Abstract

The burden of chronic wounds to patients and the challenge they present to health care workers is enormous. Acute wounds heal in an orderly manner by coagulation, followed by an inflammatory phase with re-formation of the extracellular matrix, cell proliferation and finally remodelling with scar formation. In chronic wounds one or more of these stages becomes unregulated with the major difference appearing to be the destruction of the extracellular matrix (ECM) by matrix metalloproteinases (MMPs) and increased inflammatory activity which is frequently caused by infection. The important targets appear to be the MMPs which can be inhibited by both endogenous and synthesised inhibitors which have been shown to reduce MMP activity. Interestingly, doxycycline and tetracyclines have recently been shown to also inhibit MMP activity. In vitro, these also appear to have the ability to disrupt bacterial biofilms which are often present in chronic wounds. However, although the efficacy of these antibiotics has been demonstrated in periodontal disease, studies are needed to test their efficacy in chronic wounds.

Table I: Characteristics of chronic wounds compared to acute wounds and their implications. Adapted from Rayment and Upton, 20091 and Schultz et al, 20052

<table>
<thead>
<tr>
<th>Chronic wound characteristic</th>
<th>Implication</th>
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<tr>
<td>Abundant granulated tissue and fibrosis</td>
<td>Scar contraction</td>
</tr>
<tr>
<td>Increased proteolytic activity – MMPs, neutrophil elastase</td>
<td>Excess ECM degradation, fibronectin staining absent</td>
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<tr>
<td>Decreased α1-protease inhibitor (TIMP)</td>
<td>MMPs uninhibited → ↑ ECM degradation</td>
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<tr>
<td>Increased pro-inflammatory cytokines (TNF-α, IL-1β)</td>
<td>Uncontrolled inflammation</td>
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<tr>
<td>More B-lymphocytes, monocytes and macrophages, few T-helper cells</td>
<td>Inappropriate immune response</td>
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<tr>
<td>Presence of biofilm</td>
<td>Bacterial infection → ↑ inflammatory cytokine expression</td>
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<tr>
<td>Tissue hypoxia</td>
<td>Anaerobic environment, cytokine expression, ↑ free oxygen radicals</td>
</tr>
</tbody>
</table>

Introduction

The USA and Australia spend more than US$ 9 billion and US$ 410 million respectively annually treating and caring for chronic wounds in patients. Up to 15% of diabetic patients develop a diabetic foot ulcer and this is but a fraction of the total number of patients coping with chronic wounds. This debilitating condition impacts on the patient’s quality of life, increasing their risk of depression, malnutrition, and developing other co-morbidities with significant emotional and economic impact on their families. Despite treatment regimens based on the best available clinical evidence, health care professionals still face the burdensome and time consuming challenge of treating chronic wounds, often unsuccessfully.1

Acute wounds heal by a dynamic yet orderly process of
a) coagulation and clot formation;

b) an inflammatory phase where neutrophils, lymphocytes and macrophages infiltrate the wound space;

c) proliferation and repair with formation of a provisional extracellular matrix (ECM) and re-epithelisation;

d) remodelling with scar contraction and remodelling of the ECM.2

Key in this process is the reformation of the extracellular matrix which gives skin elasticity, tensile strength and compressibility. It provides a framework with signalling peptide sequences to direct cells into the wound space and activates these cells once bound to the matrix. In chronic wounds, this orderly process is disrupted with increased inflammation and ECM degradation (Table I).1,2

Matrix degradation and tissue remodelling is attributed, in part, to the expression and activity of a number of zinc-dependant endopeptidases, collectively known as matrixmetalloproteinases (MMPs).3,4 Their role in wound healing is vital and the activity of these enzymes needs to be carefully controlled as disturbances, whether an increase or decrease in their activity, may lead to aberrations in the wound healing process.1 This review summarises the role of the MMPs in the chronic wound healing process. In particular, certain antibiotics have been demonstrated to not only inhibit MMPs but also to disrupt bacterial biofilms which commonly afflict these wounds and delay the wound healing process.

MMP activity in the chronic wound

MMP expression and activity is altered in chronic wounds when compared to acute wounds1 with MMP activity up to 30 times.
higher in chronic wounds (59.9 ± 70.9 and 0.75 ± 0.76 µg MMP Eq/ml respectively, p < 0.001). Nwomeh et al reported increased MMP-1 and MMP-8 activity in chronic wounds. MMP-8 was identified as the predominant enzyme suggesting that excessive expression and activation of this enzyme may contribute to the pathogenesis of chronic wounds. Relative to systemic concentrations, MMP-2 and -9 profiles within the chronic wound varied between patients. Regulating MMP levels within tight physiological ranges would be an important goal of possible pharmacological intervention to promote appropriate wound healing.

**MMP inhibition**

Panuncialman and Falanga suggest that simply inhibiting MMPs in chronic wounds is not an appropriate strategy, as MMP activity is important for cytokine and chemokine production to attract cells into the wound area. Whereas some investigators have studied endogenous MMP inhibitors, such as α2-macroglobulin and tissue inhibitors of metalloproteinases (TIMPs), others have studied exogenous MMP inhibitors that could modify MMP activity. Ågren et al created suction blisters on human test subjects and showed delayed healing with the non-selective MMP inhibitor ilomastat, which reduced MMP-2 and keratinocyte migration into the wound space but increased levels of MMP-9 in the treated wounds compared to the control wounds. Others have also demonstrated the importance of MMPs in promoting cell migration using MMP inhibitors. The inhibitor BB-3103 has been used to demonstrate the importance of MMPs in promoting cell migration using MMP inhibitors.

Early work investigating the effects of tetracyclines, and chemically modified tetracycline (CMTs) derivatives, including their non-microbicidal activities, on MMP activity has been reviewed. Doxycycline reduced MMP activity in endotoxin induced periodontal breakdown in rats. Both this tetracycline and CMTs at therapeutic concentrations inhibited catalytic activity of MMP-8 and -9 without affecting MMP-2 activity or endogenous TIMP-1 and -2 in cultured human endothelial cells. Topical doxycyclines reduced proteolytic activity in wound fluid of chronic venous leg ulcers and established chronic ulcers from diabetic patients with wounds persisting for more than six months but these were not as effective as ilomastat.

The possibility that the low dose antibiotic treatment may cause bacterial resistance was excluded as none of the test wounds were found to be infected. Comparison of several antibiotics on MMPs in gingival crevicular fluid present in periodontal disease, decreased MMP levels in patients treated with tetracycline and minocycline but not in those in the control or metronidazole treated groups. TIMP levels were increased in all antibiotic test groups.

Bacterial colonisation of a wound would inevitably trigger the inflammatory cascade. Increased MMP-9 activity and concentrations have been reported in pulmonary tissue with associated hospital-acquired pneumonia. The pro-inflammatory cytokines IL-1β and TNF-α have been implicated in the modulation of MMP activity. Tasaki et al showed these cytokines stimulated the release of MMP-1 and this effect could be overcome by inhibitors of the cytokine action in human pancreatic periacinar myofibroblasts. TNF-α was shown to be converted to its mature form by the MMPs and was necessary for leukocyte migration into damaged tissue. IL-1β released from macrophages enhances MMP-1, -3, -7, -9 precursor formation.

**Treatment of biofilms**

Chronic wounds have a high infection rate with a host of colonising organisms which may form a layer of multi-cellular colonies within an exopolysaccharide (EPS) matrix barrier. Such biofilms occur in up to 80% of chronic wounds and form in almost any moist environment which has the appropriate nutrients to sustain the colony. The insoluble EPS barrier resists entry of substances such as antibiotics and disinfectants and effectively prevents individual bacterium being washed out. Once the bacteria establish themselves in the biofilm they are capable of producing “virulence factors” which defend against the body’s polymorphonuclear leukocytes. Although conventional antibiotics are effective against free-living (planktonic) bacteria, they are frequently ineffective against bacteria in such biofilms.

Decreasing the bacterial burden of chronic wounds would uncouple the self-perpetuating inflammation within these wounds and allow host defences to control the infectious burden. Strategies for controlling biofilms rely on chemical biocides or antibiotics that kill the attached microorganisms and/or remove them from the surface. Antibiotics have been widely used to treat chronic wounds with little evidence as to optimal treatment regimens for biofilms. The bacteria in the biofilms have become resistant to a range of antibiotics including ampicillin, streptomycin, tetracyclines, gentamicin presumably as a result of the antibiotics not effectively penetrating the EPS matrix layer.

Other non-traditional substances have been tested to eliminate the biofilm. Initial results on silver based dressings which appear to disrupt the biofilm, have been positive. Other substances disrupt biofilm formation or attachment, or the formation of bacterial substances which defend against the host’s polymorphonuclear leukocytes.

Chloramphenicol and tetracycline are being re-examined as potential drugs for treating chronic infections. The efficacy of tetracycline and chloramphenicol in destroying biofilms created in vitro cultures were evaluated by Liaqat et al. Used together, this antibiotic combination significantly decreased both loosely attached and tightly bound cells in biofilms. Tetracycline alone was able to decrease in biofilm formation in the bacterial isolates (Klebsiella spp, , Achromobacter spp, P aeruginosa and Bacillus pumilis) within 3–5 days, with chloramphenicol showing similar efficacy within 5–8 days. Studies in the patient setting are yet to be carried out.

In summary, chronic wounds present an enormous challenge to health care workers. The major difference between the acute and chronic wound appears to be in the integrity of the extracellular matrix and the activity of inflammatory pathways. The main culprits here appear to be the MMPs and infection. Pro-inflammatory cytokines produced in response to infection stimulate MMP production. Both endogenous and synthesised MMP inhibitors effectively reduce MMP activity.
Chronic wounds frequently become infected with the formation of a biofilm against which most conventional antibiotics are ineffective. Although novel agents such as silver can disrupt biofilms, well known antibiotics such as doxycycline and tetracyclines appear to have the dual ability to disrupt biofilms as well as inhibit MMP activity. However, although their efficacy in periodontal disease has been demonstrated, studies are needed to test their efficacy in other chronic wound settings.

References