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Review

The specific role of extracellular matrix metalloproteinases in the pathology and therapy of hard-to-heal wounds

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Matrix metalloproteinases (MMPs) are zinc-dependent endoproteases responsible for the metabolism of extracellular matrix (ECM). MMPs can degrade the various ECM components as a variety of non-ECM molecules. Hyperactivity of MMPs and improper regulation or inhibition could lead to certain disorders, like non-healing chronic wounds. In chronic wounds, unlike in acute ones, there are always higher levels of MMPs due to the accompanying inflammation. Different proteases are responsible for this condition; nonetheless, blocking MMPs can help restore the wound's healing ability. The level of MMPs can help indicate the prognosis of chronic wounds. In some cases, the healing process is delayed by microbial wound infections. Bacterial proteases may up-regulate the levels of MMPs produced by host cells. That means that both host MMPs as proteases secreted by the infecting bacteria need to be targeted to increase the healing capacity of the wound. MMPs activity modulating treatments by superabsorbent polymer dressings can improve healing rates of chronic wounds. The main goal of this review was presentation the specific role of metalloproteinases in the pathology and therapy of hard-to-heal wounds.

Keywords: matrix metalloproteinases, healing, chronic wound, inflammation, bacterial proteases, superabsorbent polymer dressings

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Abbreviations: AA, acetic acid; DFUs, diabetic foot ulcers; DUs, pressure ulcers; ECM, extramolecular matrix; GAG, glycosaminoglycan; HPV, Human Papilliomavirus; HSV, Herpes Simplex Virus; MDROs, multidrug resistant organisms; MMPs, matrix metalloproteinases; MRSA, methicillin-resistant S. aureus; NOSF, Nano Oligo Saccharide Factor; ODC, octenidine dihydrochloride; ORC, oxidized regenerated cellulose; PTX, pentoxifylline; TLC, lipido-colloid technology; VLUs, venous leg ulcers

INTRODUCTION

Matrix metalloproteinases (MMPs) belonging to the zinc-dependent endoproteases family are crucial molecules responsible for extracellular matrix (ECM) metabolism by the ability to degrade all kinds of extracellular matrix proteins. Besides degradation of the various ECM components like collagens, fibronectin, or elastin, MMPs can also process several non-ECM molecules, including cytokines, growth factors, their receptors, and ligands. In this way, MMPs are involved in the most physiological and pathological remodeling processes in tissues (Trojanek *et al.*, 2014; Trojanek *et al.*, 2020). The excessive production and hyperactivity of MMPs, and lack of proper regulation and inhibition could lead to certain disorders, like non-healing chronic wounds. Therefore, this article presents how the level of MMPs can be used to indicate the prognosis of chronic wounds and how protease-modulating treatments can improve healing rates of chronic wounds with examples of specific wound dressings.

In a physiological state, most MMP expressions remain at a very low level, sometimes even close to zero. Their induction occurs in response to tissue damage, enhancing the process of extracellular remodeling. During the extracellular matrix degradation, also many growth factors are released. These growth factors can then be bound to different matrix components, thereby regulating their availability and activity in the tissues. For the proper process of wound formation, a balance between the synthesis and degradation of the extracellular matrix components is necessary. In the pathological state, the components of the ECM are accumulated. Through uncontrolled MMP expression and activation, fibrosis is formed, which may significantly delay or even entirely disturb the healing process (Lazaro *et al.*, 2016). In chronic wounds, unlike in acute ones, there are always higher levels of MMPs (especially gelatinases) due to the accompanying inflammation. It was reported that MMP-9 and MMP-8 were synthesized by inflammatory cells and presented in excess could affect the healing process (Westby *et al.*, 2020). Not only metalloproteinases were responsible for this condition (Harding *et al.*, 2018; Westby *et al.*, 2018), but blocking MMPs can help restore the wound's ability to heal, especially in combination with a good standard of care (including compression therapy, cleaning and removal of hyperkeratosis). The healing process is additionally complicated by the possibility of infection and coexisting diseases such as diabetes, cirrhosis or liver failure, renal failure, active tumor, tumor treatment, anemia, malnutrition, obesity, smoking, a prolonged hospitalization period and the advanced age of the patient (Anderson & Hamm, 2012).

WOUND HEALING STAGES

Wound healing is a physiological process after an injury to restore tissue structure and function. This process may be divided into several phases: fibrin clot formation, inflammatory response, granulation and epithelial formation, angiogenesis and fibroblast proliferation, regeneration of a functional connective tissue matrix with remodeling, and scar tissue formation (Lazarius *et al.*, 1994). The fibrin clot stores cytokines and growth factors released from injured cells and platelets during the activated coagulation cascade, initiating an inflammatory response. Incoming neutrophils secrete pro-inflammatory cytokines, activating local keratinocytes and fibroblasts. After completion of the inflammatory state, the granu-

lating tissue starts forming. Proteases are active in all phases of wound healing (homeostasis, inflammation, proliferation, and remodeling) and, therefore, could play several roles in the normal wound healing process (Velnar *et al.*, 2009; Patel *et al.*, 2016). During wound healing, MMPs participate mainly through the degradation of ECM, enabling cellular migration, facilitating the fresh epidermis formation and angiogenesis, the new extracellular matrix formation, and remodeling of wound scar tissue (Le *et al.*, 2007).

CONCISE MMPs CHARACTERISTIC

Currently, there are twenty-three variable matrix metalloproteinases in humans like MMP-1 to MMP-28 but not included four: MMP -4, -5, -6, -22 (discovered simultaneously by different research teams). Typically, the MMPs family consists of several distinct domains conserved between MMP family members. These domains are predomain, propeptide, catalytic, and hemopexin domains. The propeptide domain consists of approximately 80 aa and contains a highly conserved sequence: PR**C**G-VPG constituting the so-called "cysteine switch". This regulatory element contains a conserved cysteine residue, which interacts with the zinc in the active site and prevents binding and cleavage of a substrate, keeping the enzyme in the inactive site. The catalytic domain consists of about 170 aa and contains two zinc ions (catalytic and structural) and 1-4 calcium ions Ca^{2+}. In the active center, the zinc ion is coordinated by a very conserved three-histidine sequence (for zinc chelation), creating a zinc-binding sequence HEXXHXXGXXH. A typical MMP contains a linker peptide or flexible hinge known as a hinge region of variable 75aa length and haemopexin-like-C-terminal domain (Hpx) with a sequence similar to hemopexin related with hem metabolism of approximately 200 aa. The hemopexin domain is a place of interaction between MMPs with their endogenous tissue inhibitors-TIMPs. Only two among all MMPs do not obtain this domain: MMP-7 and MMP-26 (belong to matrylisin), and MMP-12 lost it after activation. Three repeats of fibronectin type 2 in the middle of the catalytic domain enhance substrate binding by gelatinases and are the most important for effectively degrading type IV collagen, elastin and gelatin. Membrane-type MMPs (MT-MMP-1 – MT-MMP-6) contain furin cleavage sites in their propeptide domains (Fig. 1).

Based partly on the historical assessment of substrate specificity and partly on the cellular localization the MMPs family can be divided into seven groups: collagenases, gelatinases, stromelysins, matrilysins, metalloelastases, membrane-type MMPs, and other MMPs (Table 1).

MMPS IN WOUND HEALING

According to the Wound Healing Society, chronic wounds can be classified into four categories: pressure ulcers (PUs), diabetic foot ulcers (DFUs), venous leg ulcers (VLUs), and arterial insufficient ulcers (Simönes *et al.*, 2018). MMP activity is strictly controlled during the post-injury processes. One example of this process disruption stuck in the inflammatory phase and thus preventing the transition to the phase of granulation tissue formation is hard-to-heal chronic wounds. In this state, there is a significant increase in the activity of MMPs, which later self-perpetuates and may increase through the secretion of cytokines. An increase in the activity was found for MMP-2, -8, -9, and -14, while a decrease for TIMP-1 and-2, compared to the healing group. Poor healing was correlated with increased MMP-9 expression in chronic venous wound biopsy specimens and elevated levels of MMP-9/TIMP-1 in the wound exudates. However, significant differences were found between MMP-1 and other proteases. Because of heterogeneity in protease activity, MMP-1 may be associated with more healing and other proteases with less healing. A higher level of MMP-1 in healing wounds permits the proliferative phase to be completed and allows the progression of the healing process (Westby *et al.*, 2020). Limited evidence suggests correlations between elevated levels of MMPs and delayed healing in PUs (Ladwig *et al.*, 2002), in DFUs (Liu *et al.*, 2009), as well as in VLUs (Serra *et al.*, 2013). Possibly, the association of MMP level with delayed wound healing may be a general wound phenomenon; however, differences between wound types have also been observed (McCarthy & Percival, 2013; Lazaro *et al.*, 2016,). The treatment strategy for such ulcerative wounds has been directed towards decreasing the concentration level of metalloproteinases and regulating their activity. The use of proper inhibitors has become one of the goals of modern wound healing therapy. MMP inhibitors, depending on the type of influence, can be divided into two types – direct or indirect. The most essential direct inhibitors are endogenous tissue MMP inhibitors – TIMPs. Four types of these multifunctional, evolutionarily stable proteins are known: TIMP-1, TIMP-2, TIMP-3, and TIMP-4, with a mass of 22-29 kD. TIMPs differ in specificity and block active MMPs by creating stable and reversible coordination bonds in a stoichiometric ratio of 1:1 or 2:2. The inhibition mechanism involves blocking the access of the substrate to the catalytic site of MMPs (Brew & Nagase, 2010). In the fluid of chronic wounds, TIMP-2 was significantly lower. Also, instead of the concentration of MMPs or TIMPs individually, the MMPs/TIMPs ratio seems to predict better wound healing (Lading *et al.*, 2002). Other direct MMP inhibitors include a broad spectrum of inhibitors like batimastat, marimastat, and ilomastat. Whereas tetracyclines, heparin, glycosaminoglycan (GAG)-sulodexide, pentoxifylline (PTX), reactive oxygen species, and superabsorbent polymers belong to non-direct inhibitors, they can reduce MMP expression indirectly by affecting the inflammatory cascade with immunomodulating or proteolytic actions (Krejner *et al.*, 2016).

Recently, the composite of hybrid dressings has been developed. They combine the properties of different materials (further discussed). Most of these dressings contain gel-forming superabsorbent polymers (sodium polyacrylate or sodium carboxymethylcellulose), which absorb excessive amounts of wound exudates. However, they can decrease the concentration of locally active bacterial proteases and endogenous proteolytic enzymes, including MMPs (McCarthy & Percival, 2012).

A complicated wound is a unique entity and is defined as a combination of an infection and tissue defect. Some researchers believe that every wound is contaminated regardless of the cause, location, size, and management (Velnar *et al.*, 2009).

WOUND HEALING AND INFECTION

Under normal conditions, the human body can heal wounds on its own, so maintenance of the wound in clean and moist conditions and usually used disinfectants are sufficient. However, in some cases, the healing process is delayed, or complications could happen entirely

Figure 1. Diagram of the MMPs domain structure.

Table 1. Extracellular matrix metalloproteinases.

Class	Common name	MMP number	Collagen substrate	Various substrate
Collagenases	Interstitial collagenase	MMP-1	I, II, III, VII, VIII, X	Gelatin, MMP-2,-9, proteoglycans, fibronectin, laminin, pro-TNF
	Neutrophil collagenase	MMP-8	I, II, III, V, VII, VIII, X	Gelatin, fibronectin, proteoglycans ADAMTS-1, pro-MMP-8
	Interstitial collagenase	MMP-13	I, II, III, IV, V, VII, IX, X	Gelatin, laminin, proteoglycans, fibrinogen, proMMP-9, -13
	Collagenase 4 (*Xenopus*)	MMP-18	I	Gelatin
Gelatinases	Gelatinase A	MMP-2	I, II, III, IV, V, VII, X, XI	Gelatin, fibronectin, laminin, elastin, proMMP-9, -13, IGFBPs, IL-1b, TGF-b, a1-antiprotease
	Gelatinase B	MMP-9	I, IV, V, VII, X, XI	Gelatin, elastin, laminin, fibronectin, vitronectin, CXCL5, IL-1b, TGF-b, plasminogen
Stromelysins	Stromelysin 1	MMP-3	III, IV, V, VII, IX, X, XIV	Gelatin, fibronectin, laminin, pro MMP-1, -7, -8, -9, -13, proTNFa, E-cadherin, L-selectin, tenactin
	Stromelysin 2	MMP-10	I, III, IV, V, IX, X	Gelatin, laminin, casein, MMP-1, -8, fibronectin, proteoglycans
	Stromelysin 3	MMP-11	IV	Gelatin, fibronectin, laminin
	Stromelysin 4 or RASI-1	MMP-19	native type IV	Gelatin, laminin, entactin, fibronectin, aggrecan, fibrinogen
Matrilysins	Matrilysin 1or PUMP-1	MMP-7	I, IV	Gelatin, laminin, elastin, fibronectin, proteoglycans, proMMPs, proTNFa, E-cadherin
	Matrilysin 2 or Endometase	MMP-26	I, IV	Gelatin, laminin, elastin, fibronectin, proteoglycans, proMMPs, proTNFa, E-cadherin
Metalloelastase	Macrophage metalloelastase	MMP-12	IV	Elasin, fibronectin, gelatin, proteoglycans, plasminogen
Membrane-type MMPs	MT-MMP-1	MMP-14	I, II, III	Gelatin, fibronectin, laminin, vitronectin, proteoglycans,pro-MMP-2, -13
	MT-MMP-2	MMP-15		Fibronectin, tenascin, enactin, laminin, proMMP-2
	MT-MMP-3	MMP-16	III	Gelatin, fibronectin, vitronectin, MBP, proMMP-2
	MT-MMP-4	MMP-17		Gelatin, fibrinogen, proMMP-2
	MT-MMP-5	MMP-24		Gelatin, fibronectin, chondroitin/ dermatan proteoglycan, proMMP-2
	MT-MMP-6 or Leukolysin	MMP-25	IV	Gelatin, laminin, fibronectin, MBP, fibrin/ogen
Other MMPs	Enamelysin	MMP-20	XVIII	Amalogenin, ameloblastin, aggrecan, laminin, pro-MMP-20
	X-MMP *Xenopus*	MMP-21		Gelatin, aggrecan, casein
	C-MMP Chicken	MMP-22		No matrix substrate defined
	CA-MMP *Femalysin*	MMP-23		Gelatin, casein, fibronectin
Cysteine Array MMP	CA-MMP *Gallus*	MMP-27		Gelatin, casein, pro-MMP-27
	CA-MMP *Epilizin*	MMP-28		proTNFb, casein

disturbing healing. These cases include wound infections or chronic wounds. Microorganisms naturally occurring on the skin cause the most significant risk of wound infections. Among them, Gram-positive bacteria such as *Staphylococcus aureus* or *Streptococcus* spp. occur frequently and after penetrating the wound, significantly delaying or preventing healing. Also dangerous are Gram–negative organisms, like *Escherichia coli* and *Pseudomonas aeruginosa*, as well as anaerobic bacteria – *Bacteroides* spp. *and Clostridium* spp. Wounds can also be infected with fungi

(the most common is *Candida* spp.), viruses like Human Papillomavirus (HPV) or Herpes Simplex Virus (HSV), and parasites; in each case, the wound will heal pathologically (Ding *et al*., 2022; Diban *et al*., 2023).

General therapy of infected wounds based on culture with antibiogram is conducted with clinically available antibiotics, including aminoglycosides, β-lactams, cephalosporins, quinolones, and tetracyclines, which interfere with different bacteria structures or metabolic pathways, and for this reason are often included in wound dressings (Simönes *et al*., 2018; Kaiser *et al*., 2021). Unfortunately, wounds can become colonized (infection exists, but microbes do proliferate in high numbers) with multidrug-resistant pathogens termed MDROs (Multidrug-Resistant Organisms). They increase the risk of complications because antibiotics have very poor or no effect. The only method seems to be topical antiseptics, which, when used early enough, can stop spreading the infection with fewer side effects than local antibiotics. These are elementary silver, iodophors, octenidine dihydrochloride (ODC), acetic acid (AA), and plant-derived substances like Manuka honey, curcumin, essential oils and many other natural products (Chen *et al*., 2023).

Chronic wound infections occur due to microorganisms creating biofilm, which adheres to the skin around the wound. Biofilm is a polymicrobial population less susceptible to the human immune defense system, displaying a high level of antibiotic tolerance with intense inter-bacterial communication through quorum sensing (Preda & Săndulescu, 2019; Kaiser *et al*., 2021). Most chronic wounds are colonized with bacteria. Infections refer to the invasion of tissue by bacteria leading to a clinically evident pathogenic inflammatory response and tissue damage (Persival *et al*., 2012; Suleman, 2016). The infections may, in some cases, e.g., with a weakened immune system, spread to the surrounding tissues (muscles, bones, joints) and even to the blood, causing systemic infections. Sepsis during chronic wound infection is an infrequent complication, but the possibility of its occurrence should be considered.

Chronic wounds are susceptible to colonization by numerous bacterial species, like *S. aureus* (93.5%), *Enterococcus faecalis* (71.1%), *P. aeruginosa* (52.2%), coagulase-negative *Staphylococci* (45.7%), *Proteus* species (43.1%), and anaerobic bacteria (39.1%). These bacterial species create a biofilm on the wound surface and may secrete bacterial proteases, essential for bacterial growth and virulence (Oldak & Trafny, 2005). Extracellular bacterial proteases can evade the host's immune response and target the immune mediators (Suleman, 2016). Moreover, proteases in wounds could originate from the host or bacteria. It was supposed that host MMPs together with bacterial proteases probably play synergistically causing tissue breakdown on the wound bed. Bacterial proteases may up-regulate the levels of MMPs produced by host cells. Many pathogenic bacteria secrete a range of proteases, of the serine, cysteine, and metallo-type that act as virulence factors. That means both host MMPs and those derived from infecting bacteria need to be targeted to improve the healing capacity of the wound (McCarthy *et al*., 2012; McCarthy & Percival, 2013).

CHRONIC WOUND TREATMENT

The wound needs to be debrided and dressed correctly. Correct debridement means the removal of nonviable, infected, and hyperkeratotic tissue– it helps to convert a chronic wound into an acute one, which can then progress through the normal stages of healing (Lazaro *et al*, 2016).

In 2002, a group of wound treatment experts described a simple model of chronic wound care. This model with the acronym TIME, which is described as: **T**issue assessment and management, **I**nfection/Inflammation control and management, **M**oisture imbalance and management, **E**dges of wound observation and management (Leaper *et al*, 2012). However applying the above recommendation did not prevent some wounds from failing to heal, and additional efforts were required to resume the healing process.

SELECTED WOUND DRESSING

Wound dressings reduce excess inflammation, allowing chronic wounds to heal more readily. Non-healing wounds often become locked up in the inflammatory phase and cannot progress to produce granulating tissue. Persistent inflammation, in turn, enhances MMP activity. Therefore, one of the main goals of local treatment of wounds, regardless of their etiology, is decreasing the MMPs activity and sequestering host proteases within the wound environment (Krejner *et al.,* 2016; Westby *et al.,* 2020).

Wound dressings have progressed significantly for years, from traditional dry-type gauze or bandages to very sophisticated materials like hydrogels, foams or films. Their application's primary purpose was to maintain a sterile, hypoallergenic, moist, and thermally suitable environment to protect against bacterial infection and promote new tissue growth (angiogenesis) (Verdolino *et al*., 2021). Some types of dressing especially deactivate elevated MMP activity. The goal was to invent a cost-reasonable dressing, which modulates inflammation and promotes healing. It could be distinguished into several kinds of dressings, which work based on the inactivation of the protease action: skin substitute dressing, collagen-based dressing, cellulose-based dressing, and synthetic dressing based on lipid-colloid technology (TLC).

The first was the skin substitute dressing named Puracol ultraECM, a decellularised porcine mesothelium matrix. It demonstrated high angiogenic potential in vitro and MMP inhibition abilities. Simultaneously, it allowed for a high recovery of growth factor – FGF, VEGF, and TGF-β necessary for quick wound healing (Capella-Monsonis *et al*., 2020).

The second type was a collagen-based dressing, which compiled the essential requirements for a good dressing: biocompatibility, a lack of toxicity, and a biodegradable material. It could be used for treating chronic wounds, as it demonstrated a decrease in healing times by the ability to inactivate proteases and keep moisture in the wound bed (Holmes *et al*., 2013; Pallaske *et al*., 2018). BIOSTEP Collagen Matrix dressing possesses type I collagen and gelatin (denatured collagen). The addition of EDTA to this type of dressing allows binding and irreversibility inactivation of both collagenase (MMP-1), like gelatinases (MMP-2; MMP-9) (Finnegan & Percival, 2015).

The next type was a cellulose-based wound dressing representing the Promogran™ Matrix family, which contained oxidized regenerated cellulose (ORC) besides collagen. This type of dressing presented both antimicrobial and anti-inflammatory properties. The studies proved that the ORC/collagen dressing and silver-ORC (Promogran Prisma™ Matrix) represent antimicrobial properties inhibiting MMP-2 and MMP-9 expressions in wound fluid, and increasing healing rates. Importantly, tEDTA demonstrates antimicrobial and antibiofilm properties, but clinical trials using a synthetic inhibitor MMP (Promogram) showed only 13% effectiveness (Cullen *et al*., 2001; Verdolino *et al*., 2021).

Table 2. Summary of MMPs and their role in wound healing – an overview of findings.

Main points	References
In chronic wounds, there are always higher levels of MMPs due to accompanying inflammation.	Patel *et al*., 2016; Lazaro *et al*., 2016; Westby *et al*., 2018
Poor healing is correlated with increased MMP-9 expression and elevated levels of MMP-9/TIMP1 in wound secretion probes.	Ladwig *et al*., 2002; Serra *et al*., 2013 Westby *et al*., 2020; Chen *et al*., 2023
During infection, bacterial proteases may up-regulate the levels of MMPs produced by host cells.	McCarty *et al*., 2012; Suleman *et al*., 2016; Westby *et al*., 2020
The treatment strategy for chronic wounds is directed towards decreasing the concentration level of MMPs and regulating their activity.	McCarty & Percival, 2013; Krejner *et al*., 2016
The essential requirements for wound dressing were maintaining a sterile, hypoallergenic, moist, and thermally suitable environment promoting new tissue growth with antimicrobial and antibiofilm properties.	McCarty & Percival, 2013; Kaiser *et al*., 2021
The modest wound dressing type additionally inactivated the protease action and allowed for high growth factor recovery, which was necessary for quick healing.	Meaume *et al*., 2012; Meaume *et al*., 2017; Sigal *et al*., 2019; Verdolino *et al*., 2021

Another type was the synthetic dressing Urgostart from a polyester mesh saturated with a sucrose octasulfate potassium salt (Nano Oligo Saccharide Factor – NOSF) embedded lipido-colloid matrix (Technology Lipido-Colloid, TLC). Manufacturer's patents protected NOSF and TLC compositions. Oligosaccharides (NOSF) reduce MMP levels and restore growth factor biological functions, whereas the TLC matrix creates a moist wound environment. NOSF also restores angiogenesis by migration and proliferation of endothelial cells (growth factors protection against MMPs activity). Their mechanisms of action are still unknown. NOSF probably mechanically blocks, i.e. captures and immobilizes MMP molecules, that are massively secreted during prolonged healing (causing a state of chronic inflammation) and contributes to the reduction of the proteolysis (degradation, enzymatic breakdown) of the ECM components (i.e., collagen fibers) by surrounding epithelial cells (fibroblasts and keratinocytes) and macrophages (Lazaro-Martines *et al*., 2019). Two prospective, multicentric clinical studies named NEREIDES and CASSIOPEE were focused on patients with non-infected, moderate to strongly exudation leg ulcers of venous or mixed origin treated with a dressing and compression system for 12 weeks. Ensure the rapid transition from the debridement stage to the granulation stage, and finally to closure of the wound. Poly- absorbent fibers of the new type of dressing inhibit the proteases in excess accumulating, restore the impaired biological functions, and stimulate angiogenesis through migration and proliferation of endothelial cells (Sigal *et al*., 2019).

Both TLC-NOSF and ORC/collagen matrix dressings improved venous leg ulcer healing (1% of the adult population in the Western world suffers from leg ulcers). Four randomized controlled trials assessing these two MMP devices were ongoing (Schultz *et al*., 2008; Sigal *et al*., 2019).

Urgo-Clean Ag, which combines TLC technology with silver ions, exhibits a broad spectrum of antimicrobial activity, including *S aureus*, *P. aeruginosa*, vancomycin-resistant *Enterococci* and methicillin-resistant *S. aureus* (MRSA). Additionally, after 24 hours, according to Hieu et al. in 2021, UrgoClean Ag reduced the biofilm population by more than 99,99% (4,6 log reduction).

The application of TLC-NOSF technology to lipocolloid dressings (according to Explorer –NTC01717183 and Challenge clinical trials results) in the experts' opinion significantly shortens the healing time of chronically, non-infected wounds, which reduces the cost of treatment and improves the efficiency and quality of patients' life (*Meaume et al*., 2012; Meaume *et al*., 2017; Edmonds *et al*., 2018).

It should be mentioned that the specific clinical study refers to optical and magnetic stimulation (COMS) therapy on wound-healing-related parameters (tissue oxygenation and water index) that were analyzed by hyperspectral imaging on 11 patients with chronic leg and foot ulcers of different etiology NCT03112395 (according to ICHGCP) (Traber *et al.,* 2023).

SUMMARY

A brief summary of the above observations on the role of MMPs in wound healing is presented in Table 2.

CONCLUSIONS

Generally, MMPs play a crucial role in all stages of wound healing, but it was suggested that non-healing wounds may be associated with prolonged high activity of proteases in the later stages of the wound healing process. This persistent proteolytic activity is due to damage to newly formed tissue and the degradation of growth factors necessary for healing. The primary focus in the future will be finding the selective inhibitors of distinguished members; most clinical trials on small spectrum inhibitors until now had negative results due to the widespread inhibitory effect connected with similar structures of all MMPs. It should be assumed that detailed knowledge of protease substrates, especially non-matrix molecules, could help to understand MMP's involvement in physiology and pathology and find the appropriate therapy for hard-to-heal wounds.

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