Proteases made easy

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Introduction

The impact of proteases on wound healing is widely accepted. In particular, the functions that proteases perform during each stage of normal wound healing and the potentially damaging effects of elevated protease levels are well recognized. In recent years, research has emerged providing additional guidance for choosing treatments where protease activity is impairing healing, in particular, the importance of choosing strategies that can deal with different types of proteases by combining functionalities. This Made Easy explores existing knowledge of the major protease groups that affect wound healing and documents the impact recent findings should have on informed treatment choices for patients with chronic wounds.

Authors: Schultz G (USA), Cullen B (UK). Full author details can be found on page 5.

What environment is required for a wound to heal?

Complete wound healing is achieved via a system of integrated cellular and biophysical processes that must all occur in the appropriate sequence and time frame[1]. These processes fall within the phases of rapid hemostatis, inflammation, proliferation and remodeling, as described in Table 1.

Why do some wounds fail to heal?

Within this complex healing environment, there are many points of regulation, which precisely control the biological processes necessary to achieve normal wound repair. However, many wounds do not achieve re-epithelialization within 4 weeks, which is the widely accepted optimal healing time. Indeed, an alteration in any of these physiological processes can lead to the formation of a chronic wound, with wound healing vulnerable to interruption at each of the given stages by internal and external factors, related to[1]:

- **the patient – medical history, underlying pathologies and comorbidities**
- **the wound – size, depth, duration, or local factors such as desiccation, maceration, necrosis, pressure or edema**
- **the environment or systems of care within which patients are being treated – related to clinical competency or service delivery.**

When healing stalls, regardless of the reason, wounds can become chronic or may never achieve complete healing[4]. All stalled wounds share a similar biochemical profile, often demonstrating:

- **elevated inflammatory markers**
- **elevated protease activity**
- **low levels of growth factor activity**
- **reduced numbers of cells in the wound[5].**

In general, chronic wounds are characterized by a prolonged inflammatory phase, which ultimately results in elevated protease activity and the subsequent degradation of growth factors and other positive wound healing factors; the overall consequence is impaired healing. This hostile biochemical environment must be corrected if wound healing is to occur[6].

Table 1: The phases of wound healing[2,3]

Phase	Processes
Rapid hemostasis	■ Blood vessels constrict and fibrin clots form ■ Proinflammatory cytokines and growth factors are released by the blood clots and surrounding tissues ■ Epithelial cells are stimulated, followed by recruitment of fibroblasts, neutrophils and monocytes
Appropriate inflammation	■ Inflammatory cells migrate into the wound site – sequential infiltration of neutrophils, macrophages and lymphocytes
Proliferation/regrowth of the epithelial surface	■ Epithelial cells proliferate and migrate over a provisional matrix within the wound ■ Fibroblasts and endothelial cells support capillary growth and formation of collagen/granulation at the wound site
Remodeling: collagen synthesis, cross-linking and alignment	■ Remodeling begins once fibroblast cells have secreted a collagen framework upon which to develop

Proteases made easy

What are proteases?

Proteases or proteinases are synonymous terms that define enzymes involved in degrading proteins such as collagen and other elements of the extracellular matrix (ECM). They are generally classified into four major groups based on functionality; however, only two of these groups, the serine proteases and matrix metalloproteinases (MMPs), are considered important extracellularly (i.e. outside the cell) as they function optimally under physiological conditions and at a pH ~7.5[7].

At each of the physiological stages of normal wound healing, controlled proteases – such as MMPs – are necessary for wound healing: removal of the denatured matrix (debridement), contraction of the ECM, epithelial migration, migration of epidermal cells and remodeling of the scar (Figure 1). However, these proteases must always be present in the right places, at the right times and at the right levels.

Since these proteases are destructive in nature under normal physiological conditions, they are subject to strict regulation[7]. When the critical balance between proteases and their control system is upset, a pathological condition can occur; elevated levels of active proteases have been shown to be associated with many chronic conditions and diseases[8]. In addition, the presence of bacteria can exacerbate the hostile wound environment by producing virulence factors such as bacterial proteases[6].

How can proteases disrupt wound healing?

Although wound healing requires protease activity, the delicate balance between tissue breakdown and repair can be disturbed if protease levels become abnormally elevated, as depicted by the hypothesis of chronic wound pathophysiology in Figure 2.

While an unbalanced proteolytic environment endures, wound chronicity is perpetuated: key functional molecules that are required to stimulate cell growth and production of new tissue – such as collagen, ECM proteins and growth factors – are degraded, and so the negative proinflammatory cycle continues[9,10].

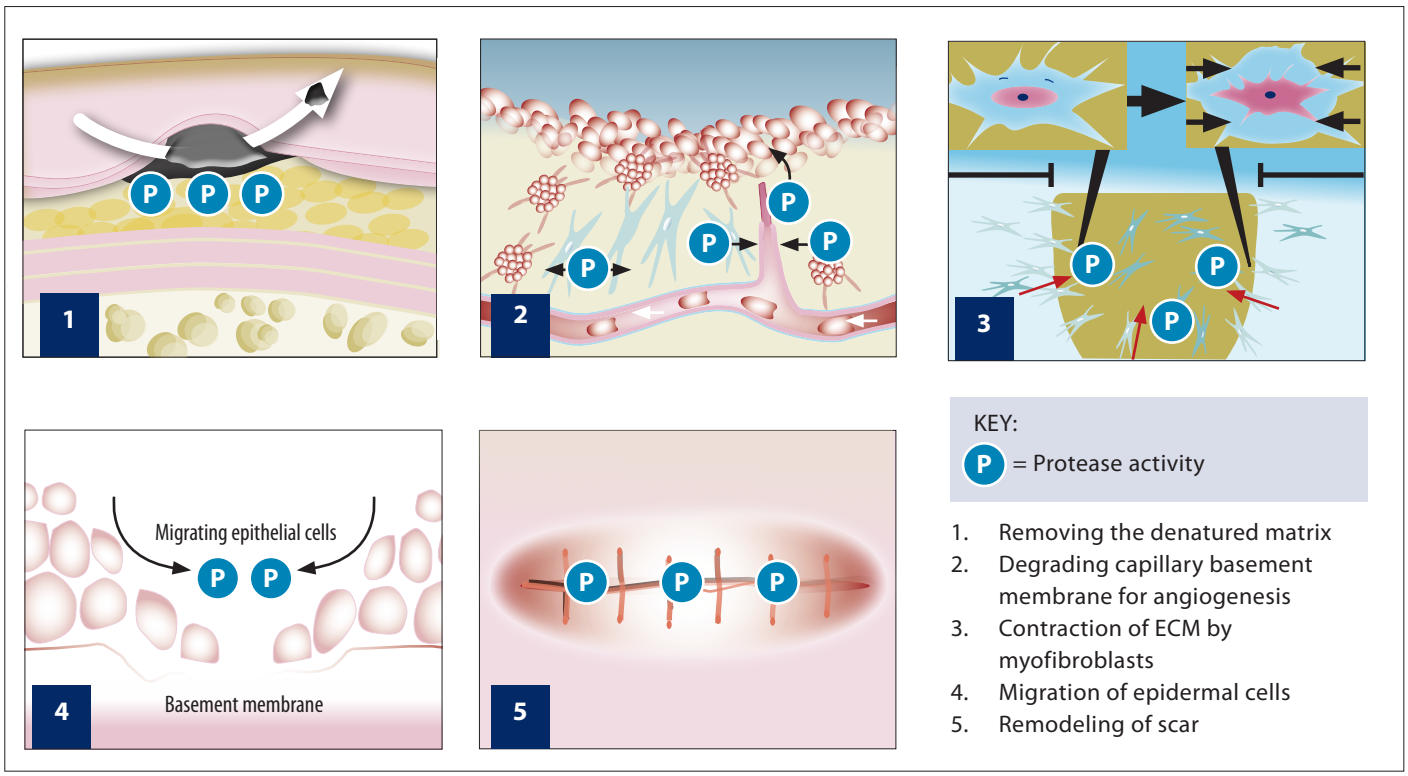

Figure 1: Proteases and the physiological stages of wound healing: debridement, angiogenesis, contraction, epithelial migration and scar remodeling

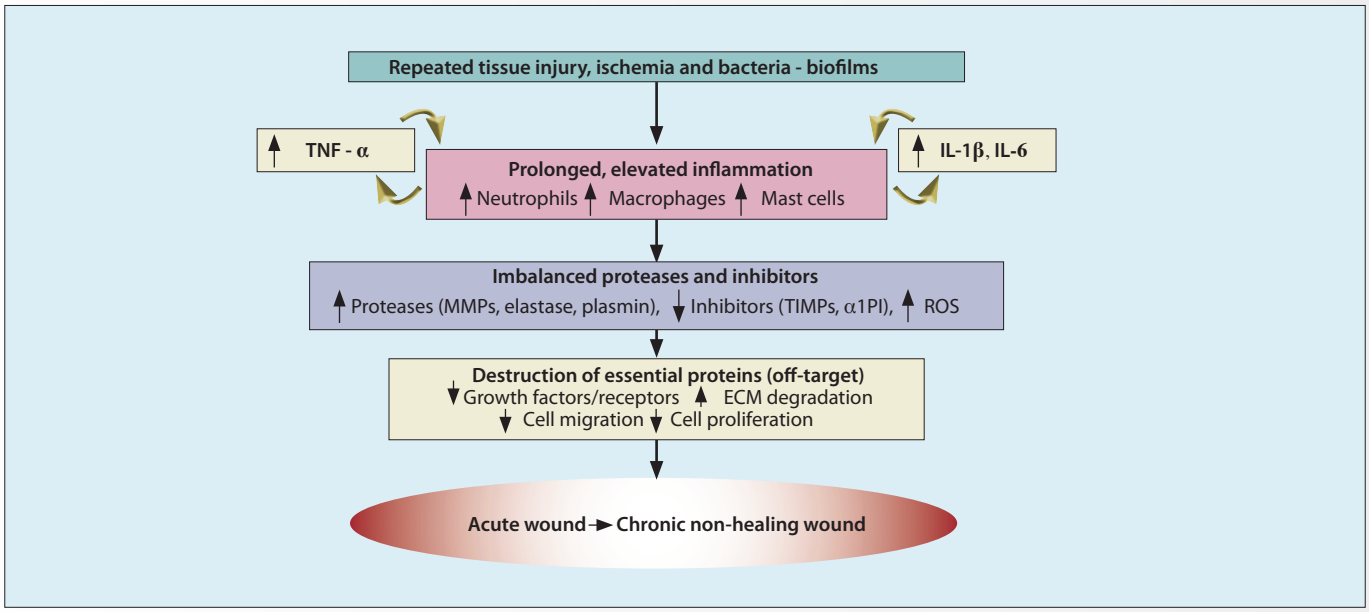

Figure 2: Hypothesis of chronic wound pathophysiology[11]

BOX 1: THE MAJOR PROTEASES INVOLVED IN WOUND HEALING

MATRIX METALLOPROTEINASES

Much is known about the critical role MMPs play in wound healing. Moreover, there is a substantial body of evidence demonstrating that MMPs are elevated in chronic wounds, and that elevated and prolonged expression of MMPs leads to impaired wound healing. When MMPs are elevated or present in the wound for too long they start to damage proteins that are not their normal substrates, resulting in destruction of essential growth factors, receptors or ECM proteins[12;5].

SERINE PROTEASES

The serine proteases, which are expressed by neutrophils such as elastase (i.e. human neutrophil elastase [HNE]), cathepsin G, and urokinase-type plasminogen activator, are also involved in the wound healing process. In addition to MMPs, serine proteases – such as elastase – are often present in the proteolytic environment of a chronic wound[12].

BACTERIAL PROTEASES

Evidence exists to support the contribution of bacteria to the proteolytic wound environment via the production of their own proteases[13]. Bacterial proteases are virulence factors that are secreted by a number of different types of bacteria commonly found in wounds. Virulence factors are molecules produced by pathogens that facilitate colonization, replication and spread of bacteria within a host[13]. Production of bacterial proteases can enhance the activity of proteases produced by the host, with host and bacterial proteases working in synergy to amplify the hostile wound environment[14,15].

Proteases and wound healing: developments in our understanding

Many published studies have documented that excessive protease activity in chronic wounds creates a negative healing environment[16], but few studies have documented which proteases are in excess and at what levels they become detrimental. Levels of both MMPs and serine proteases in chronic wounds have been measured and found to be significantly elevated compared with levels in acute wounds, while MMP8, MMP9 and HNE, all of which are inflammatory-derived proteases, predominate in chronic wound fluid[17,18]. Therefore, it is widely accepted that the chronic wound environment is proinflammatory and contains an excess of active proteases, which contribute to wound chronicity (Box 1).

However, recent research has shown that it is important to look at a combination of proteases that may be in excess rather than one individual protease, and that there may be several contributing factors resulting in elevated protease activity in non-healing chronic ulcers (Box 2 and Box 3)[19,20]. While more research is needed to determine why different proteases are elevated in some non-healing wounds rather than others, when an excess of any of the aforementioned proteases is present, the probability of healing is greatly reduced unless appropriate interventions are in place[21].

BOX 2: DIAGNOSTIC MARKERS FOR CHRONIC WOUND SUBPOPULATIONS

Many developments have been made in recent years in the field of advanced chronic wound treatment. For example, advanced therapy now includes the use of growth factors, extracellular matrixes and engineered tissue-based products[22]. Clinical studies have shown that these treatments tend to be most effective within chronic wound subpopulations. This suggests that there are subgroups within the whole chronic wound population that fail to heal as a result of different or multiple underlying biochemical defects.

As such, it is critical to develop an improved understanding of chronic wound pathophysiology, so targeted approaches to treatment can be instigated. This will also require the stratification of patients according to biochemical diagnostic markers rather than etiology alone, which is a new approach for wound care, but one that has been validated in other fields such as oncology[23].

BOX 3: QUANTIFYING THE LEVEL OF PROTEASE ACTIVITY THAT IS DETRIMENTAL TO HEALING

BACKGROUND

Although it is well known that elevated protease activity can impede wound healing, less is known about the levels of protease activity required to have a detrimental effect. A study published in 2016 explored the relationship between inflammatory protease activity and wound healing status, particularly with regards to the levels of HNE and MMP activity that correlate to non-healing[20].

METHODOLOGY

Patients (n=290) over 18 years of age with a wound that had been present for at least 4 weeks were assessed by a clinical investigator between May 16, 2011 and January 16, 2012. A chronic wound was defined as one that had failed to progress through a normal, timely sequence of repair and where comorbidities were interfering with the normal healing process, which included delayed, stalled, hard-to-heal, recalcitrant, difficult and complex wounds. Etiologies within the study included diabetic foot ulcers, pressure ulcers, venous leg ulcers, surgical wounds and traumatic wounds.

Healing status was defined by percentage wound area reduction during the first 4 weeks of treatment, with wounds initially assessed to determine whether they were on a healing or non-healing trajectory (the percentage area reduction required to show healing varied depending on the wound type). To determine protease levels, wounds were swabbed using a technique designed for optimum assessment of protease activity (the Serena technique). Wound fluid was eluted from the swabs and extracts were used immediately for protease testing.

OUTCOMES

HNE values

The median HNE value for all wounds was 2.6 mU/110 µL (range 0-108; n=286). HNE levels were substantially higher for some wound etiologies, including pressure ulcers (5.0 mU/110 µL) and arterial ulcers (11.1 mU/110 µL).

MMP values

The median value for all MMPs measured for all wounds was 12.6 U/110 µL (range 0-476; n=286). Compared with HNE values, there was less variation between wound types, although MMP values were found to be higher in arterial wounds (23.5 U/110 µL).

SUMMARY

Results suggest that chronic wounds with values of HNE >5 and/or MMP >= 13, respectively, should be considered to have impaired healing and treated as such. In this study, it was estimated that 66% of the non-healing chronic wounds met these criteria and had elevated protease activity. Receiver operating characteristic curve analysis was conducted to determine the classification of non-healing wounds in the context of these HNE and MMP values.

Interestingly, the median value of all wounds for each individual protease was below this threshold, however, 66% of wounds were estimated as having elevated protease activity. This shows that in some non-healing wounds, the excess protease activity was predominantly due to HNE activity, whereas in others, it was mainly due to MMP activity. As such, to deal effectively with excess protease activity in all non-healing wounds it is necessary to be able to reduce both HNE and MMP activity.

What are the wider implications of excess protease activity and disrupted wound healing?

Abnormal protease activity can affect wounds in all of the complex stages of healing, but the effects of elevated protease activity are not purely physiological. Indeed, where stalled wound healing is not recognized and treated as quickly as possible, there can be numerous wider implications, impacting patients' quality of life and increasing the burden to patients and their caregivers, as well as leading to financial challenges for healthcare systems and society as a whole[24].

Living with a stalled wound can have a negative impact on physical, mental and social aspects of patient wellbeing, especially where wounds persist for months or years. In wound care, outcomes are often measured in terms of wound progress (i.e. percentage change in wound size), but patients with chronic wounds often report concerns with pain, malodour, excessive exudate, appearance of the wound and comfort of the dressing applied[25]. Activities of daily living are also often impacted, with patients unable to continue living their lives – for example, in terms of work and productivity – as they could before their wounds presented or became chronic[26].

Wound care is an important element of healthcare provision, but it can often be both expensive and labour-intensive. As such, if healing problems related to excess protease activity are not adequately identified and addressed, costs to healthcare providers can be compounded as chronicity worsens. Indeed, the prevalence and incidence of stalled wounds are predicted to continue rising from current levels, due to the presence of an aging population and increasing levels of damaging comorbidities such as diabetes. Inevitably, there are pressures to reduce increasing costs of care, especially for chronic wounds[26].

As such, optimizing the healing process will be of major importance as it regards chronic wound care. Effective treatment is vital to address costs to the patient, society and healthcare systems of delayed wound healing. It is important to identify appropriate advanced wound care therapies, as well as addressing issues of service delivery and clinician competency. Individualized wound management plans should be developed based on assessment and monitoring of healing progress[24]. As with any scientific endeavor, it will be critical to consider the implications of new research in the ongoing provision of effective, advanced wound management that addresses both patient and budgetary needs.

How does this knowledge impact choice of treatment?

One strategy employed to overcome the chronicity of non-healing wounds relies upon wound bed preparation (debridement, control of infection or inflammation, moisture control, and wound edge management), in conjunction with the use of advanced therapies[27].

Based on current wound care knowledge, an ideal intervention should have the ability to simultaneously control MMP activity, HNE activity and bacterial protease activity. Some studies have shown that dressings that foster an optimal moist wound environment may also be effective in 'kick-starting' the wound healing process[27].

Whereas studies of dressing materials such as collagen have demonstrated an effect *in vitro* in reducing excess MMP activity, a lesser effect has been observed in other protease activities, such as HNE and bacterial protease activity. Other studies involving oxidised regenerated cellulose material indicate that it breaks down to simple sugars, glucose and glucoronic acid when in contact with wound fluid, an effect that works to reduce pH[28].

As discussed, such a reduction in pH has been shown to minimize the activity of extracellular proteases such as MMPs, HNE and bacterial proteases. *In vitro* studies have demonstrated that the combination of these materials has the effect of reducing excessive levels of protease activity found in wound fluid, whether this is of MMP, HNE or bacterial origin[29,30].

Author details

Schultz G[1], Cullen B[2].

1. Greg Schultz, Institute for Wound Research, Department of Obstetrics and Gynaecology, University of Florida, USA
2. Breda Cullen, R&T Director, Systagenix Wound Management

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Supported by an educational grant from Systagenix, An Acelity Company. The views expressed in this Made Easy do not necessarily reflect those of Acelity, A Systagenix Company.

Summary

The impact of proteases on wound healing, including the negative effect of abnormally elevated protease activity, has been widely accepted for some time. As understanding of the underlying pathophysiology of non-healing wounds improves, so also will the ability to combine appropriate technologies and materials to increase product functionality and ensure better outcomes for patients. In terms of excess protease activity, utilizing a strategy that deals with just MMPs addresses only a small part of the problem. In instances where there are other proteases in excess, other modes of action are also needed. It is this combined functionality that will make the difference in terms of the success of advanced chronic wound treatments in the future.