Practical context of enzymatic treatment for wound healing: A secreted protease approach (Review)

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Received October 1, 2019; Accepted February 14, 2020

DOI: 10.3892/br.2020.1300

Abstract. Skin wounds have been extensively studied as their healing represents a critical step towards achieving homeostasis following a traumatic event. Dependent on the severity of the damage, wounds are categorized as either acute or chronic. To date, chronic wounds have the highest economic impact as long term increases wound care costs. Chronic wounds affect 6.5 million patients in the United States with an annual estimated expense of $25 billion for the health care system. Among wound treatment categories, active wound care represents the fastest-growing category due to its specific actions and lower costs. Within this category, proteases from various sources have been used as successful agents in debridement wound care. The wound healing process is predominantly mediated by matrix metalloproteinases (MMPs) that, when dysregulated, result in defective wound healing. Therapeutic activity has been described for animal secretions including fish epithelial mucus, maggot secretory products and snake venom, which contain secreted proteases (SPs). No further alternatives for use, sources or types of proteases used for wound healing have been found in the literature to date. Through the present review, the context of enzymatic wound care alternatives will be discussed. In addition, substrate homology of SPs and human MMPs will be compared and contrasted. The purpose of these discussions is to identify and propose the stages of wound healing in which SPs may be used as therapeutic agents to improve the wound healing process.

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Abbreviations: ECM, extracellular matrix; FMC, fish mucus cathepsin; FMM, fish mucus meprins; FMMPs, fish matrix metalloproteinases; FMSP, fish mucus serine proteases; MaPs, maggot proteases; MMPs, matrix metalloproteases; SPs, secreted proteases; SVMPs, snake venom metalloproteases; SVSPs, snake venom serine proteinases; TGF-β1, transforming growth factor-β1; VEGF, vascular endothelial growth factor

Key words: enzymatic wound treatment, fish epithelial mucus, maggot secretory products, matrix metalloproteases, snake venom proteases

1. Introduction

A wound of the skin is generally described as the interruption of the epithelial surface caused by a physical or thermal challenge (1). Skin wounds have been extensively studied as their healing represents a critical step in achieving homeostasis following a traumatic event. Dependent on the severity of the damage, wounds are categorized into either acute or chronic (2). To date, chronic wounds have the highest economic impact as long term treatment increases wound care costs (3). It is estimated that 1-2% of the population of the developing world will experience a chronic wound in their lifetime (4). According to Brem *et al* (5), in 2007 chronic wounds had affected 6.5 million patients in the United States, with an annual estimated health care expense of $25 billion (6). However, to date, the actual cost of chronic wound care in the United States is unknown (7). There has been a relatively high increase in the incidence of chronic wounds, and this may be closely associated with the increase in factors which impair wound healing, such as diabetes, obesity, or therapeutics such as chemotherapy, steroids and non-steroidal anti-inflammatory drugs (6).

The cost of chronic wound care represents a complicated scenario for patients and health care systems, leading to a necessity for the development of healing solutions which are both quicker and more cost-effective. To date, the available wound treatment therapeutics are: dressings, such as antimicrobial, films and alginate; hydrocolloids, collagen products, gauze composites and hydrogels; and active wound care (8). Active wound care represents the fastest growth category (20.6% compound annual growth rate between 2016-2022) as it is an alternative that has a more specific action and is more cost-effective (9). Within the active wound care category, proteases from a range of sources have been employed as successful agents in debridement (10), enhancing wound healing (11), coagulation (12) and keloid scar treatments (13). Of these, debridement comprises the principal dermatological application in enzymatic wound care, a proven and well-established principle (14).

The wound healing process is predominantly mediated by matrix metalloproteinases (MMPs) (15-17). Dysregulation of MMPs results in defective wound healing, which has made them targets of study in cases of chronic wounds, diabetic foot injury, keloid healing and burned skin (10). The topical application of non-human proteases has demonstrated beneficial therapeutic effects in events where MMPs fail due to dysregulation, for example in hemostasis (18), wound closure (19) and debridement (20).

Debridement is the most widely explored enzymatic wound care application, in which the most frequently used proteases are collagenases, serine proteases and cysteine proteases. The therapeutic activity of animal secretions from fish epithelial mucus (21), maggot (*Lucilia sericata*) secretory products (22) and snake venom (23) have also been demonstrated. These secretions contain different types of proteases capable of degrading the same substrates as MMPs. Besides these, no further use cases, sources or types of proteases for wound healing were found based on the currently available literature.

Through the present review, the context of enzymatic wound care alternatives will be discussed, along with a comparison of substrate homology of secreted proteases (SPs) and human MMPs. This review will aid in the identification of which stages of the wound healing process SPs may be used as therapeutic agents.

2. Chronic wound healing management: Practical context of traditional and enzymatically based debridement approaches

Debridement is the first step to enhance repair of chronic wounds. According to the European Wound Management Association, this procedure is considered a basic necessity to induce the physiological process of tissue repair (24). Through debridement, necrotic tissue is removed by external means to create a stable and healthy scaffold for re-epithelialization (25). In healthy individuals under normal circumstances, debridement is performed naturally following clot formation by neutrophil-derived MMPs and other components (26). However, when the MMP machinery fails, there is an accumulation of devitalized tissue. As a consequence, the steadiness of prolonged catabolism diminishes re-epithelialization and results in chronic wounding (27).

This failure represents an important baseline to treat chronic wounds, as devitalized epithelium builds up a physical barrier that precludes the healing process by interfering with the repair machinery, mimicking signs of infection, providing nutrients to anaerobic pathogenic agents, such as *Clostridium perfringens* or *Bacteroides* sp., and promoting cytokine production that in severe cases generates a septic response (28).

Debridement can be performed through autolytic, surgical, biological or enzymatic means (28). Of these, autolytic debridement is the most conservative treatment strategy. It enhances the action of endogenous phagocytic cells and proteases such as MMPs through dressings that provide the ideal catalytic conditions for removal of necrotic tissue (29). Among the dressings available for autolysis, films (polydimethylsiloxane), gauzes, hydrocolloids, hydrogels, alginates, hydrofibers and foams have been proposed (25,30). This strategy is selective, painless, inexpensive and suitable for most types of wounds (31). However, this process is slow, dependent on suitable reaction conditions and on the physiological response of the patient, and carries the risk of skin degradation due to prolonged exposure to moisture (maceration) (32) within the surrounding skin (28).

Surgical debridement strategies are performed by excising necrotic tissue until only healthy skin regions are exposed (33). Available variants of surgical debridement include ultrasound debridement, plasma-mediated bipolar radio-frequency ablation, versa-jet (fluid jet technology) and hydrosurgery (34,35). Surgical debridement is the fastest and most effective route of treatment, but is an expensive method that requires a sterile surgical environment, trained practitioners, and specific instruments, and is contraindicated for patients with clotting disorders (28,36).

By contrast, biological debridement promotes the removal of devitalized epithelium through the digestive action of *Lucilia sericata* sterile maggots (31). Maggots are caged in wound-sized hydrocolloid dressings that are placed in the affected area (37). The secretion of several components including proteolytic enzymes, such as trypsin and chymotrypsin serine proteases, then catalyze non-viable skin into a liquid feedstock that facilitates maggot feed (38). This alternative has proved to be efficient in several types of chronic wounds (39) and ulcers (40,41) by providing quick wound debridement, reduction in the use of biofilms, disinfection from bacteria (40,42-45) and improved pain control (46). However, due to the negative image several societies impose on maggots, this alternative has not been well accepted by patients and practitioners (47). Furthermore, it is contraindicated for the treatment of fistulae, exposed vessels and wounds in proximity to vital organs (42).

A potential compromise is enzymatic debridement, in which proteases from different sources (bacterial, vegetal or animal) is applied to the wounded area to remove necrotic tissue (48,49). Enzymatic debridement is selective and suitable for infected wounds (36), without the need for complex equipment or application procedures. This alternative also takes less time and requires fewer applications to accomplish debridement compared with dressings used for autolytic treatments (50). Other reported enzymatic wound healing approaches are anti- or pro-coagulation through venom toxins

from *Bothrops* sp. (51,52). These enzymes may frequently be inhibited by salts, temperature and hydrogen peroxide, which are common elements of aseptic solutions. A stinging sensation and exudate may also be observed as an after-effect of enzymatic treatment (36).

From these four mentioned alternatives, three are directly dependent on proteases to perform the debriding activity. The direct or indirect use of proteases is therefore the second most commonly used tool after surgical debridement. In the current literature, the most commonly used proteases in direct enzymatic debridement are bromelain, papain and bacterial collagenases (53). Other enzymes have been demonstrated to intervene as anti- or pro-coagulation agents and in non-specific wound healing from animal secretions. The most common commercially and non-commercially available proteases associated with wound healing are listed in Table I.

Animal secretions with high quantities of protease content, including fish epithelial mucus and snake venom, have been reported to enhance wound healing. Wound healing properties were reported for the secreted mucus of the fish species *Netuma barba* (54), *Channa striatus* (55) and *Clarias gariepinus* (56). A reduction in healing time of almost 60% was achieved following the topical application of mucus preparations in the wounds of mice, rats, guinea pigs and humans (57). For snake venom, anti- or pro-coagulation and epithelial cell migration properties were observed with the toxins from the venom of *Bothrops moojeni, B. atrox* (51), *B. alternatus* (18) and *B. jararaca* (58).

Thus far, the primary application of proteases in wound treatment has been debridement. Information regarding the use of proteases being used for other wound healing treatments is scarce, suggesting that relatively little attempt has been made to propose the use of proteases in different stages of the wound healing process (57,59). Several therapeutic benefits have been described from animal secretions, but studies on their possible use in wound healing stages are limited. It may be beneficial to determine whether the existing types of SPs present in animal secretions with reported therapeutic effects (maggots, fish and snakes), can mimic human MMPs.

3. MMPs in skin wound healing: Comparison and substrate homology with proteases secreted from other animals

Wound healing is the process by which an epithelial discontinuity is closed and is divided into four major steps: Hemostasis, inflammation, cell migration-proliferation and skin remodeling (60,61). The interaction and co-ordination of several elements such as cytokines, growth factors, coagulation elements, extracellular matrix (ECM) components, parenchymal cells and MMPs (62,63) enable the correct progression of these major steps (Fig. 1).

It has been reported that MMPs predominantly mediate the wound healing process and are involved in several events in each stage, including ECM degradation (64), cell proliferation/migration, mesenchymal cell differentiation (65), wound contraction, angiogenesis and re-epithelialization (66-68). At present, 25 different MMP variants have been identified in the human genome (64,69). Of these, 11 are responsible for skin remodeling and wound healing (Table II).

The presence of SPs has been reported in the secretions of fish (70), maggots (71,72) and snake venom (73). As MMPs are one of the primary participants of the wound healing process, a similarity may exist in the catalytic mechanisms of SPs and MMPs. This similarity may explain the therapeutic effect provided by these secretions.

Maggot therapy efficiency in the treatment of necrotic, infected chronic wounds is due to the activity of several SPs. This secretion consists of serine proteases (trypsin-like and chymotrypsin-like) and metalloproteases (71,72). As a secretion, maggot proteases (MaPs) contribute to the wound healing process, primarily in fibroblast stimulation and bacterial disinfection. MaPs degrade fibrin clots and fibronectin (74), enhancing fibroblast metabolism and migration (22,75). In addition, MaPs increase TGF-β (transforming growth factor-β) signaling in wounds treated with maggots (76), which enhances endothelial cell and keratinocyte migration, thus promoting wound closure. Furthermore, MaPs inhibit neutrophil migration and decrease the production of pro-inflammatory mediators in neutrophils and monocytes (44,77), leading to recruitment of pro-angiogenic growth factors (78) and healthy granulation tissue (79). MaPs are also considered antimicrobial enzymes (80), capable of eliminating *Staphylococcus aureus* and *Pseudomonas aeruginosa* (44,81) as well as degradation of biofilms produced by *S. epidermidis* and *S. aureus* (41).

From MaPs, only a chymotrypsin-like protease has been isolated from maggot secretions, which exhibited clotting and proteolytic activity in fibronectin, suggesting its use in hemostasis and for temporary collagen-rich replacement of ECM (74,82). These proteases also reduce biofilms in patients with leg ulcers (40,41).

Similar to maggot secretions, fish mucus and snake venom have been hypothesized as wound healing treatment agents. In traditional medicine, they have been used as a therapy for skin burns and hemostasis (51,55,56,83). Fish epithelial mucus consists primarily of glycoproteins and immune biomolecules (84). Immune components, metalloproteases, serine proteases, and cathepsins B, D and L, have been identified in fish epithelial mucus (85,86). Enzymatic components from crude secretions contribute to accelerated clot formation and agglutination of red cells (87).

In the case of metalloproteases, fish matrix metalloproteinases (FMMPs) 9 and 13 and fish mucus meprins (FMM) have been described as components of fish mucosal secretions (88,89). FMMPs 9 and 13 have analogous variants in human tissue, which participate in wound contraction and re-epithelialization (66,90). FMMs can degrade collagen IV, fibrillar procollagen and fibronectin (91-93), which are also degraded by MMPs 3, 10, 11 and 12 (Table II). These proteases are involved in wound contraction, monocyte/macrophage metabolism and re-epithelialization (66).

Cathepsins are a family of proteases that have been identified in fish epithelial mucus, and these cathepsins in fish mucus have not been characterized. It is hypothesized that the cathepsins in fish mucus may exhibit a therapeutic effect on wound healing based on the available data regarding their properties on human skin. These proteases are normally present in lysosomal vesicles, but their presence has also been demonstrated extracellularly (94). In human physiology, they participate in wound healing during hemostasis (95), ECM

Table I. Applications of proteases in wound healing treatments classified by their reported therapeutic effect.

A, Debridement and skin burns

Author, year	Enzyme	Source	(Refs.)
Ford *et al*, 2006	Papain + urea (Accuzyme SE)	*Carica papaya*	(152)
Ford *et al*, 2006	Papain, Urea, Chlorophyllin Copper Complex Sodium (Panafil SE)		(152)
Muhammad *et al*, 2014; Yaakobi *et al*, 2007	Papain/Chymopapain		(20,153)
Klasen, 2000	Collagenase	*Clostridium* sp.	(14)
Smith & Nephew, Inc., 2014	Collagenase (Santyl®)	*C. histolyticum*	(154)
Giudice *et al*, 2017	Bromelain (NexoBrid)	*Ananas comosus*	(155)
Gorecki and Toren, 2005	Bromelain cysteine protease		(156)
Klein and Houck, 1980	Bromelain cysteine protease		(157)
Niehaus *et al*, 2012	Debrilase	*Lucilia sericata*	(158)
Niehaus *et al*, 2012	Serine protease		(159)
Rosenberg, 2012	Bromelain, trypsin enzyme H-4, collagenase, papain/papain-urea	Several	(160)
Freeman *et al*, 2012	Collagenase, elastase, papain, bromelain, hydrolase, streptokinase		(161)

B, Anticoagulation and procoagulation

Author, year	Enzyme	Source	(Refs.)
Waheed *et al*, 2017	Moojenin (Defibrase®)	*Bothrops moojeni*	(51)
Waheed *et al*, 2017	Batroxobin (Reptilase)	*B. atrox*	(51)
Chan *et al*, 2016	Thromboplastin-like and thrombin-like (Hemocoagulase)		(52)
De Marco Almeida *et al*, 2015	Venom	*B. alternatus*	(18)
Yaakobi *et al*, 2004	Collagenase	Non specified	(162)
Rodeheaver *et al*, 1974	Trypsin/ADAMS SVMP	Bovine	(163)
Glyantsev *et al*, 1996	Collagenase	Crab (specie non specified)	(27)
Ferreira *et al*, 2017	Buffalo cryoprecipitate and Serine protease	*Crotalus durissus terrificus*	(59)

C, Enhancing wound healing

Author, year	Enzyme	Source	(Refs.)
Fierro-Arias *et al*, 2017	Collagenase	*C. histolyticum*	(13)
Gao *et al*, 2015	rMMP8 and MMP9 inhibitor	Non specified	(164)
Pasha *et al*, 2015	Cream/composite	*Channa striatus*	(143)
Rilley and Herman, 2005	Collagenase	*Clostridium* sp.	(19)
Ferreira *et al*, 2018	Jararhagin	*B. jararaca*	(58)
Mukherjee *et al*, 2017	Mucus	*Echinoida* sp.	(83)
Costa-Neto, 2004	Globe eye	*Netuma barba*	(54)
Manan Mat Jais, 2007	Mucus	*C. striatus*	(55)

MMP, matrix metalloproteinase; SVMP snake venom metalloprotease; rMMP, recombinant MMP.

remodeling (96) and keratinocyte migration (97). Cathepsin-L substrate affinity has been described for laminins, fibronectin, elastin and collagen (98,99). Cathepsin-D has affinity for fibronectin, proteoglycans, and collagens I and II (100), while substrate affinity of Cathepsin-B has been described primarily for collagen II, IX and XI (101). These substrates are also

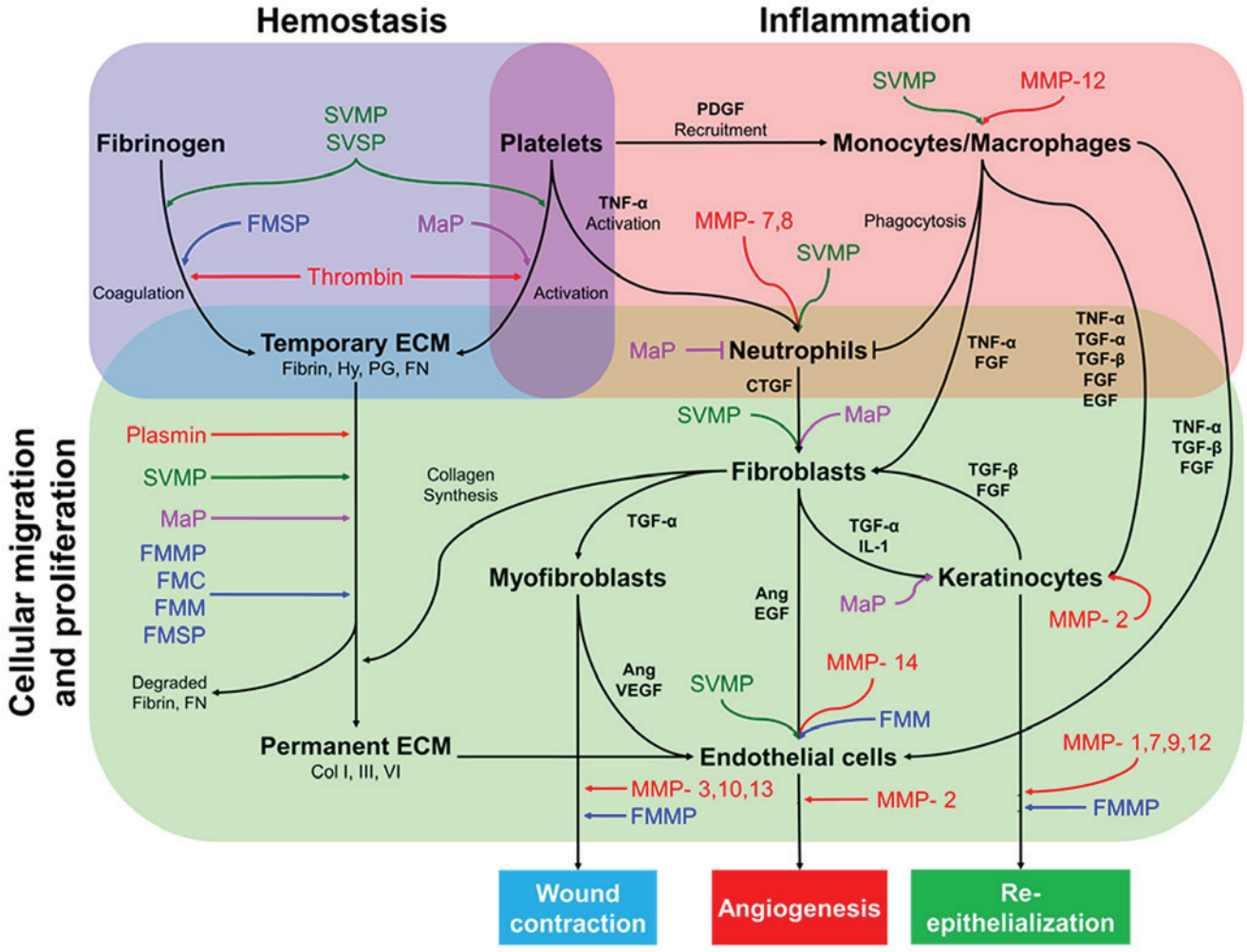

Figure 1. Simplified diagram of the interactions between different cell types during wound healing, the contribution of MMPs and proposed wound healing mechanisms of SPs. Skin injury repair begins with hemostasis, a process which stops blood loss and provides a temporary matrix facilitating further steps in wound healing. Fibrin-rich ECM formation stimulates neutrophil-activated monocyte recruitment through TNF-α and PDGF. Both neutrophils and monocytes produce several growth factors, such as TNF-α, TGF-α, TGF-β, EGF and FGF, to enhance migration and proliferation of fibroblasts, endothelial cells, and keratinocytes to the site of injury. Fibroblasts stimulate other cells to produce collagen deposits in the ECM, wound contraction, angiogenesis and re-epithelization. Studies suggest that SPs, such as FMC, FMMP, FMM, FMSP, MaP, SVMP and SVSP, may behave similarly to endogenous MMPs during these stages. Ang, angiopoietin; CTGF, connective tissue growth factor; Col, collagen; ECM, extracellular matrix; EGF, epidermal growth factor; FGF, fibroblast growth factor; FMC, fish mucus cathepsin; FMMP, fish mucus matrix metalloprotease; FMM, fish mucus meprin; FMSP, fish mucus serine protease; FN, fibronectin; Hy, hyaluronan; IL-1, interleukin-1; MaP, maggot protease; MMP, matrix metalloproteinase; PDGF, platelet-derived growth factor; PG, proteoglycan; SVMP, snake venom metalloprotease; SVSP, snake proteinase; TGF, transforming growth factor; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor.

target proteins for MMPs 1, 8, 13 and 14 (66,102), which supports the reported role of cathepsins in wound contraction and hemostasis.

Additionally, fish mucus serine proteases (FMSPs) are present in mucosal secretions (103), albeit with only poor substrate characterization thus far. Nevertheless, this family of proteases has reported activity on collagen, elastin, fibrin and fibrinogen (104,105). Thus, this protease may be useful during hemostasis, generating platelet aggregation and fibrin clot formation (106). Additionally, FMSPs degrade fibrin, which may assist in the change of ECM from temporary to collagen-rich, resulting in cellular proliferation and migration (107). This family of enzymes also interferes with the maturation of MMPs (66) and the desquamation processes (108).

Snake venoms, particularly from the *Viperidae* family, are rich in proteases. There secretion is comprised of two types of proteases: Snake venom metalloproteases (SVMPs) and snake venom serine proteinases (SVSPs) (73). These enzymes catalyze a broad range of ECM components, coagulation factors and proteins involved in platelet aggregation (109,110).

SVMPs can intervene in hemostasis, as these hydrolyze glycoprotein Ib and factor X, which promote coagulation (110-112) and platelet aggregation (113,114), respectively. During inflammation, SVMPs enhance the infiltration of inflammatory cells (115,116) as well as increasing neutrophil and macrophage numbers (117-119), which increases soluble collagen levels and enhances angiogenesis through increasing vascular endothelial growth factor (VEGF) and TGF-β1 release (58). During cell migration and proliferation, it has been demonstrated that SVMPs degrade fibrin and fibronectin (112,120), resulting in the change from temporary to collagen-rich ECM. SVMPs also activate migration of skin fibroblasts (121) and endothelial cells (111,122-124). In

Table II. Classification and function of human MMPs involved in skin remodeling and wound healing.

Family	Type	Function	Source	Substrates	Dysregulation effects	(Refs.)
Collagenases	1	Promotes re-epithelialization when cleaving native col 1	Interstitial fibroblasts	Collagen I, II and III	In high levels generates chronic wounds	(66,165-171)
	8	Regulation of neutrophil chemotaxis and effectors of inflammatory process	Neutrophils		Increased levels fibroblast lack apoptosis	
	13	Maturation of granular tissue and wound closure	Stromal fibroblasts Human chondrocytes	Collagen I, II, III, V and XI	Leads to arthritis, fibrosis, atherosclerosis and cancer	
Gelatinases	2	Cleaves γ2 of laminin 332 promoting keratinocyte migration, angiogenesis regulation by p10 angiogenic cytokines	Fibroblasts, endo thelial cells alveolar epithelial cells	Collagen (IV, I), γ2 laminin 332, gelatin	Chronic wounds when MMP-2 is in high levels	(66-68,102, 170,172-175)
	9		Keratinocytes	Elastin, aggrecan, fibronectin and vitronectin	Wound closure impaired in MMP9 -/-	
Stromelysins	3	Regulates wound healing (wound contraction), activate pro-MMPs and releases bioactive cytokines (HB-EGF, FGF)	Dermal fibroblasts and basal keratinocytes	Collagen (II, III, IV, IX, X) proteoglycans, laminin and fibronectin	Increased expression has been reported in dystrophic epidermolysis bullosa	(66-68,102, 170,172-175)
	10	Enhance migrating cell front in keratinocytes	Colocalized with MMP1 in leading edge of the wound keratinocytes	Collagen III, IV and V	Disorganized cell migration, degradation of new matrix, aberrant cell to cell contact and increase in cell death of wound edge	
	11	Activation of pro-MMPs antiapoptotic	Peritumoral fibroblasts	α-I-antiprotease collagen VI	In increase expression promotes tumor development	
Matrylisins	7	Wound re-epithelialization and neutrophil migration enhancing through chemokine processing.	Stromal fibroblasts in mucosal epithelia	Pro-MMP-1, gelatin, collagens	Innate immunity defects decreased re-epithelialization in lung injury	(19,176-178)
Membrane bound	14	Regulates epithelial cell prolif eration by altering KFG receptor and activates pro MMP2	Cell membrane of keratinocytes of the migrating front	Collagen (I, II, III), gelatin, fibronectin, laminin	Defective collagen I production, loss of MMP2 and impaired wound healing	(66,102)

Table II. Continued.

Family	Type	Function	Source	Substrates	Dysregulation effects	(Refs.)
Other MMPs	12	Elastin degradation and microphage migration	Macrophages	Collagen (1, IV), elastin, fibronectin, laminin, vitronectin, proteoglycan	Increased angiogenesis because of decreased angiotensin	(173,177, 179-182)

MMP, metalloproteinase; HB-EGF, Heparin-binding EGF-like growth factor; FGF, fibroblast growth factor; KFG, KGF, Keratinocyte growth factor.

addition, SVSPs exhibit proteolytic activity on Factor V and fibrinogen, promoting fibrin clot formation (125-127). SVSPs also promote aggregation of platelets (128).

Following analysis of reported interventions of SPs in wound healing, it could be presumed that they can intervene as helpers in several intermediate steps of the wound healing processes including coagulation, ECM degradation for re-epithelialization, or wound contraction, among other steps. The hypothesized mechanisms of SPs during the process of wound healing are presented in Fig. 1. Study of these variants may assist in the development of novel specific alternatives for active chronic wound healing care.

4. Potential of SPs as novel alternatives for wound healing care

Substrate homology analysis among MMPs and SPs suggest that animal enzymes may act similarly to the ones physiologically present in human skin. As presented in Fig. 1, previously compared SPs may be used to facilitate several steps involved in the process of wound healing, or to compensate for the physiological variants when they do not function properly. To understand this from a clearer perspective, it is important to comprehend in which of the most common chronic wounds types SPs may serve as suitable co-adjuvants.

In the current literature, chronic wounds have been classified into pressure ulcers, venous ulcers or diabetic ulcers (129,130). Pressure ulcers are caused by pressure, shear force, friction or a combination of these (131). The prevention and cure of pressure ulcers is associated with daily movement of extremities and frequent body positioning during hospitalization (132). In this case, the use of proteases may serve as palliative care in bed preparation for wounded patients as opposed to assisting the metabolic processes of wound healing.

Chronic venous ulcers are associated with inflammation, mechanical damage and erratic structural remodeling of the vein. Pathological hemodynamics results in changes to microcirculation; this produces thrombosis, proinflammatory activity and impaired MMP-3 activity (133), leading to cell dysfunction and finally to ulceration (134). For ulceration and potential necrosis, maggot therapy has shown efficacy (40,41) by decreasing inflammation and neutrophil migration (77,135). It also degrades eschar, debrides the wound and serves as a bacterial disinfectant (40,42-45). Furthermore, fish mucus proteases have been shown to exhibit antibacterial activity (55,136), which may be useful for bacterial disinfection of ulcers.

Diabetic foot ulcers are wounds that manifest after a cascade of metabolic dysregulations initiated by long-term hyperglycemia (137). As a result of prolonged exposure to high blood sugar levels, there is a decrease in fibrinolytic activity, thus increasing blood viscosity and coagulation in this type of wound (138). In addition, hyperglycemia results in a reduction of growth factors and receptor levels (such as TGF-β1), accompanied by a prolonged inflammatory phase due to upregulation of MMP-9 (139,140), which interrupts the inflammatory and proliferative phases of wound healing (141).

As an alternative therapy for diabetic foot ulcers, maggot treatment has demonstrated improved efficacy and efficiency compared with conventional methods (142). Furthermore,

MaPs (74), FMMPs (91) and a certain type of SVMP (112,120) have been reported to exhibit fibrinolytic activity which may ameliorate the characteristic viscosity of diabetic ulcers. Additionally, it has been reported that TGF-β signaling is increased in the presence of MaPs (76) and SVMPs (58), and this may also assist wound healing in this type of ulcer. However, certain SVMPs can promote coagulation (110-112,120); thus, meticulous care must be taken to separate and study each component embedded within the secretion instead of applying it as a whole.

In another report, fish mucus application enhanced the healing of laparotomy wounds (143). Therefore, SPs may be used to reduce the time taken for wound healing or for the removal of necrotic tissue, depending on the wound pathophysiology.

Despite the positive effects of SPs in wound healing, further research must be performed to determine the specific mechanisms of action, regulation, site delivery and bioavailability of proposed proteases before they may be recommended as feasible pharmacological candidates for treatment of chronic wounds. The application of SPs may be limited however, as its use for treatment of burn wounds exhibits highly variable results in patients (14).

It is also important to determine how SPs may affect other wound healing mechanisms when used as an adjuvant with other healing methods such as skin transplants. In this procedure, lost skin is covered with healthy tissue or artificial composites (144,145) that provide the necessary elements (cells, growth factors, MMPs and scaffolds) for the healing process (146). The success of a skin transplant is primarily dependent on angiogenesis between the skin graft and the injury, which is predominantly mediated by MMP-2, 9 and 14 (147). Thus, SPs have been proposed as potential adjuvants to increase tissue compatibility during skin transplants.

Nevertheless, studies on SP-aided transplants is still ambiguous. For example, the use of botulinum toxin A during skin transplantation in murine models enhances the expression of VEGF and prolonged the survival of skin grafts (148). By contrast, Kucukkaya *et al* (149) demonstrated that the same toxin reduces wound-graft contraction. Thus, the effects of SPs on skin transplants requires additional studies to determine its benefits during skin transplantation.

5. Future perspectives

Studies and development of less expensive wound healing treatment alternatives must be encouraged. Treatment of all types of even the most common chronic wounds still incur a high cost, and the reported care expenses are $50,000 for a diabetic ulcer (25), $500-$70,000 dollars for a pressure ulcer (150) and $390-$50,967 dollars per venous ulcer (151). The proposal of proteases obtained from animal secretions is a promising area to explore, as these act on specific substrates involved in the wound healing process. Furthermore, it is important to determine the molecular events specific to each chronic wound case, as these may represent key tags on how the proposed SPs may intervene. Under these conditions, active wound care represents a viable solution if its use is based on specific requirements. Importantly, SP characterization is crucial to dispense with the use of secretions in wound repair, and instead use only the SPs. This may also allow heterologous production, immobilization or improvement of the therapeutic properties of the characterized SPs through mutagenesis. In addition, time-efficient diagnostic tests on for detection of molecular targets in skin wound healing may be developed to guide practitioners on which tool to use for chronic wound care, resulting in improved wound healing and thus restoration of homeostasis.

Acknowledgements

Not applicable.

Funding

The present study was funded by CONACYT (grant nos. 886264 and 548216).

Availability of data and materials

Not applicable.

Authors' contributions

All authors (MIAR, DMM, CLC, JMAY, JB and MLS) contributed to writing, editing and revising the manuscript. All authors approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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