Investigations on pH-values in milieus of chronic wounds during modern wound therapy

**Dissemond J, Witthoff M, Grabbe S.**

247 pH values were measured in 39 different patients with chronic wounds. These patients had been treated in the outpatient clinic at the department of dermatology, University of Essen, Germany for 12 months. A wound was defined as chronic if there was no objective tendency for healing within three months. The study demonstrated that chronic wounds had pH values from 5.45 to 8.65, with a mean of 7.42.

The activity range of a selection of proteolytic enzymes is below.

<table>
<thead>
<tr>
<th>Proteolytic Enzymes</th>
<th>pH range of activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNAase</td>
<td>Activity: pH 4.5 – 5.5</td>
</tr>
<tr>
<td>Fibrolysin</td>
<td>Activity: pH 7.0 – 8.0</td>
</tr>
<tr>
<td>Collagenase</td>
<td>Optimum: pH 6.0 – 8.0</td>
</tr>
<tr>
<td>Krill-enzymes</td>
<td>Optimum: pH alkaline</td>
</tr>
<tr>
<td>Papain</td>
<td>Activity: pH 3.0 – 12.9</td>
</tr>
<tr>
<td>Streptodornase</td>
<td>Max. activity: pH 7.5</td>
</tr>
<tr>
<td>Streptokinase</td>
<td>Activity: pH 7.3 – 7.6</td>
</tr>
<tr>
<td>Sutilain</td>
<td>Optimum: pH 6.0 – 8.0</td>
</tr>
</tbody>
</table>

The lowering of pH values in chronic wounds by the application of CADESORB®

**Koeber A, Freise J, Grabbe S, Dissemond J.**

This poster presentation looked at the pH values of chronic wounds before and after the application of CADESORB to determine if the reduction in pH seen *in-vitro* could be demonstrated *in-vivo*.

The results were as follows.

<table>
<thead>
<tr>
<th>Number of Patients</th>
<th>10 (3 female / 7 male)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean pH value of wound before CADESORB</td>
<td>7.70</td>
</tr>
<tr>
<td>Mean pH value of wound after 24 hours CADESORB</td>
<td>6.69</td>
</tr>
<tr>
<td>Mean pH value CADESORB</td>
<td>4.35</td>
</tr>
<tr>
<td>Mean pH value of CADESORB after 24 hours</td>
<td>4.18</td>
</tr>
</tbody>
</table>

The study showed a reduction in the wound pH over 24 hours. The authors proposed that lowering the pH in chronic wounds could lead to an acceleration of the wound healing process. Moreover they thought it conceivable to shift the pH in a wound environment towards a range less favourable to bacteria.
Proteases and pH in chronic wounds

Greener B, Hughes AA, Bannister NP, Douglass J.

Our understanding of chronic wounds has progressed substantially in recent years due largely to painstaking research into the cellular and molecular environment of the wound bed. This has led to more rational approaches to treatment based on a clearer understanding of the effect that bacteria and moisture have on the wound bed. Control of bacteria and other pro-inflammatory elements is therefore an obvious approach to treatment. There is no doubt that proteolytic activity is a significant factor in most chronic wounds, which is in many cases due to uncontrolled inflammation, associated with infiltration of neutrophils. It is also well documented that proteolytic activity is sensitive to the environmental pH. With these factors in mind, we suggest that reduction of pH at the wound bed could be a simple and effective approach to reducing protease activity and promoting healing.

Cytokine and protease levels in healing and non-healing chronic venous leg ulcers


Leg ulcers present a common and recurring problem in older people creating discomfort and distress for the patient and a great cost to the health care services. Cultured keratinocyte grafts have been used by many investigators to stimulate healing of chronic venous ulcers. It has been proposed that they may do this by producing cytokines which modulate the healing process. However, the types and levels of cytokines in the leg ulcer fluid before and during healing are not known. Wound fluid was collected from venous leg ulcers in 18 patients beneath occlusive Tegaderm™ dressing for 4 to 6 hours. The leg ulcers were divided on clinical criteria into ‘healing’ and ‘non-healing’. PDGF-AB, GM-CSF, IL-1α, IL-1β, IL-6 and bFGF were measured by ELISA and the levels of IL-1α, IL-1β and IL-6 were also measured using biological assays. The effect of leg ulcer wound fluid on fibroblast and keratinocyte proliferation was measured indirectly by 3H-thymidine incorporation and MTT assay. Total protein, albumin levels, fibronectin degrading activity and collagenase activity, both active and latent were measured. No statistically significant differences in the levels of cytokines or collagenase were identified between healing and non-healing leg ulcers in the sample of leg ulcers studied. However, this study does give valuable information concerning the levels of cytokines and collagenase in chronic leg ulcer wound fluid.
Up-regulation of elastase in acute wounds of healthy aged humans and chronic venous leg ulcers are associated with matrix degradation


Chronic wound healing states are often associated with aging, and despite the increased number of aged patients with non-healing wounds, controversy still exists concerning the effects of age on wound repair. Our previous work showed that in both venous ulcers in humans and acute wounds in aged animals, fibronectin, an early component in granulation tissue, is deficient compared to normal skin and acute wounds in healthy young animals, respectively. In the present study, we have determined the protease responsible for fibronectin degradation by analysing tissue taken from the margins of chronic venous ulcers and standardised acute cutaneous wounds collected from a large cohort of “Health status”- defined aged human subjects (screened as per the SENIEUR protocol). When tissue samples were subjected to fibronectin zymography, the main protease involved in the breakdown of fibronectin in both venous ulcers and acute wounds of elderly subjects was found to be a serine protease with a molecular weight of approximately 30 kd. This protease was identified as neutrophil elastase by immunoblotting. In tissue biopsies, elastase was localised to granulocytes by immunocytochemical techniques and shown to be present in greater quantities in venous ulcers and acute wounds of elderly subjects relative to those of young subjects. The highest quantities were found in acute wounds of elderly women. Our results suggest that the process of aging in healthy human subjects is associated with an up-regulation of elastase during acute wound healing and that an abnormality in down-regulation of this protease could be partially responsible for the transition to chronic wound healing states in the aged.

Topical pH and burn wound healing: a review. Beyond occlusion: wound care proceedings


The accumulated data in the literature indicate the correlation of wound healing to topical pH levels. In addition, pH of various levels directly affects the healing process of colonic anastomoses, skin graft take, burn wound healing, and some basic enzymatic and cellular activities directly involved in the healing process. It is postulated, therefore, that wound healing could be controlled in part, by changing the pH levels at the healing site.
Topical acidification promotes healing of experimental deep partial thickness skin burns: a randomised double-blind preliminary study


The effects of three buffered solutions with pH values of 3.5, 7.42 and 8.5, respectively, on the healing rate of deep partial skin thickness burns, was followed for 21 days in 16 guinea-pigs. Two symmetrical burns were inflicted on the back of each animal and then each individual wound was dressed with an irrigation disc dressing; solutions were coded (no. 1 to no. 3) and the animals were randomly divided and blindly treated as follows: Group A, solution no. 1 v. solution no. 2 (n = 4); Group B, solution no. 2 v. solution no. 3 (n = 4); Group C, solution no. 1 v. solution no. 3 (n = 4); Group D, non-irrigated disc dressings (n = 4). The solutions were applied to the surface of the burn wounds at a rate of 0.15 ml/cm\(^2\). Dressings were changed every 7 days to assess contraction and epithelialisation by a sonic digitiser. On post-burn day 21 the newly formed scar tissue was measured in all wounds. After computation of the healing rate at the end of the study, the data were then related to the coded treating agent. Contraction did not differ in all test groups during the study. Epithelialisation was significantly faster in the pH 3.5-treated burns than in the other treated wounds (P less than 0.001). The present study indicates that topical acidification of experimental deep partial skin thickness burns promoted healing. The precise mechanism should be elucidated.

Chemical acidification of wounds. An adjuvant to healing and the unfavourable action of alkalinity and ammonia


The authors ask whether there could be a single factor, which might account for delayed healing of contaminated wounds in a diversity of clinical situations? The paper discusses the fact that one requirement of all healing tissues is an available supply of oxygen. Oxyhaemoglobin releases its oxygen more readily in an acid environment. The adverse affect of alkalinity can comparably deprive wounds of oxygen by stabilizing oxyhaemoglobin. Some bacteria produce ammonia which is in itself, necrotising but which could impair oxygenation of the tissues by raising the pH. Even small changes in pH could induce wide changes in wound oxygen concentration.
Studies performed by Leveen et al demonstrated a five-fold increase in oxygen as a result of a reduction of 0.9 pH units. Acidification was found to increase the partial pressure of oxygen in surface wounds by virtue of a shift in the oxyhaemoglobin-haemoglobin dissociation curve. The studies showed that acidity should be constantly maintained if it is to have beneficial effects on healing and that the superior ammonia binding capacity of polycarboxylated polymers makes them the acidifying agents of choice.

The paper discusses the role pH plays in the histotoxicity of ammonia, stating that high concentrations of ammonia do not destroy erythrocytes in an acid medium and finishes by concluding that chemical acidification of wounds is most effective in minimising the toxicity of the ammonia formed by urease producing organisms.

The pH of varicose ulcer surfaces and its relationship to healing

Wilson IAI, Henry M, Quill RD, Bryne PJ. VASA, 1979; 8: 339 – 42

Leveen et al (1973) demonstrated experimentally that prolonged chemical acidification of wound surfaces increased their healing rate by increasing cellular oxygen availability.

This principle was applied to the problem of varicose ulcers. The aims were:

1. To study the effect of different preparations on ulcer surface pH
2. To determine if the acidification of ulcers promotes healing

Two preparations were used, one a buffered emulsified ointment (pH 6.0) was compared with a commercial product comprising of malic, benzoic and salicylic acids (pH 2.8).

Both presentations showed a significant difference in pH at 2 and 4 hours but only the buffered product still showed a significant difference in pH after 24 hours. This confirmed the idea that buffering an acidic preparation would prolong the effect of lowering the pH at the ulcer surface.

To investigate the relationship between prolonged acidity and healing rate, 36 patients were admitted to a controlled clinical trial utilising both previous preparations. The healing rates achieved with the buffered material were significantly better than those achieved with the unbuffered ointment (despite the difference in initial pH).

The authors suggested that the healing rate was influenced by the buffered preparation and so supports the idea that prolonged chemical acidification of the ulcer surface does increase the healing rate. The effect of pH on tissue oxygen was thought to be one means by which this may occur.
The Wound Milieu in Venous Ulcers – Further Observations

Roberts G, Chumley A and Mani R.

Aim: To identify the significance of surface pH and temperature in venous ulcers.

In a randomised controlled study on patients with venous ulcers, we measured wound pH and temperature in order to gain further understanding of the wound milieu. We measured surface pH on the wound and control sites using a protocol as previously published by us.

Patients (N =25) with venous ulcers were screened for the study with prior approval from the Ethical Committee for this hospital and after written informed consent from participant. From this group, patients (N= 16, 8M, 8F age range 52 – 85 years) were recruited. Patients were randomly assigned to wound dressing (X) or control, and 4-layer bandaging (PROFORE™). Surface pH and temperature measurements were made at all visits once wound dressings were taken off with patients lying supine in a room temperature of 21-24°C.

Healers had significantly lower mean wound surface pH 6.91 against 7.42, p=0.01). Healers also had higher wound temperatures 31.1-30.67°C, p>0.05, NS). Wound pH was negatively correlated with surface temperature.

Wound pH and temperature measurements are simple and reliable. These data suggest that wound pH decreases in healing wounds, which is in accord with our previous observations. A lowering wound pH and a higher wound temperature would appear to be conducive to healing.

Extracellular matrix and keratinocyte migration

O'Toole EA. Clinical and Experimental Dermatology, 2001; 26: 525-530

We are just beginning to understand some of the cellular mechanisms involved in human keratinocyte migration on extracellular matrix. Extracellular matrix components have differing effects on keratinocyte mobility. Signalling through integrin receptors and secretion of collagenase are both components of this process. An understanding of the effect of extracellular matrix on keratinocyte migration has direct relevance to the problem of wound re-epithelialisation and will assist in the development of therapeutic efforts to enhance wound healing artificially.
Matrix metalloproteinases in repair
Parks WC. Wound Rep Reg. 1999; 7: 423-432
During repair, many different matrix metalloproteinases are produced by multiple cell types residing in various compartments within the wound environment. This diversity of enzymes, coupled with discreet cellular expression, implies that different matrix metalloproteinases serve different functions, acting on a variety of substrates, during wound healing. With few exceptions, however, the actual function and spectrum of functions of matrix metalloproteinases in-vivo is not known. Even with the advent of genetically defined animal models, few studies have rigorously addressed the substrates and role of matrix metalloproteinases in wound repair. Before we can understand the role of matrix metalloproteinases in ulceration and disease, we need to determine the function these enzymes serve in normal tissues and repair.

Proteolytic activity in burn wound exudates and comparison of fibrin degradation products and protease inhibitors in exudates and sera
Proteolytic (caseinolytic) activity in burn wound exudates was screened over the range pH 5.3 to 8.4. Although activity was greatest at pH 8.4 in four of seven exudates, individual differences indicated that different proteases predominate in the local environment of the wound. Paired exudate and serum samples were compared with regard to fibrin degradation products and three protease inhibitors: antithrombin III, a1-protease inhibitor, and a2-antiplasmin. The concentration of fibrin degradation products was higher in exudates than in paired sera, indicating the wound as the source of circulating fibrin degradation products rather than intravascular coagulation followed by fibrinolysis. In contrast, all three protease inhibitors exhibited higher concentrations in serum than in the paired exudate. The serum/exudate ratio for AT III differed significantly from that for a1-protease inhibitor and a2-antiplasmin, and the ratio of two inhibitors in serum differed from the ratio of the same two inhibitors in the exudate in two of three comparisons. These findings emphasise the importance of exudate examinations as a reflection of events in the wound itself. The importance of microenvironments is invoked to account for the significant exudate fibrin degradation products titers, which are seen despite the presence of antithrombin III, which could inhibit coagulation, and the presence of a2-antiplasmin, which could inhibit fibrin degradation.
Proteolytic events of wound-healing – coordinated interactions among matrix metalloproteinases (MMPs), integrins, and extracellular matrix molecules

Steffensen B, Häkkinen L, Lurjava H.

During wound-healing, cells are required to migrate rapidly into the wound site via a proteolytically generated pathway in the provisional matrix, to produce new extracellular matrix, and, subsequently, to remodel the newly formed tissue matrix during the maturation phase. Two classes of molecules co-operate closely to achieve this goal, namely, the matrix adhesion and signalling receptors, the integrins, and matrix-degrading and processing enzymes, the matrix metalloproteinases (MMPs). There is now substantial experimental evidence that blocking key molecules of either group will prevent or seriously delay wound-healing. It has been known for some time now that cell adhesion by means of the integrins regulates the expression of MMPs. In addition, certain MMPs can bind to integrins or other receptors on the cell surface involved in enzyme activation, thereby providing a mechanism for localised matrix degradation. By proteolytically modifying the existing matrix molecules, the MMPs can then induce changes in cell behaviour and function from a state of rest to migration. During wound repair, the expression of integrins and MMPs is simultaneously up-regulated. This review will focus on those aspects of the extensive knowledge of fibroblast and keratinocyte MMPs and integrins in biological processes that relate to wound-healing.
Analysis of the acute and chronic wound environments: the role of proteases and their inhibitors


To assess the differences in proteolytic activity of acute and chronic wound environments, wound fluids were collected from acute surgical wounds (22 samples) and chronic wounds (25 samples) of various aetiologies, including mixed vessel disease ulcers, decubiti and diabetic foot ulcers. Matrix metalloproteinase (MMP) activity measured using the Azocoll assay was significantly elevated by 30 fold in chronic wounds (median 22.8g MMP Eq/ml) compared to acute wounds (median 0.76g MMP Eq/ml) (p< 0.001). The addition of the matrix metalloproteinase inhibitor Illomostat decreased the matrix metalloproteinase activity by approximately 90% in all samples, confirming that the majority of the activity measured was due to matrix metalloproteinases. Gelatin zymograms indicated predominantly elevated matrix metalloproteinase-9 with smaller elevations of matrix metalloproteinase-2. In addition tissue inhibitor of metalloproteinase-1 levels were analysed in a small subset of acute and chronic wounds. When tissue inhibitor of metalloproteinase-1 levels were compared to protease levels there was an inverse correlation (p = 0.02, r = 0.78). In-vitro degradation of epidermal growth factor was measured by addition of 125I labelled epidermal growth factor to acute and chronic wound fluid samples. There was significantly higher degradation of epidermal growth factor in chronic wound fluid samples (mean 28.1%) compared to acute samples (mean 0.6%). This also correlated to the epidermal growth factor activity of these wound fluid samples (p< 0.001, r = 0.64). Additionally, the levels of proteases were assayed in wound fluid collected from 15 venous leg ulcers during a non-healing and healing phase using a unique model of chronic wound healing in humans. Patients with non-healing venous leg ulcers were admitted to the hospital for bed rest and wound fluid samples were collected on admission (non-healing phase) and after 2 weeks (healing phase) when the ulcers had begun to heal as evidenced by a reduction in size (median 12%). These data showed that the elevated levels of matrix metalloproteinase activity decreased significantly as healing occurs in chronic leg ulcers (p< 0.01). This parallels the processes observed in normally healing acute wounds. This data also supports the case for the addition of protease inhibitors in chronic wounds in conjunction with any treatments using growth factors.
The proteolytic environment of chronic wounds

Yager DR, Nwomeh BC. Wound Rep Reg, 1999; 7(6): 433-441

A consistent feature of chronic leg and pressure ulcers is chronic inflammation associated with an elevated infiltration of neutrophils. Neutrophils and their proteases have been implicated in mediating the tissue damage associated with a variety of chronic inflammatory disease. This review discusses our current understanding of the proteolytic enzymes found in chronic wounds and attempts to relate this information to the abundant presence of neutrophils. In addition, the implications that the proteolytic environment may have for current and future treatment strategies of chronic non-healing wounds are discussed.

Wound fluids from human pressure ulcers contain elevated matrix metalloproteinase levels and activity compared to surgical wound fluids

Yager DR, Zhang LY, Liang HX, Diegelmann RF, Cohen IK.

Fluid from acute surgical wounds and from non-healing pressure ulcers was examined for the presence of several matrix metalloproteinases. Gelatin zymography demonstrated the presence of two major gelatinases with apparent molecular masses of 72 kDa and 92 kDa and two minor gelatinases with apparent mobilities of 68 kDa and 125 kDa. Antigen-specific sera identified the 72-kDa protein as matrix metalloproteinase-2. The same sera also reacted with the 68-kDa protein, which is consistent with it being an activated form of matrix metalloproteinase-2. Antigen-specific sera identified the 92-kDa and 125-kDa proteins as matrix metalloproteinase-9. Levels of matrix metalloproteinase-2 and matrix metalloproteinase-9 were elevated more than 10-fold and 25-fold, respectively, in fluids from pressure ulcers compared with fluids from healing wounds. Examination of total potential and actual collagenolytic activity revealed that fluid from pressure ulcers contained significantly greater levels of both total and active collagenase compared with that of acute surgical wounds. In addition, an enzyme-linked immunosorbent assay demonstrated that fluids from pressure ulcers contained significantly more collagenase complexed with the inhibitor, tissue inhibitor of metalloproteinases. Together, these observations suggest that an imbalance exists between levels of matrix metalloproteinases and their inhibitors in the fluids of pressure ulcers and that this is primarily the result of elevated levels of the matrix metalloproteinases. The presence of excessive levels of activated forms of matrix-degrading enzymes at the wound surface of pressure ulcers may impede the healing of these wounds and may be relevant to the development of new rationales for treatment.
Wound Management and Dressings


From the evidence currently available, it would appear that dressings that directly or indirectly reduce the pH of wound fluid may help to prevent infection, and will be more likely to produce conditions that are conducive to rapid healing than other materials which produce a more alkaline local environment.