]. This protective organ's primary functions include regulating body temperature, permitting sensations like heat, cold, and touch,and the last one is protected from the outer environment. Each layer of skin has its distinctive feature. They have their separate role and functions to perform[4] for the tensile potency of the skin dermis layer along with the extracellular matrix (ECM) [5]. This cushioning effect is more potential in the presence of ECM. It is an absolute 3D structure made up of connective tissues fat cells, and complex proteins such as collagen, proteoglycans, and glycoproteins (almost 300 different types of proteins) [6].

The proper functioning of the skin is very important for the body as it is the first line of defence against

invading pathogens [7][3]. Damage to any of the layers of human skin may alter the proper functioning of the skin which may result in instability. Skin damage is also easily accessed by environmental pathogens. Breakdown in the protective or defensive function of the skin may be considered a wound that has to be healed in a well-planned manner. Thus, wound healing is required as a necessary physiological protective mechanism of the human body. The integrity of the skin is supremely important while maintaining the physiological homeostasis and feasibility of the internal tissues as well.

The skinis directly involved in the healing of cutaneous wounds while it also plays an indirect role in the healing of internal or chronic wounds [8-11]. Categorically, wounds can be differentiated into various types that are: Superficial wounds (loss of epidermis layer only), partial wound (loss of epidermis and dermis layer) and full thickness wound which involves the loss of the dermis subcutaneous fat and sometimes even bone. Thus, superficial wounds do not possess any threatening damage whereas partial and full-thickness wounds may involve the loss of the dermal and subcutaneous layer as well. Apart from these categories based on the degree of damage and the time needs to be healed, wounds are divided into two subcategories which are acute and chronic wounds.

The normal healing process pursues a systematic and organized sequence of four dissimilar yet overlapping stages that are hemostasis, inflammatory phase, proliferation phase, and remodeling phase [12][4].While acute wounds heal in a synchronized manner of the healing cascade, on the other hand in the case of chronic wounds-compliance of some wounds to the healing pattern may lead to a delay in their healing. This may occur because of the prolonged residency of one or the other healing phases.The interruption in any phase of this dynamic process of healing may lead to the onset of chronic ulcers eventually. For the skin's normal functioning, the damage must be repaired immediately to prevent further impairment due to the chances of microbial infections. [13-16]. Additionally, functional coordination among all four partially overlapping phases is mandatory to commence the progression of tissue restoration. For the healthy progression of wound healing, ECM has to be remodeled efficiently as it is responsible for the tensile strength of the skin and in this process endopeptidases such as MMPs are thoroughly involved.

The prominence of this review article is to emphasize the role of MMPs that are systematically involved in the wound healing process. At present, 24 known MMPs are likely to be present in the human body [17]. Recent research works also identified some additional MMPs. MMPs are generally endopeptidases that are competent in eliminating all the components of the ECM. ECM generally promotes all the cellular responses in the process of wound healing including cell adhesion, migration and tissue remodeling. Removal of all the damaged proteins and cells within the ECM is the main line of action of the MMPs. Their engagement is seen in all four phases of wound healing. During the inflammatory phase, they are capable of removing the damaged proteins and temporary ECM [18-22].While during the proliferation phase, MMPs help in degrading the capillary basement membrane, they promote angiogenesis during the wound-healing cascade. The respective role of MMP in wound healing can be studied by carefully evaluating all the events that participated in this sequential process. The first and initial phase (hemostasis) is tissue injury which involves the epidermal layer, platelets and TGF-β. Immediately after the hemostasis inflammatory phase begins which involves neutrophils, macrophages, and the mediators released by these cells such as reactive oxygen species (ROS), tumor necrosis factors (TNFs),vascular endothelial growth factors(VEGFs), platelet-derived growth factors(PDGFs) and others. Granulation and formation of new blood vessels (angiogenesis)are also the two most crucial events in the wound-healingprocess[23].

Besides immunological cells such as neutrophils and macrophages,nonimmunological cells namelykeratinocytes, fibroblasts andendothelial cells are involved in these events.Further, re-epithelialization and tissue remodeling generally involve keratinocytes and collagen fiber cross-linking. All the phases must have to be integrated timely and sequential manner to heal the wound completely [24]. Here, MMPs play a critical role in every phase of wound healing. Thiscomprehensivereview article discusses all the possible rolesof MMPs and tissue inhibitors of matrix metalloproteinases (TIMPs) that are involved during every step of the wound healing process. Finally, the review highlights the latest advances in the respective field regarding the regulation and mediation of MMPs in the human skinandtheir possible application to other branches of medical sciences as well.

2. THE WOUND HEALING CASCADE

Wound healing is a physiological process of repairing damaged or injured tissues, as well as the formation of new tissues with three overlapping phasesnamely the inflammatory phase, the proliferative phase and the maturation phase [25][5]. The inflammatory phase forms a clot followed by inflammatorycells building up in the wound or injury site. This phase lasts up to 2-5 days and includes vasoconstriction, platelet aggregation and clot formation followed by vasodilation and phagocytosis. The second phase lasts for about 2-3 weeks consisting of granulation, contraction and epithelialization. Thereafter comes the maturation phase in which new tissue is formed and it lasts approximately 3 weeks.

Upon injury, to the skin, the superficial wound quickly crosses the threshold of the primary stage of the healing process which is known as hemostasis. Excessive bleeding is protected by the onset of blood vessels that is further pursued by the accumulation of plateletsaround the ruptured endothelium region resulting in the formation of the plug [26][6]. A blood clot is formed upon a further cascade of events. The serine proteinase thrombin leads to the cleavage of fibrinogen into small fibrin threads which aggregate along with platelets to form a clot. The formation of a

blood clot not only stops the bleeding but also serves as a provisional matrix for the migration of cells [27].

The cells surrounding the clot release various inflammatory cytokines and growth factors (GFs) which further signal the attraction of various cells such as neutrophils, lymphocytes, monocytes and macrophages and initiate the phase of inflammation. Neutrophils, the most abundant white blood cells in circulation arrive at the site of injury only within a few hours. They are highly responsible for the release of fibronectin which possesses multiple roles [28,29]. Fibronectin and fibrin offer a provisional matrix thatinitiatesthemigration of cells and sticks them together. Their adhesion power is dependent on the intensity of injury that occurred[28]. GFs induce activation of macrophages which leads to the synthesis of ROS, multiple GFs like PDGF, VEGF, MMPs, transforming growth factor-β (TGF-β), and fibroblast growth factor (FGF) [30].

Angiotensin II is an important factor that can activate macrophages. It is mainly regulated bythereaction of the renin-angiotensin system (RAS) pathway. It generally co-exists in the fibroblasts, macrophages and endothelial cells present in human skin [31][7]. Secretion of MMPs and ROS is usually initiated by the activation of Angiotensin II along with the trigger of inflammatory cells. Stimulation of these factors initiates the further intensification of keratinocytes. They are also responsible for their migration through the injured cells as well.Themultifunctional cytokine TGF-βplaysacrucial part in the regulation and development of the ECM. Humans comprise 3 isoforms (TGF-β1–3), each presenting a distinct function in the regulation of components of ECM, the proliferation of cells and even cell death[8] [32-34].

The most renowned is the TGF-β1, whichregulatestheproduction and destruction of several constituents indulged in the healing of wounds [28]. Upon binding of TGF-β1 to its respective receptor, synthesis and production of components of ECM like collagen, fibronectin, and hyaluronic acid in different cell types, including fibroblasts are initiated [35]. Collagen is mainly synthesized by fibroblastic cells and other constituents which are accumulated within the ECM[17]. Fibroblasts are also accountable for the stimulation of ROSgenerationwhichincludes the defensive warriors of the body against any kind of microbial attack.ROS includehighlyreactive superoxide and hydroxyl molecules and relatively less reactive peroxides molecules [36].

The synthesized ROS stimulates different immunological cells for the expression of various cytokines which further increase the proteinase production andalter the fragments of the ECM [37]. The main characteristic of ROS is that it has twofold functions that are associated with it. The antimicrobial function is of great advantage to the human body as it protects the body from foreign particles. However, excessive levels of ROS may damage the constituents of ECM. This moderate equilibrium between the secretion of ROS may activate the intricate pathways in the body which activates the MMPs secretion in the wounded area. The elevated levels of ROS could harm the tissues and in turn, may alter the wound-healing process [38,39].

Dealings that are involved in the next event of the cascade are of great importance and the events are angiogenesis and granulation. In this phase, the granulation tissue is supported by theblood supply. Vascular support is increased in the affected tissue and the site surrounding it as well. This process is a reciprocating way for the human body to the excessive blood loss which occurred during the wounds. Vascular supply also allows the continuous supply of oxygen to the wounded area. Granulated tissue is generally soft pink to red in appearance which marks the presence of continuous vascular supply to the wounds [36]. Around the wounded area, the presence of fibroblasts markedly starts increasing through the process of migration [40].

The proliferation of the fibroblasts is also a distinctive feature of this phase in wound healing as it interacts with the GFs and the ECM which initiates the process of fibroblast proliferation. Integrins also play a vital role in the mediation of the whole proliferation process as they are the respective receptors for the fibroblasts andcan send signals bidirectionally. They are also responsible for the biochemical signaling of the earlier-mentioned process. The extracellular sphere of influence of integrins binds with the ECM portion and the internal surface of the receptor is accountable for several signaling procedures [41]. Exposure of the integrins and other fibroblast receptors like discoidin with collagen is responsible for the processing of MMP 2 in the wounded tissue. MMP2 allows the migration of the fibroblast during angiogenesis and ECM remodeling. The interaction of integrins with collagen is responsible for the strength of the ECM and in turn, encourages the secretion of supplementary GFs.By using a murine model Rossiter et al. corroborated that the removal of keratinocyte-specific VEGF is responsible for altered angiogenesis and delayed wound healing [42].

The final support given by the granulation tissue to the epithelial cells is the re-epithelialization phase of wound healing. Keratinocytes are generally involved during this phase and they are the main mediators that can positively influence the healing phase. Likewise to the other cells in the earlier phases of wound healing keratinocytes also undergo the same process of migration, differentiation and proliferation. Migration of keratinocytes occurs toward the center of the wound to close the wounded site completely. To do that keratinocytes must not lose their grip on the adjacent tissue.Keratinocytes also have a distinct feature of changing their shape and size before migration to accelerate the process of wound closure[9] [43-46]. Here again, integrins are involved as keratinocytes move toward the newlymolded ECM. During this process, MMPs are continuously activated through macrophages and keratinocytes to degrade the matrix components to achieve healthy healing of the tissue [47][10]. After all the complexity of the events, the wounded tissue enters the last phase which is tissue remodeling. In this phase type 3 collagen is replaced by type 1 collagen to give more tensile strength to the tissues [36].

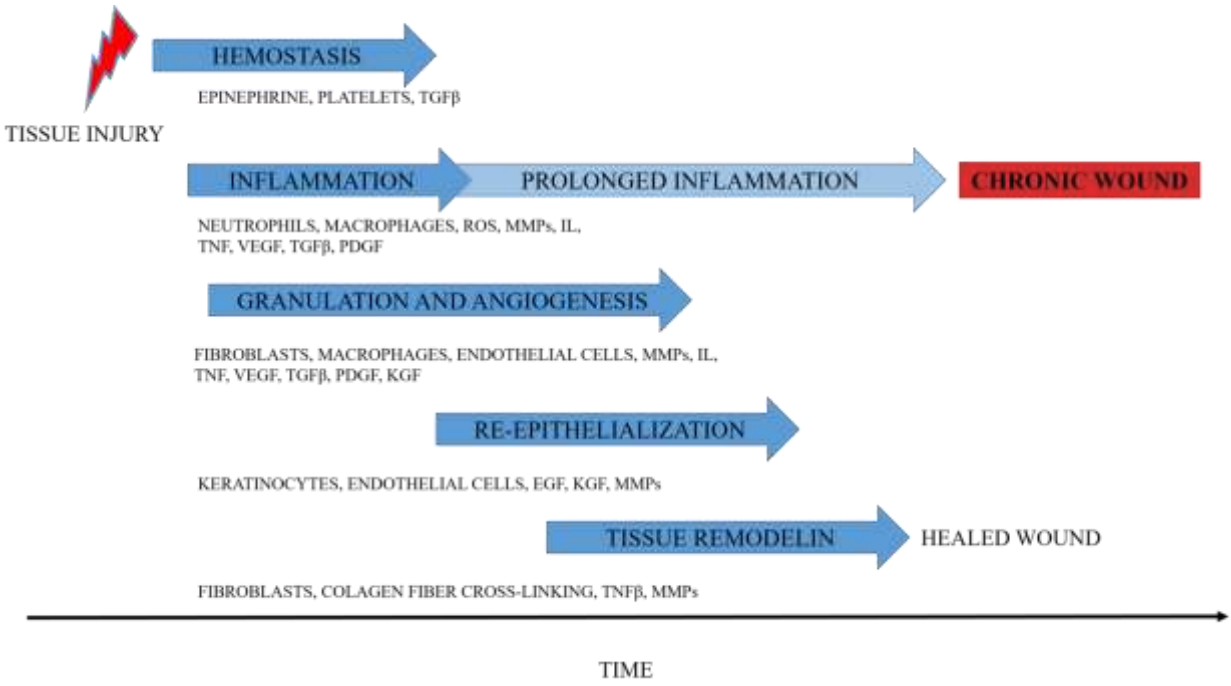

Figure1. Phases of wound healing. The phase of inflammation follows tissue damage. At this point, cells are drawn to the wound site by the release of cytokines, chemokines, and reactive oxygen species. In the proliferative phase that follows (neoangiogenesis, tissue development, re-epithelialization), fibroblasts, keratinocytes, and endothelial cells work together to produce new tissue. Tissue remodeling begins after these preliminary measures. The terms EGF, FGF, IL, KGF, keratinocyte growth factor, MMPs, ROS, transforming growth factor, platelet-derived growth factor, TGF, transforming growth factor, TNF, tumor necrosis factor, and VEGF stand for epidermal growth factor, fibroblast growth factor, and keratinocyte growth factor**.**

3. ROLE OF MATRIX METALLOPROTEINASES AND THEIR INHIBITORS IN WOUND HEALING

There are currently 28 known matrixmetalloproteinases(MMPs)out of which 24 are human enzymes. MMPs generally exist in three different categories that are inactive, active and complex MMPs. These enzymes are endopeptidases reliant on zinc at their active catalytic site whichis concealed through ECM. The primary structure of MMPs comprises a single arrangement of N-terminus, a pro-domain thatis capable of capping the active binding site and a catalytic sphere of influence [48,49].

The activity of MMPs is synchronized by a group of endogenous TIMPs. These inhibitors are further divided into4 subtypes (TIMPS 1, 2, 3, 4). The inhibitors bind specifically to block the functioning of MMPs [50][11]. MMP 7 and MMP 26, also classified as matrix-lysins exhibit a very basic structure consisting of the minimal domain asalreadydiscussed above. Thecatalytic domain is exemplified by the zinc-binding HExxHxxGxxH motif, consisting of three conserved histidines[51]. Several MMPs have a supplementary domain called the hemopexin-like domain, which is associated at the C-terminus of the above-mentioned basic arrangement Thehemopexin-like domain is supposed to impart a function in substrate identification. This association of domains for MMPs is noticed in MMP-3, -10 and -11 (also known as stromelysin-1, -2 and -3), MMP-1, -8, -13 and -18 (also known as collagenases),MMP-2, -9 (gelatinases), MMP-12 (metalloelastase), MMP-20 (enamelysin), MMP-7, -26 (matrilysins) and MMP-22 and -27[12,13] [52-54,59] (Figure 4).

4.ROLE OF MATRIX METALLOPROTEINASES IN WOUND HEALING

4.1.Role in inflammation: Inflammation is not only the body's defense mechanism but also protection against injury and damage to any tissues including skin tissue damages. Thus, the the defense cells of the body, i.e. leukocytes incursion-initiated inflammatory phase is the initial phase of wound healing.Several factors are directly or indirectly involved to manipulate this inflammatory stage of wound healing. Some of those factors released by the immunological cells include chemokines, cytokines, ECM splinters, and lipid mediators. Antimicrobial peptides are also among those influencing factors as well. Matrix metalloproteinases (MMPs) are a group of proteins that are held responsible for the secretion and pursuit of these factors from the epithelial and other cells. Studies from different labs revealed that MMPs that originated or developed from the epithelial cells normalize various events in the inflammatory stage which is also the initial stage of wound healing. The events which are to be regulated through MMPs in this phase are transepithelial migration of leukocytes and partition of signaling proteins such as chemokines. It has been validated through the work done by Parks et al. that MMPs are prominently expressed in inflammatory cells [55]. While on the other hand, stromal and epithelial

cells at the wounded site have also corroborated to express multiple MMPs counting MMP 1, 2, 3, 7, 9, 10 and 28. All these respective MMPs could mediate the activity of chemokines. This regulation could occur through distressing the production of chemokine slope or via direct proteolysis [56-59].

The chemokines are subcategorized into small families like CC chemokines and CXC chemokines. This subdivision occurs based on their discrete function of magnetizing the leukocytes resulting in their influx. Apart from this, their N-terminal cysteine residues are also a major factor that is taken into consideration while dividing these subfamilies [60,61]. The first family which is the CC chemokines plays a considerable role in the chemotaxis of monocytes. However, CXC chemokines are generally held responsible for the regulation of neutrophil chemotaxis. If the other dividing factor is also taken into consideration while comparing these two closely related chemokine subfamilies vary. The respective function or activity of these signaling proteins is downgraded by MMPs by their division or severance. CC Chemokines are responsible for down streamsignaling through the formation of receptor antagonists in the presence of MMPs. While on the other hand, CXC chemokines variably responded to the MMPs. It has been noted that some of the CXC chemokines are impervious to the presence of MMPs while some others are promptly affected by MMP's presence and quickly got under the processing.This dissimilarity elaborates on a major operative variation of humans with mice [62,63].

MMP 8 and 9 are accountable for the processing of Human CXCL8. This process is responsible for the significant amplification in the activity of CXCL8.Functional responses of MMP such as modulation in the migration of the inflammatory cells at the wounded site are different in humans and mice. This discrepancy is the core reason for the urge or needs to broaden research in this field. There is another CXC chemokine that is found to be handled or processed by several MMPs CXCL5 (LIX). MMPs that are thoroughly involved in the preparation of this chemokine are 1,2,8,9 and 13. This phase of chemokine processing is ultimately responsible for the enhancement of more inflammatory cells to be involved in the wound healing process. Furthermore,for the processing of CXCL12, several MMPs such as 1, 2, 3, 9, 13 and 14 are thoroughly involved in the process [62,64].

Results of CXCL 12 processing via MMPs are different from the previous CXCL5 processing which consequentially alleviates the efficiency of chemokines. Out of all the known MMPs,MMP1, 3 and 9 are validated to possess the extensive capability of supervising the signaling of chemokines. MMPs are generally involved in absolute chemokine breakdown and generating antagonists for chemokine receptors to enhance the performance of chemokines. Apart from MMPs, a disintegrin and metalloproteinase domain-containing protein 10 (ADAM10)areinvolved in sorting the chemokines. ADAM10 is competent in cleaving CXCL16 from the cell surface and by this means permits it to connect to its specific receptor and control T-cell commencement around the wounded site [65]. This shows that both the metalloproteinases which are MMPs and ADAMs, are thoroughly engrossed in the mediation of the chemokine. Both positive and negative regulations are carried out by these metalloproteinases and homeostasis is maintained during wound healing [66][14].

4.2. Role of epithelial repair in wound healing

Re-epithelialization is one of the most important and necessary phases of the sequential process of wound healing. The progression of building healthy tissue all over again at the denuded area is generally categorized as re-epithelialization [24]. In this tricky process, the wounded cells have to lose their grip on the ECM fragments so that they can freely move across the ECM to heal completely. Involvement of numerous MMPs can be seen in this phase of wound healing such as 1, 3, 7, 9, 10, 14 and 28 [67-70]. MMP1 which is also known as collagenase 1 is expressed at the wounded site during this particular phase of wound healing but its activity is valid till the closure of the wound after that it automatically got turns off [71,72].

Keratinocytes usually got transferred from the basal lamina at the wounded stage and then they bump into a matrix present in the dermal layer of the skin which is highly rich in type 1 collagen. MMP expression was initiated via ligation of keratinocytes to this particular ECM component with the help of α2β1 integrins. Findings from the labs of Gill et al. suggested that keratinocyte migration was assisted by MMP1 above the dermal layer matrix. This procedure was carried out by alleviating the empathy of collagen and integrin fragments [73-75]. Reduced contact between the two results in the migration of keratinocytes. MMP 10 (stromelysin-2) is confined with MMP1 howeverMMP 3 (stromelysin-10) is restricted only to the cells following the migrating overlook. Thenon-intersecting localization of MMP3 and 10, which are relatively analogous proteinases, intimates that these twoMMPs serve distinct functions in re-epithelialization. Apart from all these positive roles, MMPs are engaged in airway differentiation of the epithelial cells as well [76,77].

Confirmation of the involvement of MMP7 and MMP 9 in reepithelization originated from the human tracheal xenograft experiments in nude mice models. Their expression and activity were found to be maximum during the later phases of the wound healing progression. Specific inhibition of these respective MMPs contributes to the impairment in the differentiation of the epithelial cells. These shreds of evidence collectively prove that MMPs are necessary for wound healing for the proper differentiation of epithelial cells [78]. MMP 10 are also produced by the epithelial layer during the cell migration process revealing that these MMPs are mandatory during the cell migration. Several studies from the labs of Gill et al. corroborated that MMP 10 enhances the process of cell migration. This phase which is the repairing phase of wound healing includes various cellular responses

such as migration, proliferation, differentiation, and even cell death [79,80].

Now, it is quite evident that these events or responses either separately or collectively can generate MMPs over the years due to which the line of action of these proteinases has been deciphered now. All this evidence corroborates that practically all the MMPs have been confirmed as positive influencers of the wound healing process apart from MMP 2 and MMP 9 which may have negative or inhibitory effects on cell proliferation [81].

4.3.Role of matrix metalloproteinases in wound contraction or resolution:

Wound contraction and resolution in the last phase of wound healing. Several experiments confirmed that MMP 3 is involved in the resolution phase of wound healing. *In vivo* studies using animal models showed that mice lacking MMP 3 slowed down the process of wound healing [82,83]. Slowing down in the pace of contraction of the wound ultimately increases the size of the wound which may slow down the whole process of wound healing. In this situation, the epithelial cell might also take longer to migrate during the migration phase which is also a negative sign in the healing process. There is one other role of MMPs which was originally thought of as its main function is the elimination of the ECM fragments. ECM degradation is a vital step in the process of healthy wound healing. As of now it has been validated and examined that the primary function ofmostofthe MMPs is to activate several cellular factors such as growth factors, cytokines and chemokines. Reframing of the collagen generally consists of the mortification of the present collagen fibers to build new ones. This is the most important event in the resolution phase of wound healing.Because MMPs are capable of this process, it seems plausible that they would have a key role in collagen remodelingduring wound resolution.

5. Role of Matrix metalloproteinases inhibitors in different stages of wound healing:

As the activity of metalloproteinase in wound healing is regulated by endogenous tissue inhibitors of metalloproteinases. These inhibitors can bind at specific sites resulting in inflammation inhibition. Of note, while inflammation is a protective mechanism against invading pathogens and a requisite factor needed for wound healing, an exaggerated inflammatory reaction may be deleterious for the wound. The factors that are involved in the process of inflammation involve multiple cytokines and chemokines. One of the dominating cytokines that are involved inacute inflammation is TNF-α, activated by ADAM 17 or TACE (TNFα converting enzyme). In a similar behaviorto monocytes, TNF-α stimulates the expression of MMP9 through the activation of proinflammatory cytokine NF-κB and proteins like β38 [84]. MAP kinase pathways on inflammatory cells further regulate or alter many aspects of wound healing. Another inhibitor of MMP in wound healing is TIMP3 and in its absence, ADAM17 is enhanced which leads to a constitutively increase TNFα release [85,86]. This release further triggers inflammatory cell infiltration into the liver. Also increased levels of TNFαare responsible for the increased IL6 (interleukin 6) release. This behavior causes increased susceptibility to lipopolysaccharide (LPS), a bacterial outer membrane antigen-induced mortality [87].

TIMP 3 is recognized to work under the condition of inflammation. TIMP1 release is increased during lung infection/injury. In the absence of TIMP1, increased vascular permeability and neutrophil diapedesis into pulmonary tissue have been reported [85]. TIMP1 also is responsible for controlling leukocyte extravasation and vascular permeability by the mechanism of programmed death of endothelial cells(apoptosis) [87]. However, TIMP1 exerts an anti-apoptosis effect on cytokine-simulated endothelial cells using the phosphatidylinositol-3-kinase(PI3K) pathway. TIMP 3 as a metalloproteinases inhibitor is necessary for the regulation of the inflammation response through controlling the cytokine signaling and inflammatory cell adhesion. Hereby it may be concluded that MMP inhibitors play a very crucial role in all aspects of wound healing [88]. Moreover, TIMP3 and TIMP1 are also claimed to be capable of regulating cell migration by resisting the activity of specific MMPs. The exact role of TIMPs in the regulation of cell migration is yet to be discovered as some of the studies state that the TIMP2 accelerates the keratinocyte migration into the cell culture by in-vivo methods. In a recent study it has been stated that apart from the ability to inhibit MMPs, TIMPs also possess MMP-independent functions as well. As a piece of evidence, TIMP3 is a potent inhibitor of the angiogenesis process in wound healing. It also can directly bind to the vascular endothelial growth factor receptor and prevent the binding of VEGF to it directly [89].

The angiogenesis phase is of great importance during the cascade of healing wounds as it aids the formation of granulation tissue to enhance the vascular supply in and around the affected area. This crucial phase is regulated by the TIMP3 so it can be evaluated from this fact that this particular inhibitor of MMPS can dramatically impact the wound healing process. After all the granulation and angiogenic phase the final stage of wound healing is the closure of the wound and contraction of the wound is an important event towards the success of this closure. In the process of re-epithelialization, cutaneous wounds are treated with GM 6001 but it is also responsible for the decrease in the pace of wound contraction as well [90].

One more adverse effect of this treatment is the inability to differentiate between the granulation tissue and healthy tissue which leads to impairment in the wound restoration phase. On the other hand, the role of ECM in the wound resolution phase is an important aspect that can be carefully examined and monitored [91, 92]. Collagen is the key requirement for converting the granulation tissue into scar tissue and this process has been thoroughly regulated by the TIMP3. Fibronectin along with collagen has been inhibited and their release is further decreased in the absence of TIMP3 [93, 94].

Figure 2. Types of Open Wounds

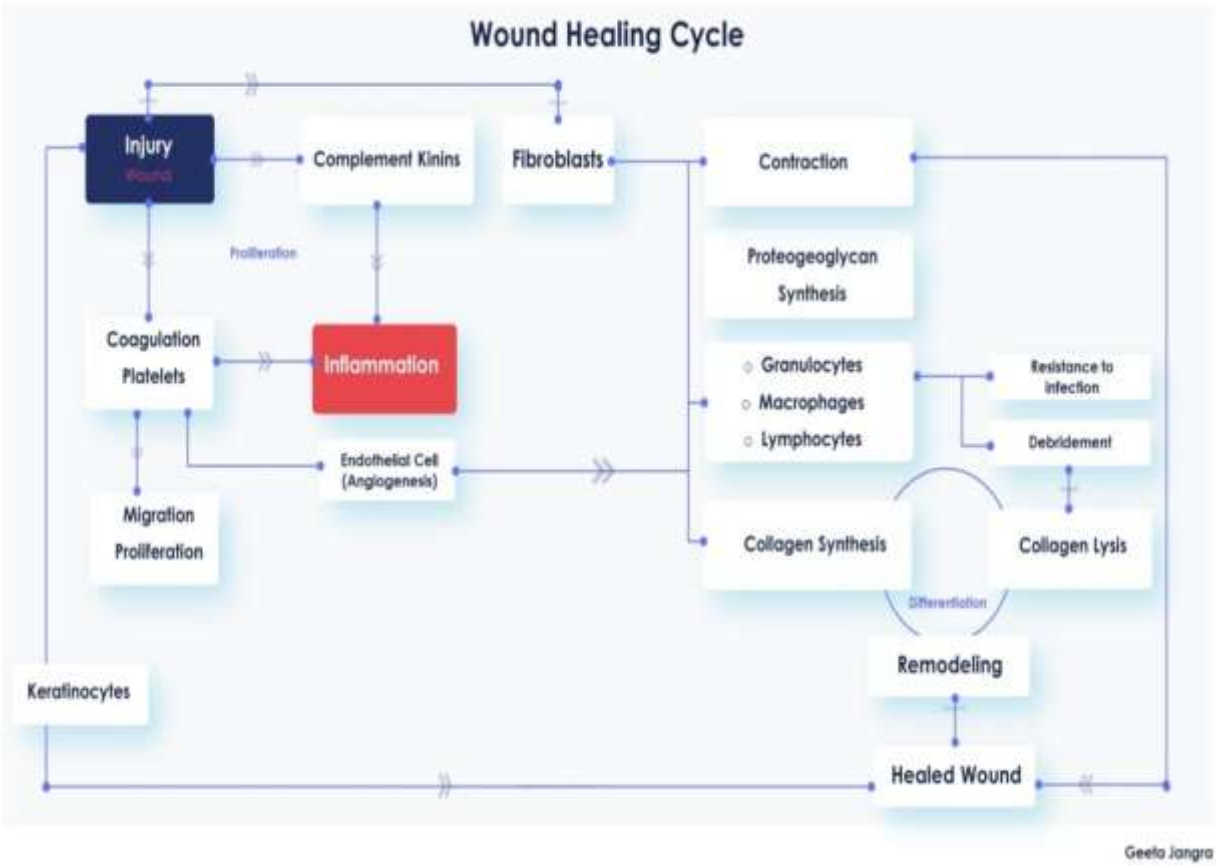

Figure 3. Wound Healing Cycle

Growing component that transforms (TGF) Beta is essential for the growth and control of the ECM (Extra cellular matrix). Humans have three TGF-ß isoforms (TGF-ß1-3), each with a unique role in controlling ECM components, cell proliferation, and even death [95].

The most well-known of these is TGF-1, which controls the production and destruction of many

substances involved in the healing of wounds. When TGF-1 binds to its corresponding receptor, it causes several cell types, including fibroblasts, to produce ECM components like collagen, fibronectin, and hyaluronic acid. Fibroblasts have specific receptors called integrins [96].

The MMP2 in the injured tissue is processed as a result of the integrins being exposed to collagen. During angiogenesis and ECM remodeling, MMP2 permits fibroblast migration, which in turn promotes the secretion of more growth factors. 3, 8, and 13 MMP-1 (also known as collagenases) [97].

TIMPs 1, 2, 3, and 4 are the four kinds that make up the Tissue Inhibitor of the Metalloproteinase (TIMPs) family. TIMPs bind MMPs in a stoichiometric 1:1 ratio, blocking the access of substrates to the endopeptidases' catalytic domain [98]. The proteins TIMP-1, TIMP-2, and TIMP-4 were produced. TIMP-3, on the other hand, is confined to the extracellular matrix since it is a membrane-bound TIMP [99].

Numerous damaging processes, including astumour cell invasion and angiogenesis, arthritis, atherosclerosis, etc., have been linked to abnormal MMP expression [100, 101]. MMPs are released from the body as inactive proenzymes that, after being cleaved by proteases at the cell membrane or in the extracellular environment, become active [102][15]. Tissue inhibitors of metalloproteinases (TIMPs) of MMPs prevent catalytic activity. Collagen and substrates with their accompanying MMPs role in wound healing and other disorders are around pH-7.4, where MMPs are most active [103, 104].

The involvement of collagen and its related MMPs in the healing of wounds and various disorders is given in the table no. 1 [105-117] :

Collagen/ Substrate/ MMPs	Involvement in potential wound healing	Part in other diseases
Collagen I,II,III,VII & X Collagenase I MMP-1	Increases the degree to which keratinocytes resting onfibrillar collagen migrate.Significantly higher amount in diabetics or those with diabetic foot ulcers.	Release the growth factors that have a negative effect on cancer patients.
Collagen I,III,IV & V Elastin, Fibrillin MMP-2 (Gelatinase A)	Increase the speed of wound healingassists in the relocation or immigration of keratinocyteshelps MMP-9 to become active.	Their level increase reveals tissues with severe colorectal cancer.
Collagen IV, V, IX, and X; fibronectin; Elastin; gelatinMMP-3 (Stromelysin 1)	stimulates MMP-9 activation, which has a favorable impact on wound recurrence and slowed healing.	Rheumatoid arthritis (RA) and ankylosingspondylitis' pathology (AS)
Collagen I, II, III MMP-8 (Collagenase-2)	Increases stimulus for subcutaneous healing when produced by neutrophils.	Serve as biological indicators for respiratory diseases such as chronic obstructive pulmonary disease and asthma.
Elastin, Fibrillin, Collagen I, III, IV, V MMP-9 (Gelatinase B)	Advances in cell migration, except for the cornea.	Controls inflammatory and fibrotic pathological re-modeling mechanisms in cardiovascular disease.
Collagen IV, V, IX and X; fibronectin; Elastin MMP-10 (Stromelysin 2)	Expressed at Keratinocytes at the exposed side of the wound.	Involved in skeletal development
CollagenIV,gelatin,fibronectin MMP-12 (Metalloelastase)	Promising monitoring of angiogenesis due to its capability of producing angiostatin	It causes the elastin in the extracellular matrix to break down, allowing immune cells that cause inflammation and granuloma development to infiltrate.
Collagen I, II,III, IV, IX, X MMP-13 (Collagenase-3)	Initiates Re-epithelialization through contracting the wound.	Involvedin the pathogenesis of osteoarthritis.

CONCLUSION

Several lines of evidence indicate that ECM proteins MMPs are involved in the healing progression of wounds or have a positive effect on the recovery from the wounds. Further, it has been deciphered that the existence of MMPs basically in three distinct forms which are pro-MMPs, active MMPs and TIMP (complexed) MMPs. However, at present, there are no such methods or mechanisms which can evenly classify these three forms of MMPs which confirms that the activity of MMPs in wound healing is still challenging. In this aspect quantitative profiling of a few active MMPs is very crucial in examining every possible role of MMPs during wound healing progression specifically during the ECM reframing phase. In medical research, the involvement of various enzymes including MMPs in wound healing can be experimentally intervened. A detailed study of all the enzymes including MMPs, other proteins, and receptor molecules involved in wound healing is highly essential before introducing any kind of novel drug or molecule as a therapeutic intervention. Following *in vitro* cell culture experiments future experimental work that may include *in vivo* animal models to study MMPs, ADAMs, or TIMPs must be done to enhance our knowledge regarding the mechanism of action of these molecules including MMPs and their respective inhibitors. Comprehensive knowledge of all the parameters essential for wound healing is necessary for successful therapeutic intervention against it.

FUTURE PERSPECTIVES

Recent research indicates activation of GFs and detachment of plasma membrane as some of the important cellular events involved in the various MMP-dependent wound healing process. These are the predominant functions of MMPs as substrates of MMPs are nonmatrix molecules. Currently, many novel techniques are being used to study the substrates of MMPs in detail. This could be the ground of future research of meta analyzing which would be able to differentiate downstream effects and substrate profiles precise to each protease. For studying or analyzing the enhanced role of MMPs in different physiologies and pathologies efficient analysis of protease substrate is very important. Almost all the variants of the MMP family are actively involved in several biological events in the body. However, few of them are restricted or specified to certain cells only like MMP 20 (specified for dental tissue). Their constitutive physiological expression is normally low, with transiently higher rates due to homeostatic matrix remodeling or specific developmental events. These short expression peaks underline their stringent regulation under physiological conditions and highlight their important role in tissue homeostasis and development. Uneven regulation of the functions of MMP specifically in the pre-chemo phase of cancer is a matter of conflict for MMP as a promising therapeutic target. The 1st clinical trial which used broad-spectrum MMP inhibitors failed. Thus, the main ground on which future research will rely is the blueprint or draft of the desired therapeutic agents in the inhibition of selective MMP variants instead of broad-spectrum MMP inhibitors as used earlier. Triumphant management of wounds is still a very challenging issue that has to be supervised with more efficacy and persistence to prevent the entry of acute wounds into the chronic phase. Thorough experimentation and studies in this relative field are very important to address this more efficiently. Careful examination of MMPs, their regulation and specific roles concerning ECM and its components may provide a new base for further research in this respective field of wound healing. Several types of research are still underway in framing innovative methods involving MMPs to treat wounds. Finally, this promising field is capable of recognizing novel inhibitors to effectively deal with wound healing problems.

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Conflict of interest

The authors declare no conflict of interest.

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