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ORIGINAL ARTICLE

Prospective and randomised evaluation of the protease-modulating effect of oxidised regenerated cellulose/collagen matrix treatment in pressure sore ulcers

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Key words
Chronic wounds; Clinical trial; Elastase; Plasmin; Pressure sores; Proteases; Wound healing

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Abstract

In chronic wounds, excess levels and activity of proteases such as elastase and plasmin have been detected. Oxidised regenerated cellulose/collagen matrix (ORC/collagen matrix) has been reported to ameliorate the wound microenvironment by binding and inactivating excess proteases in wound exudates. In this study, the levels and activity of elastase and plasmin in wound exudates of pressure sore ulcers were measured to determine the beneficial effect of ORC/collagen matrix treatment compared with control treatment with a foam dressing. A total of 33 patients with pressure sores were enrolled in the study and were followed up for 12 weeks after treatment. Ten control patients were treated with a foam hydropolymer dressing (TIELLE®, Systagenix), and the remaining 23 patients were treated with ORC/collagen matrix plus the foam dressing (TIELLE®, Systagenix) on top. Wound assessments were carried out over 12 weeks on a weekly basis, with dressing changes twice a week. Ulcers were photographed and wound exudates were collected on admission and at days 5, 14 and then every 14 days to provide a visual record of any changes in appearance of the ulcer and healing rate and for biochemical analysis of the wound. The levels and activity of elastase and plasmin were measured in wound exudates. Statistical analysis was performed using ANOVA and Bonferroni's post hoc test with P-values $<0{\cdot}05$ considered to be significant. Compared with controls, ORC/collagen matrix–treated pressure sore wounds showed a significant faster healing rate, which positively correlated with a decreased activity of elastase and plasmin in wound exudates. No signs of infection or intolerance to the ORC/collagen matrix were observed.

Introduction

Chronic wounds are a heterogeneous group of skin lesions that do not progress through the normal healing process (1,2). They include diabetic foot ulcers, vascular insufficiency ulcers at the lower extremities as well as pressure ulcers. In general, two consistent findings within the pathogenesis of these wounds are chronic inflammation and imbalance in protease and protease inhibitor (1,2). Normal wound healing is a rigorously concerted remodelling process that consists of restorative activities (new tissue formation) as well as removal of debris. Within this complex environment, there are many points of regulation that precisely control the biological processes necessary to achieve normal wound repair (3). An alteration in any of these

Key Messages

- chronic wounds are characterised by a state of ongoing inflammation and overabundance of proteases resulting in the degradation of growth factors and fibronectin and reduction in the levels of endogenous protease inhibitors
- oxidised regenerated cellulose (ORC)/collagen matrix is known to reduce the levels and activities of destructive

proteases such as elastase and plasmin in diabetic and venous leg ulcers
- ORC/collagen matrix reduces elastase and plasmin activity and enhances wound closure significantly in pressure sore ulcers

physiological processes can lead to a non-healing state and the formation of a chronic wound (4). These chronic wounds fail to follow the normal pattern of wound repair involving inflammation, granulation and reepithelialisation, and remain in a persistent inflammatory state. The latter is characterised by complement degradation and ongoing proteolysis, as indicated by increased levels of neutrophil elastase and gelatinases (5). The resulting degradation of matrix molecules, such as fibronectin, laminin and various collagens (5), hinders new tissue formation and cell adhesion, thereby preventing the constitution of proper contacts between keratinocyte integrins and underlying matrices in chronic wounds.

Elastase is one of the most destructive enzymes in the setting of wound healing and has been well characterised in non-healing wounds (6). Elastase possesses a broad specificity, preferentially cleaving bonds that are carboxy-terminal to valine and to a lesser extent alanine (7).

Plasmin is another serine protease whose primary substrate is fibrinogen/fibrin. It is converted from plasminogen through enzymatic cleavage by plasminogen activator, which also belongs to the family of serine proteases (1). Plasmin participates in a variety of pericellular proteolytic events, such as cell migration and angiogenesis (8). It activates matrix metalloproteinases (MMP) and growth factors such as transforming growth factor (TGF-β) (9,10). Furthermore, plasmin plays an important role in vascular endothelial growth factor (VEGF) degradation, a well-described key cytokine in angiogenesis (11).

Interestingly, fibrin deposition is a common feature of non-healing wounds, such as venous leg ulcers, diabetic foot ulcers and pressure ulcers (12). However, an excessive concentration of both the serine protease elastase and plasmin in chronic non-healing wounds is to a significant degree responsible for the degradation of growth factors and fibronectin and reduction in the levels of endogenous inhibitors (6,11,13).

ORC/collagen matrix, a device composed of oxidised regenerated cellulose and collagen, has been shown in previous studies to reduce the activity of elastase, plasmin and metalloproteinase in chronic wound fluids of diabetic patients in vitro (14–19). Moreover, in a randomised controlled study, ORC/collagen matrix accelerated the healing rate in venous leg ulcers compared with control wounds (20,21).

In this pilot study, we sought to assess elastase and plasmin activity in wound fluids derived from pressure ulcers treated with ORC/collagen matrix compared with standard treatment. Furthermore, we clinically compared the healing rate of ORC/collagen matrix–treated wounds with control wounds.

Patients and methods

Patients

Patients were randomly assigned to the treatment or control group. Inclusion criteria were more than 18 years of age, chronic wounds present for at least 6 weeks, but <12 weeks prior to study enrolment, wound size more than 1 cm^2 and debridement of wound before enrolment. Exclusion criteria were systemic inflammatory diseases, presence of malignant tumours, chemotherapy and alcohol and/or drug intoxication. In average, the Campbell score of the pressure ulcers was 3–4. All study procedures were carried out according to the ethical guidelines of the Declaration of Helsinki and were reviewed and approved by the local ethics committee. Individual informed consent was obtained from each patient.

Ten patients were randomly assigned to the control group. The wounds in this group were treated with an absorbing hydropolymer dressing (Tielle®, Systagenix) alone. The ORC/collagen matrix group contained 23 patients, whose wounds were treated with the ORC/collagen and Tielle® on top. In both groups, dressing changes were carried out every 2–3 days. Wound fluids were collected from all 33 patients with pressure sores on admission, on days 5 and 14 and then every 2 weeks until week 12. The majority of patients enrolled were elderly without significant differences in the age between both groups (average 63 ± 8 years).

Chronic wound fluid collection and elution

Wound fluid was collected by absorption onto a piece of RELEASE® (Johnson & Johnson Medical Ltd., Ascot, UK) dressing, which was placed directly on the pressure ulcer. The RELEASE® dressing was cut to the size of the wound, placed in contact with the ulcer bed for 6 hours and covered with BIOCLUSIVE® (Johnson & Johnson Medical Ltd.). The dressing was then removed and frozen at −80°C until elution of the wound fluid. Wound fluids from all the 33 patients with pressure sores were collected on days 0, 5 and 14 and subsequently every 14 days thereafter for 12 weeks.

Protein

Total protein was determined according to the method of Bradford using a bovine serum albumin standard curve as control (22).

Protease activity assay

Elastase assay

The levels of elastase activity present in the wound fluid samples were measured spectroflourimetrically using substrate activity assays (Enzchek Elastase assay, Molecular Probes, Leiden, The Netherlands). The substrate comprises short peptides synthesised to mimic the appropriate enzyme cleavage site and contains a fluorescent group, which is released upon hydrolysis. Activity was expressed as either units per minute or corrected for total protein. Each sample was tested three times, and the average value was calculated.

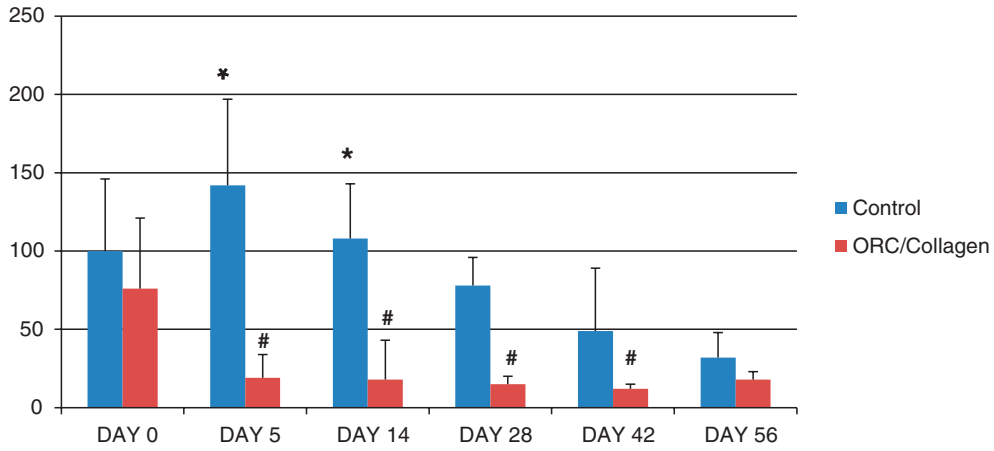

Figure 1 Elastase activity from day 0 to day 56 (*y*-axis: μU/ml/mg protein). The elastase activity was significantly reduced in the oxidised regenerated cellulose (ORC)/collagen group on day 5 and day 14 compared with the control group (*). Furthermore, elastase activity was reduced on days 5, 14, 28 and 42 compared with the baseline value at day 0 (#), ($P < 0{\cdot}05$, $n = 33$).

The resulting increase in fluorescence was monitored with a fluorescence microplate reader. A selective inhibitor of elastase, *N*-methoxysuccinyl-Ala-Ala-Pro-Val-chloromethylletone, was used for confirming the identity of the protease responsible for substrate digestion.

Plasmin activity

Plasmin activity was measured by incubating samples in 100 mM Tris buffer, pH 8·0, 0·13 M dithiobis, 20 mM Z-Lys-S-Benzyl (Enzyme Systems Products, Livermore, CA). Stock solutions of Z-Lys-SBzl were prepared in distilled water. The rate of hydrolysis of Z-Lys-SBzl was measured spectrophotometrically by allowing the released benzyl mercaptan to react directly with dithiobis. The increase in absorbance at 412 nm due to formation of the carboxy-nitrophenoxide was measured three times, the average value was calculated, and the rate of change in absorbance was determined.

Ulcer assessment

All ulcers were photographed on admission and at each wound collection time point to provide visual record of any changes in appearance of the ulcer and healing rate. The reduction in surface area of all ulcers was measured by planimetry after 8 weeks (Pharma Med Concept, Düren, Germany).

Statistical analysis

Statistical analysis was performed using ANOVA and Bonferroni's post hoc test. Results were considered significant at a *P*-value below 0·05.

Results

Elastase activity

The wound fluid of patients treated by ORC/collagen matrix showed a significant reduction in elastase activity at day 5 and at all later time points compared with initial elastase activity measured at day 0. Furthermore, elastase activity was significantly decreased in patients treated with ORC/collagen matrix compared with the control group at days 5 and 14 (Figure 1).

Plasmin activity

Plasmin activity was significantly reduced at days 5 and 14 in comparison with control wounds (*). In addition, plasmin was significantly reduced on days 5, 14, 28 and 42 in patients treated with ORC/collagen matrix in comparison with the baseline parameters of plasmin activity in the treatment group (#). At days 42 and 56, the plasmin activity in the control and treatment groups was almost equal, yet still reduced in comparison with baseline values (Figure 2).

Healing rate

After 12 weeks, wounds treated with ORC/collagen matrix showed a significant reduction in wound surface area by 65% versus 41% in the wounds of control group patients (Figure 3).

Discussion

In this study, we have measured the effect of an ORC/collagen matrix treatment on protease activity in wound fluid derived from pressure sores and compared this effect with a control treatment. We have shown that wound fluid from pressure sores contains high levels of elastase and plasmin activity, which can be reduced by treatment with ORC/collagen matrix. Furthermore, we have shown that treatment with ORC/collagen matrix leads to faster healing of pressure sores.

High protease activity may result due to one or more of the following: an increase in the expression of the protease, an increase in the extracellular activation of latent proteases or a reduction in the level of endogenous protease inhibitors. If extracellular proteolysis is excessive or poorly regulated,

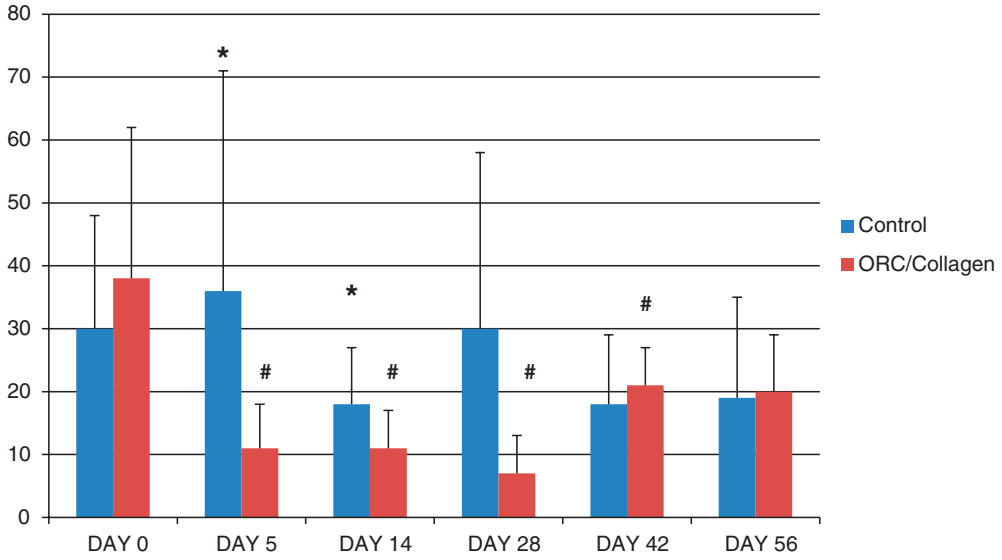

Figure 2 Activity of plasmin at different time points (*y*-axis: μU/ml/mg protein). Plasmin activity was significantly reduced on days 5 and 14 versus control wounds. In addition, the plasmin activity was significantly reduced on days 5, 14, 28 and 42 in comparison with the baseline parameters of plasmin activity in the treatment group ($P < 0{\cdot}05$, $n = 33$).

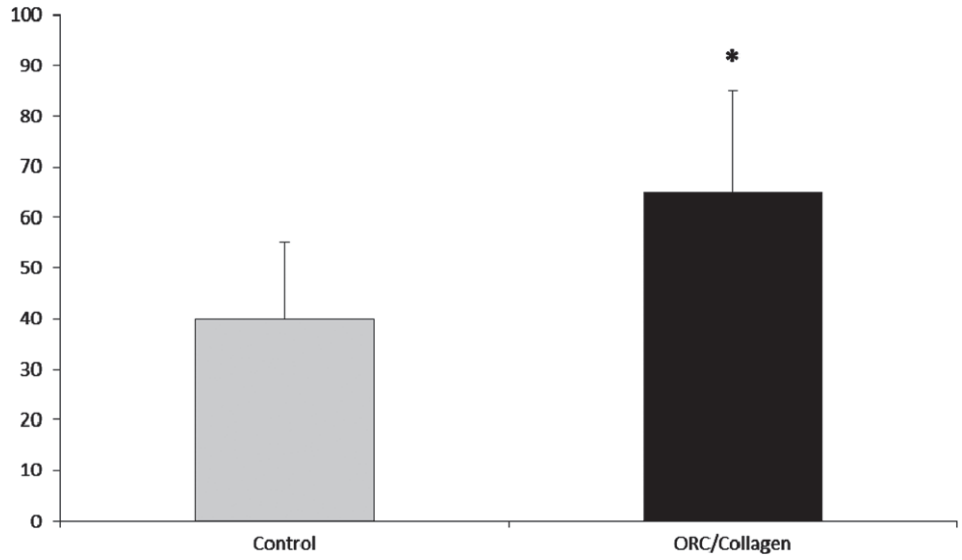

Figure 3 The wound surface area was significantly reduced ($P < 0{\cdot}05$) in the oxidised regenerated cellulose (ORC)/collagen group after 8 weeks (*y*-axis: percentage wound surface reduction) when compared with the control wounds (65% ± 13 versus 41% ± 11, $n = 33$).

it can lead to generalised tissue destruction, a characteristic of many pathological inflammatory conditions including non-healing chronic wounds (23). This hypothesis is supported by several studies in which human chronic wound tissue and fluids have been analysed (24,25). The combined use of collagen and oxidised regenerated cellulose has been shown to specifically inhibit the action of these proteases without affecting the activity of endogenous growth factor (14). Our study provides additional support to this mechanism as we found a significantly decreased activity of elastase and plasmin after 5 days of treatment with ORC/collagen matrix.

According to recent studies, high elastase activity may lead to degradation of fibronectin (26) and growth factors (6) in chronic wounds. This implies that these wounds are still in the inflammatory phase of wound repair even though all wounds had been present for at least 30 days.

Plasmin is the major fibrinolytic enzyme. It is possible that reduced plasmin activity in chronic wounds could contribute to fibrosis, which often occurs in leg ulcers (2,27). Previous studies suggested that plasmin activity is increased in chronic wound fluid (28). The relationship between elevated levels of plasmin and reduced levels of VEGF was shown by Lauer *et al.* (11). The results indicated that chronic wound

fluid contains enzymatic activity, in particular serine proteases, which might affect VEGF stability (11). VEGF plays an important role in wound healing, particularly during angiogenesis (11). In our patient group treated with ORC/collagen matrix, we found an accelerated healing of the pressure sores. This may be supported by reduced plasmin activity and increased VEGF levels.

The mechanisms responsible for the increased generation of plasmin in the chronic wound environment remain unclear. Previous studies have demonstrated that epidermal keratinocytes can regulate plasmin activation through the expression of mediators critical for plasmin activation, including the urokinase-type plasminogen activator and its receptor (29).

In previous studies, ORC/collagen reduced the activity levels of elastase and plasmin. However, the results of these studies are limited as they did not investigate these effects in vivo. In our study, we used a Z-Lys-SBzl assay, which is very sensitive for plasmin studies (30).

Previous clinical trials with ORC/collagen matrix have shown accelerated healing in venous leg ulcers and diabetic foot ulcers (14–21,31). In our present study, we observed a similar clinical effect in pressure sores.

Other advantages found when using ORC/collagen matrix include its ease of application. As the product is bio-absorbable, there was no need to remove the product at dressing changes. Furthermore, there was no infection observed in the ORC/collagen matrix group.

In summary, we have shown that ORC/collagen matrix treatment of pressure sores significantly reduces elastase and plasmin activity in wound exudates, thereby rebalancing the wound microenvironment and significantly improving the rate of healing.

Acknowledgements

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