MMPs made easy

Volume 1 | Issue 1 | November 2009 www.woundsinternational.com

Wounds International

Introduction

This article describes what MMPs are and the importance of their role in normal and disrupted wound healing. In particular, it discusses the relevance of MMPs to clinical practice, including current and potential interventions aimed at modulating their activity.

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Full author details can be found on page 5.

What are MMPs?

The matrix metalloproteinases (MMPs) are part of the larger family of metalloproteinase enzymes that play an important part in wound healing[1,2].

Enzymes are proteins that facilitate biological reactions, but are not themselves used up or changed in the reactions. They generally act on a limited number of molecules (known as the enzymes' substrates) and physically change them into other substances. Proteinases (also known as proteases) are enzymes that act on proteins, usually by cutting up the protein molecule.

Natural substrates for the different MMPs vary substantially, but include important extracellular matrix (ECM) proteins such as collagen, gelatin and proteoglycans. The MMPs degrade these proteins by cutting them into pieces. Different MMPs may act sequentially and on different parts of the same substrate.

Why are they called matrix metalloproteinases?

The name 'matrix metalloproteinase' (or 'matrix metalloprotease') indicates the key properties shared by the MMPs. They all:

- **preferentially breakdown proteins comprising the extracellular matrix of tissues**
- **require a metal ion (zinc) at the active centre of the enzyme.**

How are MMPs produced?

In normal wound healing, MMPs are produced by:

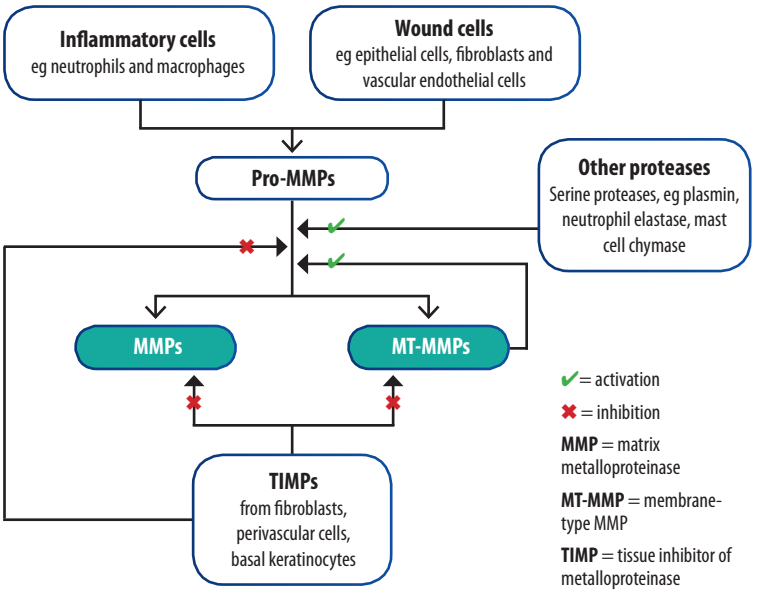
- **activated inflammatory cells (neutrophils and macrophages)**
- **wound cells (epithelial cells, fibroblasts and vascular endothelial cells).**

When first synthesised, MMPs are in a latent (inactive or pro-MMP) form. They are activated by other proteases that clip off a short section of the molecule. This opens up the active centre of the MMP molecule and allows the MMP to bind to its protein substrate(s). Other molecules called 'tissue inhibitors of metalloproteinases' (TIMPs) can inhibit activated MMPs and block the activation of pro-MMPs (Figure 1).

So far, 23 human MMPs have been identified. MMP-1, MMP-2, MMP-8 and MMP-9 have been the particular focus of research in relation to wounds.

While most MMPs are secreted into the surrounding ECM, some MMPs remain associated with cell membranes, and are known as 'membrane-type' MMPs (MT-MMPs). This group of MMPs is thought to play an important role in activating pro-MMPs, as well as activating pro-TNF (tumour necrosis factor – an important mediator involved in inflammation and cell death).

Figure 1 Overview of the production, activation and inhibition of MMPs



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MMPs in normal wound healing

MMPs play essential and beneficial roles in at least five major processes in normal wound healing (Box 1).

Box 1 MMPs in normal wound healing

Role of MMPs	Main phase of healing
▪ Removal of damaged ECM and bacteria	Inflammation
▪ Degradation of capillary basement membrane for angiogenesis ▪ Migration of epidermal cells	Proliferation
▪ Contraction of scar ECM ▪ Remodelling of scar ECM	Remodelling

Removal of damaged ECM

MMPs break down the damaged ECM that occurs at the edge of acute skin wounds. This enables new ECM components (eg collagen, fibronectin, and proteoglycans) synthesised by wound cells to integrate correctly with intact ECM components at the wound edges.

In addition, MMPs help to slough out biofilms. Biofilms consist of a gelatinous matrix produced by bacteria that shields the microbes from the immune system. The MMPs secreted by inflammatory cells surrounding biofilms digest (loosen) the attachments between the bacterial biofilms and the wound bed.

Angiogenesis

MMPs degrade the basement membrane that surrounds capillaries. This allows vascular endothelial cells to migrate from capillaries near the wound and to establish new blood vessels into the wound bed[3,4].

Migration of cells

MMPs (especially MMP-1) are required for migration of epithelial cells, fibroblasts, and vascular endothelial cells across or through the ECM. When epithelial cells at the edge of a wound begin to proliferate and migrate as a sheet across the wound bed, the epithelial cells just trailing behind the leading edge of the sheet secrete MMP-1. This partially digests the type 1 collagen and weakens the attachment of the cells' membranes to the matrix, allowing the cells to move across the collagen matrix[2,5,6].

Contraction

MMPs secreted by myofibroblasts are necessary for contraction of newly synthesised scar ECM. Large excision wounds in humans can contract up to about 20% of the initial wound area[7,8].

Scar remodelling

Repair of skin wounds initially produces a highly disorganised scar matrix. However, wound cells continue to produce low levels of MMPs long after the initial scar is formed. These MMPs slowly remove the disorganised ECM, which is gradually replaced by ECM with a more normal and more highly organised structure[1,9,10].

Why do MMPs sometimes cause problems?

Although MMPs have the important role of breaking down proteins so that new tissue forms, when MMPs are present in a wound bed at too high a level, for too long a time, and in the wrong places, they begin to degrade proteins that are not their normal substrates. This can result in 'off target' destruction of proteins, such as growth factors, receptors and ECM proteins, that are essential for healing, and so ultimately impair healing.

Substantial evidence has amassed that MMPs in general are highly elevated in wounds with delayed healing compared to acute healing wounds[11-22]. The potentially damaging effects of these high levels is compounded by the fact that TIMP levels in chronic wounds are generally slightly lower than in acute wounds.

How do we know about the effects of high levels of MMPs on healing?

Proteases came onto the wound healing radar when it was discovered that the ECM of wounds that were not healing did not contain intact fibronectin, a molecule necessary for cell adhesion and growth factor signalling[23]. Moreover, intact fibronectin reappeared in the wound bed as a wound began to turn the corner towards healing[23].

Further work demonstrated that the neutrophil-derived protease elastase was the biggest contributor to fibronectin degradation in non-healing wounds, and that fibronectin degradation products stimulate the release of MMPs[24,25].

Several groups have gone on to show that the amount of active MMP-9 is inversely correlated with wound closure rate, ie high levels of active MMP-9 correlate with lower wound closure rates[15,19,20].

It is important to note, however, that while most non-healing wounds in these studies had elevated MMP-9 activity levels, not all did, meaning that excessive MMP-9 activity is an important contributor to delayed healing, not necessarily the only cause.

How else are MMPs involved in delayed healing?

Protease inhibitors

Significantly, several MMPs are able to deactivate alpha-1 protease inhibitor (α1-PI) and alpha-2 macroglobulin (α_2M), which are two important natural inhibitors of the (non-MMP) serine proteases elastase and plasmin. High levels of MMPs in wounds can therefore indirectly lead to high levels of elastase (which degrades elastin, a major constituent of elastic tissue fibres) and plasmin (which digests fibrin, a protein found in blood clots).

Inflammatory cytokines and free radicals

Once activated at wound sites, inflammatory cells release cytokines (cell-to-cell signalling molecules) such as TNF, IL-1 and IL-6. When in excess, these cytokines stimulate production of abnormally high levels of proteases (including MMPs) and free radicals, which further fuel the inflammatory process[26,27].

Free radicals, such as hydrogen peroxide, kill bacteria and clean the wound. However, when free radicals are in excess, they can cause tissue damage. Free radicals have been implicated in the development and the persistence of venous leg ulcers and it has been shown that scavenging the free radicals using antioxidants improves healing in such wounds[28].

Growth factors

Many investigators have shown that growth factors (eg platelet derived growth factor (PDGF), epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF)) incubated in exudate from non-healing wounds are rapidly degraded and that such degradation can be prevented by the addition of a protease inhibitor to the exudate[29-31]. This suggests that in non-healing wounds the growth factor degradation may be due to excessive levels of proteases.

Bacterial proteases have also been shown to rapidly degrade growth factors[32]. The extent of growth factor degradation is dependent upon the type of bacteria, as each species produces different levels and types of proteases. This variance is associated with the virulence of the bacteria and may explain why certain bacteria even in small quantities can be extremely detrimental to the wound healing process.

Cell numbers

Degradation of growth factors removes the signal that stimulates proliferation of the cells required for tissue replacement (fibroblasts, endothelial cells and keratinocytes). Indeed, *in vitro* studies have shown that exudate from healing wounds stimulated cell division; in contrast, exudate from non-healing wounds inhibited cell division[33,34].

Bioburden

The normal host response to bacterial contamination of a wound is to elicit an inflammatory response that allows inflammatory cells to infiltrate and clean the wound in an effort to prevent infection. However, if pathogens are in excess they can cause problems, initially delaying healing but eventually leading to a wound infection[35].

Recent research suggests that a high percentage (about 60%) of wounds with delayed healing have bacterial biofilms and that bacteria in biofilms are very resistant to killing by host antibodies, inflammatory cells, antibiotics and disinfectants[36-38].

Consequently, it seems probable that in many cases, acute wounds become colonised by bacteria that transform in a matter of days into persistent biofilm bacteria and establish a long-term inflammatory source. The inflammatory cells activated in response to the biofilm secrete reactive oxygen species (free radicals) and proteases, including MMPs, in an attempt to destroy the bacteria. Unfortunately, the proteases also destroy pro-healing factors and ECM components in the wound bed, disrupting the wound healing process.

How do we know when raised levels of MMPs are causing healing problems?

The ability to heal is affected by a wide range of intrinsic and extrinsic factors. For example, increased age, medication (eg steroids), poor nutrition, comorbidities (eg diabetes, venous disease, peripheral arterial disease) and wound bioburden can each interfere with wound repair processes[39-41].

> *"The process of healing is powerfully programmed and very difficult to obstruct, but it has its enemies."* [42]

Regardless of the underlying cause of the delay, wounds with delayed healing generally share similar biochemical characteristics, including[43,44]:

- **elevated inflammatory markers**
- **high levels of proteases, including MMPs**
- **diminished growth factor activity**
- **reduced cell numbers in the wound.**

As described, these characteristics result in a hostile wound environment in which new tissue and growth factors are degraded and the wound is perpetuated. Wounds in this situation are often referred to as being 'stuck' in the inflammatory phase of healing, where they can remain for months and even years[45].

Breaking the vicious circle – stimulating healing

Management of a patient with a wound that is not healing requires an integrated, multidisciplinary approach that systematically works to alter the inflammatory phase of healing. A good analogy is to liken the wound to being stuck in a vicious circle (Figure 2): the clinician needs to use interventions to break out of the circle and move the wound on to the next phase of healing.

Breaking out of the circle will involve alleviating any environmental, systemic, local or wound related factors that might contribute to the delay in healing. The overall aim is to tip the balance in favour of the repair processes.

At the level of the wound, breaking out of the circle (Figure 3) and stimulating healing will involve:

- **treating the cause – ie reducing the inflammation**
- **managing the consequences – ie reducing protease activity whilst maintaining a moist wound environment.**

Reduction of excessive protease activity is focused on the wound and may be achieved by:

- **removing proteases – eg by absorption of protease-rich wound fluid into dressings or by removal with negative pressure wound therapy**

Figure 2 Cullen's circle – the vicious circle of delayed wound healing

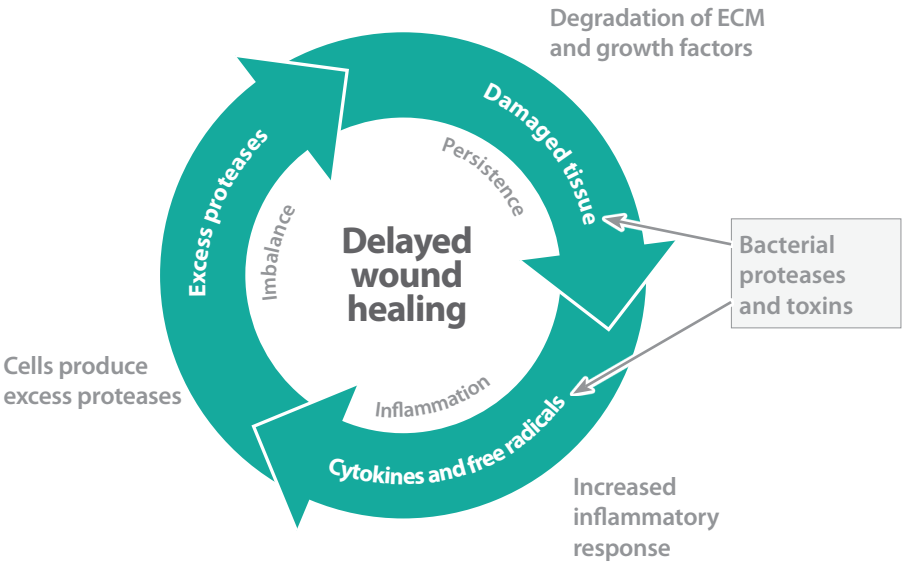
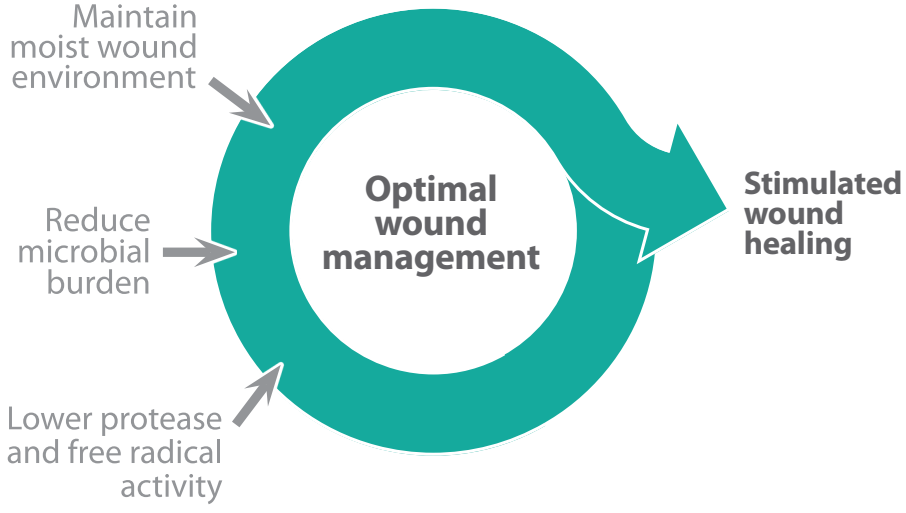


Figure 3 Breaking out of the circle to encourage healing



- **reduction of protease activity – eg by collagen-based dressings**
- **inhibition of MMP synthesis – eg by dressings containing polyhydrated ionogen, an emerging approach that awaits full clinical evaluation[46,47].**

When indicated, wound bioburden can be reduced with antimicrobial dressings (eg silver- or iodine-based technologies) and antibiotics[35]. However, antibiotics and antimicrobials are less effective at treating bacteria in a biofilm, and physical removal by debridement is currently the only demonstrated method for removing biofilm burden.

MMP modulating dressings

A number of dressings are currently being marketed as modulating protease activity. Reducing the excessive protease activity in the wound is thought to convert the wound to a healing state. Products designed to reduce excessive proteolytic activity and rebalance the wound environment ideally need to inactivate both host- and bacteria-derived MMPs and other proteases.

Modulation of MMP activity by dressings seems to be achieved by one of three approaches, as described above. A significant amount of research has focused on dressings that act to reduce levels of MMPs by absorbing wound exudate and holding the proteases within the dressing structure. In effect, this binds and inactivates the excess MMPs present in the wound environment.

Many studies have been published on the first MMP modulating dressing (Promogran – containing oxidised regenerated cellulose (ORC) and collagen) and latterly a version which contains silver (Promogran Prisma)[48]. These illustrated the ability of this dressing to reduce protease activity, scavenge free radicals and control bacterial levels[48,49]. While many of the initial studies involved *in vitro* assays, these dressings have also been assessed and shown to remove these negative factors in fluids from wounds with delayed healing (*ex vivo* studies)[50]. These *ex vivo* evaluations offer important benefits over *in vitro* assays as they more closely reflect the true biochemical nature of the wound. A randomised controlled clinical study has also shown the ability of collagen/ORC dressings to reduce proteases and that this was correlated to a positive effect on healing[51,52].

It is also important to recognise that as new products, such as protease-modulating dressings, are developed to correct a specific biochemical defect, they may not be applicable for all problematic wounds, but rather to a sub-group with that particular biochemical imbalance.

Measuring MMPs

At present, the clinician has no means of measuring MMP activity in wound fluids or biopsies. Prototype devices are under development. It is hoped that measurement of MMP activity will provide critical information on the healing trajectory of a wound and the suitability of the wound for advanced biological therapies.

Supported by an educational grant from Systagenix. The views expressed in this 'Made Easy' section do not necessarily reflect those of Systagenix.

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Summary

It is well established that MMPs are required at the right amount, in the right place, and in the right time frame (duration) for a wound to heal. They play key roles in debriding damaged/devitalised ECM, angiogenesis, re-epithelialisation, wound contraction, and scar remodelling. However, there is strong clinical evidence that chronically elevated levels of MMPs and other proteases prevent wounds from healing, and that treatments that lower MMP activities promote healing of wounds that have stalled[53-55].

To cite this publication

Gibson D, Cullen B, Legerstee R, Harding KG, Schultz G. MMPs Made Easy. *Wounds International* 2009; 1(1): Available from http://www.woundsinternational.com

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