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Biological wound matrices with native dermis-like collagen efficiently modulate protease activity

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Biological wound matrices with native dermis-like collagen efficiently modulate protease activity

Objective: When the delicate balance between catabolic and anabolic processes is disturbed for any reason, the healing process can stall, resulting in chronic wounds. In chronic wound pathophysiology, proteolytic imbalance is implicated due to elevated protease levels mediating tissue damage. Hence, it is important to design appropriate wound treatments able to control and modulate protease activity directly at the host/biomaterial interface. Here, we investigate collagen-based wound dressings with the focus on their potential to adsorb and inactivate tissue proteases.

Method: We examined the effect of six collagen-based dressings on their ability to adsorb and inactivate different granulocyte proteases, plasmin, human neutrophil elastase (HLE), and matrix metalloproteases (MMP)-1, -2, -8, and -9, by an integrated approach including immunoelectron microscopy.

Results: We observed a reduction of the proteolytic activities of plasmin, HLE, and MMP-1, -2, -8, and -9, both on the biomaterial surface and in human chronic wound fluid. The most pronounced effect was observed in collagen-based dressings, with the highest content of native collagen networks resembling dermis structures.

Conclusion: Our data suggest that this treatment strategy might be beneficial for the chronic wound environment, with the potential to promote improved wound healing.

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chronic wound • biomaterials • matrix metalloproteases • native collagen networks • protease activity • serine proteases • wound dressing

The biological response in tissues and organs is a well-coordinated, dynamic and interactive series of events. It is characterised by a balanced degradation and regeneration of cells, and the extracellular matrix (ECM) surrounding them. The molecular mechanisms underlying these processes have become an intensive area of research in the last decades. Changes of cells and their microenvironment influence the structure of the ECM in a complex bidirectional process. This mechanism is also referred to as 'dynamic reciprocity'[1] and plays an important role in wound healing.

Tissue regeneration after injury is an intricate process where devitalised cellular and tissue structures are replaced.[2] It comprises extensive changes in cellular responses as well as in ECM composition. In general, wound repair is divided into different, well-defined, predictable phases: haemostasis, inflammation, granulation and epithelialisation, followed by maturation and remodulation of the scar tissue.[3–5] The intimate relationship between cells and their surrounding framework is commonly thought to play a pivotal role in regulating regenerative processes.

Following the haemostasis stage, granulocytes and macrophages infiltrate the wound and initiate the inflammatory phase. During this stage, the wound is cleared from damaged and tissue debris. It involves ECM degradation in the wound area, predominately by tissue proteases and matrix metalloproteases (MMPs).[6–8] The MMPs are expressed in an inactive zymogen form as proenzymes which require extracellular activation by cleavage by other proteases such as elastase and plasmin. Several MMPs degrade collagen networks successively into shorter peptide fragments. In the normal wound healing process, this is followed by the proliferation stage, after about three days.[9]

If the wound healing process is disturbed for any reason, proteolytic imbalance can lead to elevated levels of proteases in the wound, resulting in increased tissue degradation. This hypercatabolic state in the wound bed is not beneficial for further healing and may contribute to the formation of a chronic wound.[9,10] To address this issue several wound dressings are designed to control the catabolic proteinase reactions and thus favour the regeneration of new tissue.[11–13] In particular, biological dressings based on native collagen networks are used to improve the wound microenvironment. The rationale behind the use of these collagen-based dressings is that proteases will be absorbed to the host/dressing interface leading to a

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Fig 1. Ultrastructure of different collagen-based dressings compared with native human dermis. Ultrathin sections of human skin biopsies (**a**,**b**) were prepared for transmission electron microscopy (TEM) and compared with MDS pure Collagen (**c**), MDS collagen alginate (**d**), Biopad (**e**), Endoform (**f**), Suprasorb C (**g**), and Promogran (**h**). On panel **a** skin fibroblasts (f) are embedded in collagen (c) and elastin (e) fibrillar networks. Similar collagen networks are shown at higher magnification in (**b**–**d**). On panels **e** and **f** fibrillar structures are less abundant (arrowheads) and absent in **g** and **h**, which exhibit an amorphous appearance. The scale bars=5µm (**a**) and 2µm (**b**–**h**)

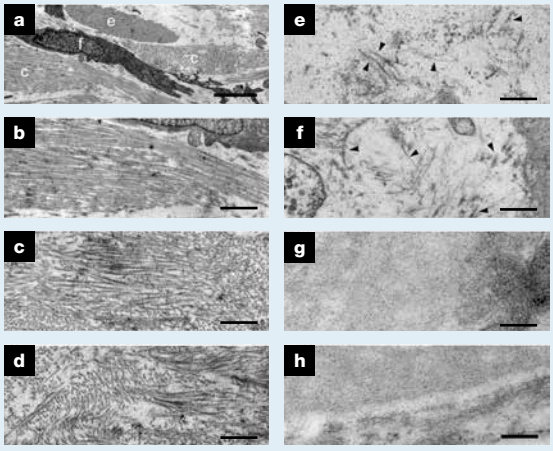
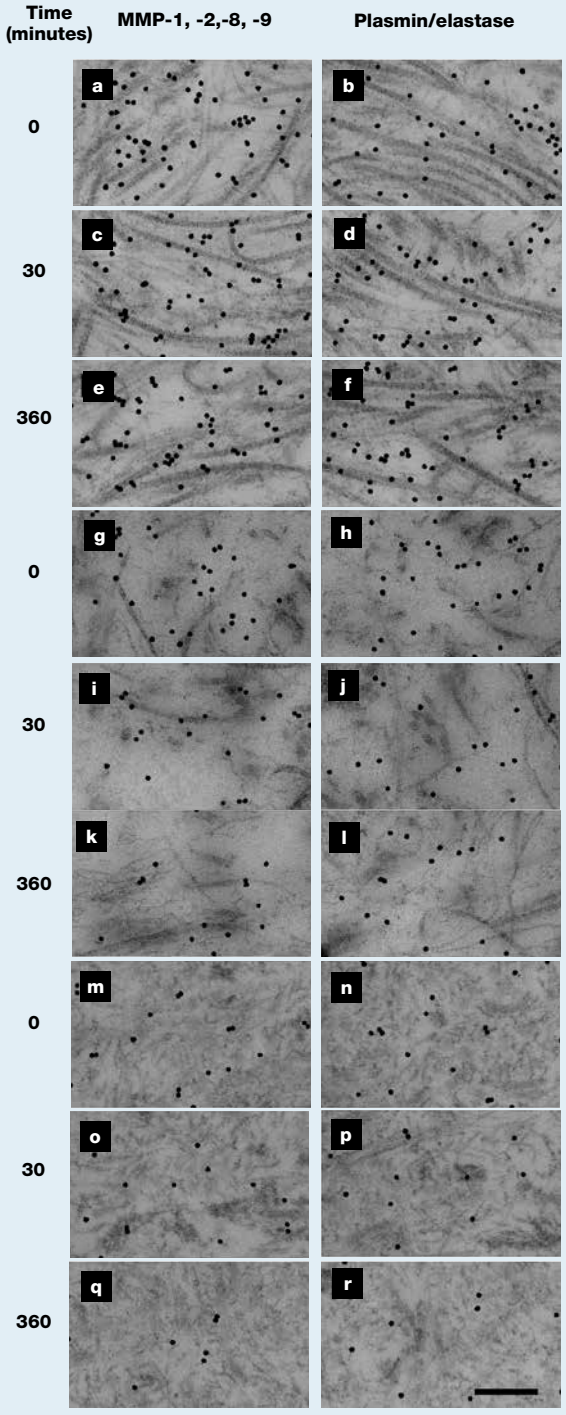


Fig 2. Binding kinetics of different proteases to the collegen-based dressings visualised by immunoelectron microscopy. MDS pure Collagen (**a**–**f**), Endoform (**g**–**l**) and Suprasorb C (**m**–**r**) were incubated with a mixture of MMP-1, -2, -8, and -9 or of human neutrophil elastase and plasmin. Incubation times were 0, 30 and 360 minutes. Bound proteases were localised with antibodies conjugated with 10nm gold on ultrathin sections. Similar observations to a–f were made with MDS Collagen Alginate, g–l with Biopad, and m–r with Promogran. The scale bar=100nm



reduction of their activity in the wound. In addition, collagen components are thought to be used as a sacrificial substrate for the proteinases resulting in less damage in the wound bed.

In order to determine the potential of a wound dressing to modulate elevated proteinase levels, attempts to measure the concentration and activity of a particular protease in a representative wound environment have been examined in different ways.[14–17] A common approach is to measure protinease levels in wound fluid after exposure to different wound dressings or wound dressing extracts *in vitro*.[14–17]

However, to our knowledge, no attempts to determine tissue proteinase levels and activities directly on a wound dressing surface have been reported. Furthermore, investigations of biological wound dressings, consisting of intact collagen fibres, which are structurally identical to native dermis structures, are not found in the literature. These parameters are of particular interest as they may more accurately reflect the *in vivo* situation in the wound bed at the host/dressing interface, complementing the body of knowledge obtained by assessment of secreted wound exudate.[14–17]

In the present study, we explore whether native collagen networks can control tissue proteases on the surface of different, commercially available, wound dressings. Employing an integrated approach, combining established methods with immunoelectron microscopy.

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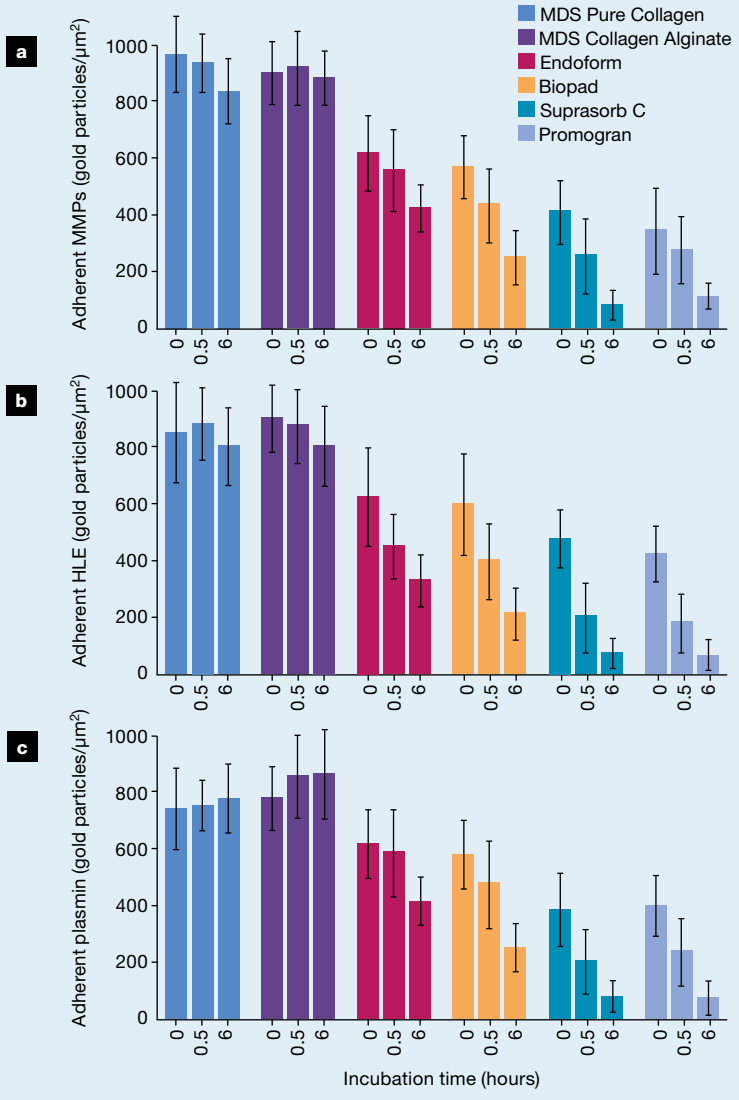
Fig 3. Quantitative evaluation of protease binding kinetics to collagen-based dressings, assessed by transmission immunoelectron microscopy. Dressing samples were incubated with proteases and visualised as shown in Fig. 2. In each case, the location of about 500 gold particles in 50 different sample profiles were evaluated. Mean± standard deviation of three different experiments



MMP—matrix metalloproteinases; HLE—human neutrophil elastase

Materials and methods

We tested six commercially available collagen-based wound dressings Biopad (BP, Euroresearch s.r.l.), Endoform (EF, Hollister Wound Care), MDS pure Collagen (MDSC) and MDS Collagen Alginate (MDSCA, MedSkin Solutions Dr. Suwelack), Promogran (PG, Systagenix Wound Management Limited), and Suprasorb C (SC, Lohmann & Rauscher International GmbH & Co KG). Skin biopsies from control subjects, served as controls for transmission electron microscopy (TEM). Wound fluid was collected from patients with chronic leg ulcers with a duration of more than three months. The project was approved by the Ethics Committee, Lund University Hospital, and in accordance with the Declaration of Helsinki Principles.[18] Informed consent was obtained from all participants, according to protocols approved by the Ethics Committee at Lund University.

Before use, MMP-2 and MMP-9 were activated with 4-aminophenylmercuric acetate (APMA, from Sigma Aldrich Chemical Company).

Transmission electron microscopy

Samples of the collagen-based dressings were punched out to 5mm diameter discs and incubated in PBS (15 minutes, 4ºC) for rehydration. They were then fixed with 2.5% glutaraldehyde in 0.1M sodium cacodylate, pH7.4 (cacodylate buffer), washed with cacodylate buffer and dehydrated with an ascending ethanol series as previously described.[19] Specimens were then embedded in Epon 812 and cut into ultrathin sections on a Reichert Ultracut S ultramicrotome (Leica Microsystems, Germany). They were examined on a Philips/FEI CM100 BioTWIN transmission electron microscope. Images were recorded with a side-mounted Olympus Veleta camera and the ITEM acquisitions software.

Radiolabelling of MMPs, HLE and plasmin

Labelling with radioactive iodine (^{125}I) was performed using IODO-BEADS (Pierce, IL, US) according to the manufacturer's instructions. Unbound iodine was subsequently removed by a PD-10 column (Amersham Biosciences, Sweden) in phosphate buffered saline (PBS) buffer containing 0.05% Tween 20 and 0.005% sodium azide. Fractions (500µl) were collected and radioactivity was measured using a automatic gamma counter (Wallac Wizard 1470). The fractions containing the radiolabelled proteins were stored at 4ºC until further usage.

Proteinase binding immunoelectron microscopy assay

The ability to bind inflammatory proteases is a prerequisite for biological wound dressings in order to quench the proteolytic burden in the wound, and, by this means, reduce tissue destruction and stimulate wound healing. Thus, we wished to determine whether the collagen-based dressings examined in this study exhibited similar properties. To this end, samples of MDSC and MDSCA were tested in immunoelectron microscopy assays for surface adsorption properties and kinetics of MMP-1, -2, -8, -9, as well as (human neutrophil elastase) HLE and plasmin. They were compared with BP, EF, SC and PG. Specimens were incubated with proteases for 0, 0.5 and 360 minutes, immersed in fixative and embedded in Epon resin.

Binding of HLE (Sigma Aldrich Chemical Company), plasmin (Sigma Aldrich Chemical Company) and MMPs (Enzo Life Sciences, US) to the biomaterial surface was visualised by immunoelectron microscopy, as modified for a previously described method.[20] Collagen-based dressing samples were punched out to

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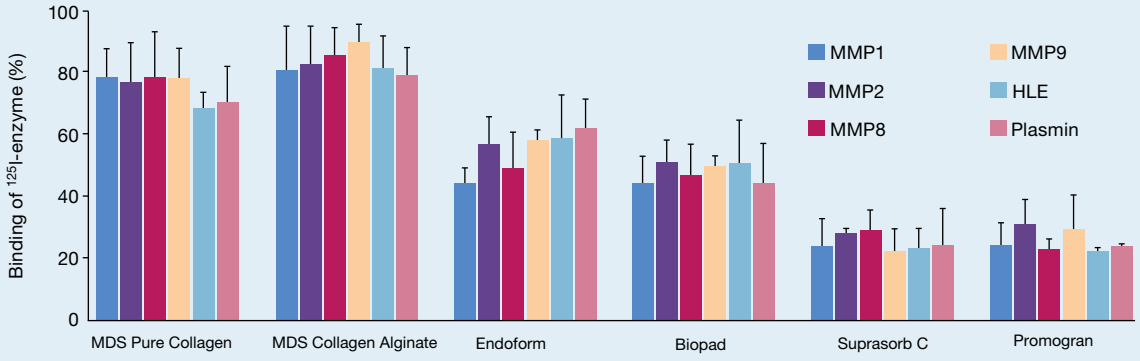
Fig 4. Quantitative evaluation of ^{125}I protease binding to different collagen-based dressing. Dressing samples were incubated with ^{125}I-labelled proteases for six hours and radioactivity was determined in a gamma counter. Means±standard deviation from three different experiments



MMP—matrix metalloproteinases; HLE—human neutrophil elastase

5mm diameter discs and rehydrated in 500µl PBS for 15 minutes at 4ºC. They were blotted to remove excess PBS and added to 70µl of proteinase (HLE, plasmin or MMPs) solution (0.5µg/ml in 50mM Hepes, 10mM $CaCl_2$, 0.05% Tween 20, pH7.5). The biologic dressing samples were incubated for 0, 30, and 360 minutes at 37ºC with shaking. Samples were washed in PBS and embedded in Epon 812 before transmission electron microscopy (TEM). Bound proteases were visualised by immunodetection with specific antibodies (Abcam PLC, UK).

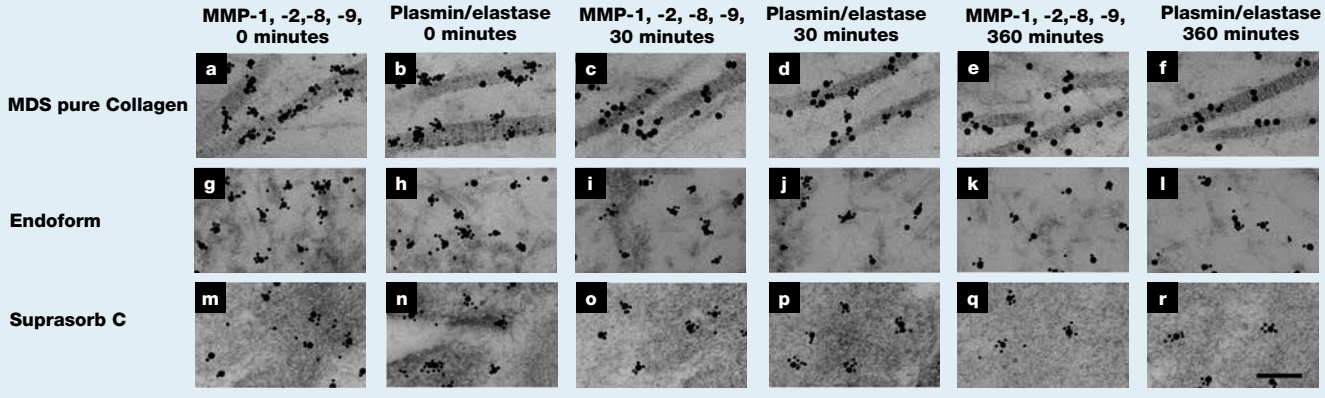
In vitro proteinase binding assay

In a parallel series of experiments, 5mm diameter discs of collagen-based dressing samples were pretreated as described previously, and incubated with approximately 100,000 counts per minute (cpm) in 70µl ^{125}I-labelled proteinase (HLE, plasmin or MMPs) solutions for 0, 30 and 360 minutes at 37ºC with shaking. Unbound proteinases were removed by washing in PBS. The membrane discs were then measured for radioactivity. Percent of binding was calculated with KaleidaGraph (Synergy Software) and values were illustrated in a line plot. Experiments were performed in quadruples at any given independent time point.

Immunoelectron microscopy measurement of proteinase activity

Direct measurements of the enzymatic activity of HLE, plasmin and MMPs at the biologic dressing surface was visualised by immunoelectron microscopy as previously described.[21,22] Punched out 5mm discs of biologic dressings were incubated with solutions of HLE, plasmin and MMP-1, -2, -8, and -9 at different time points. Specimens were then embedded in Epon 812 and sectioned. For immunostaining, gold-labelled specific substrate for the respective protease was added to the sections. Active enzymes were colocalised with their respective substrates, whereas inactivate enzymes did not bind their substrates, and therefore no

Fig 5. Proteinase inactivation kinetics on different biomaterials as visualised by immunoelectron microscopy. MDS Pure Collagen (**a–f**), Endoform (**g–l**) and Suprasorb C (**m–r**) were incubated with a mixture of matrix metalloproteinases (MMP)-1, -2, -8, and -9, or a mixture of elastase and plasmin. Protease activity was then detected by adding gold-labelled enzyme-specific substrates. Incubation times were 0, 30 and 360 minutes. Proteases were identified with antibodies conjugated with 10nm gold and substrates were labelled with 5nm gold on ultrathin sections. Similar observations to a–f were made with MDS Collagen Alginate, g–l with Biopad, and m–r with Promogran. The scale bar=100nm



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colocalisation was observed.[21,22] Specimens were visualised by immunoelectron microscopy (5nm gold, substrate) and colocalisation with gold-conjugated antibodies (10nm gold, proteases).

In vitro proteinase activity measurement

The enzymatic activities of MMPs, HLE and plasmin present on the surface of the collagen-based dressings was determined spectrofluorimetrically using substrate–activity assays. Collagen-based dressing discs were incubated in parallel with proteinases in one hour-intervals between 0 hours and six hours. At each time point, the discs were washed in PBS and then solubilised with a Polytron high speed homogeniser designed for small volumes (Kinematica, Switzerland). MMP activity was determined in MMP assay buffer (50mM HEPES, 10mM $CaCl_2$, 0.05% TWEEN20, pH7.5) by addition of the fluorogenic substrate Mca-Pro-Leu-Gly-Leu-Dpn-Ala-Arg-NH2 (MP Biomedicals, Inc, US). The reaction mixture contained 5µl solubilised biomaterial, 175µl assay buffer and 20µl substrate (final concentration: 50µm) in a microtiter well. Similarly, HLE and plasmin activities were determined using the fluorometric substrates Methoxy-Ala-Ala-Pro-Val-7-amino4-methylcoumarin (elastase substrate, MP Biomedicals, Inc) solubilised in methanol, or Methoxysuccinyl-Ala-Lys-Phe-Lys-7-amino 4-methylcoumarin (plasmin substrate, MP Biomedicals, Inc) solubilised in 1 mM HCl. The assay buffers were: 0.1M HEPES, pH7.5, containing 0.5M NaCl and 10% DMSO (HLE) and 25mM Tris/HCl, pH8.1, containing 0.5% Triton X-100 (plasmin). Production of the fluorophore was quantified every minute for a 10-minute period at 37°C, using 328nm excitation and 420nm emission wavelengths. Per cent activity was determined from the observed rate (RFU/minute) relative to the rate of an untreated sample.

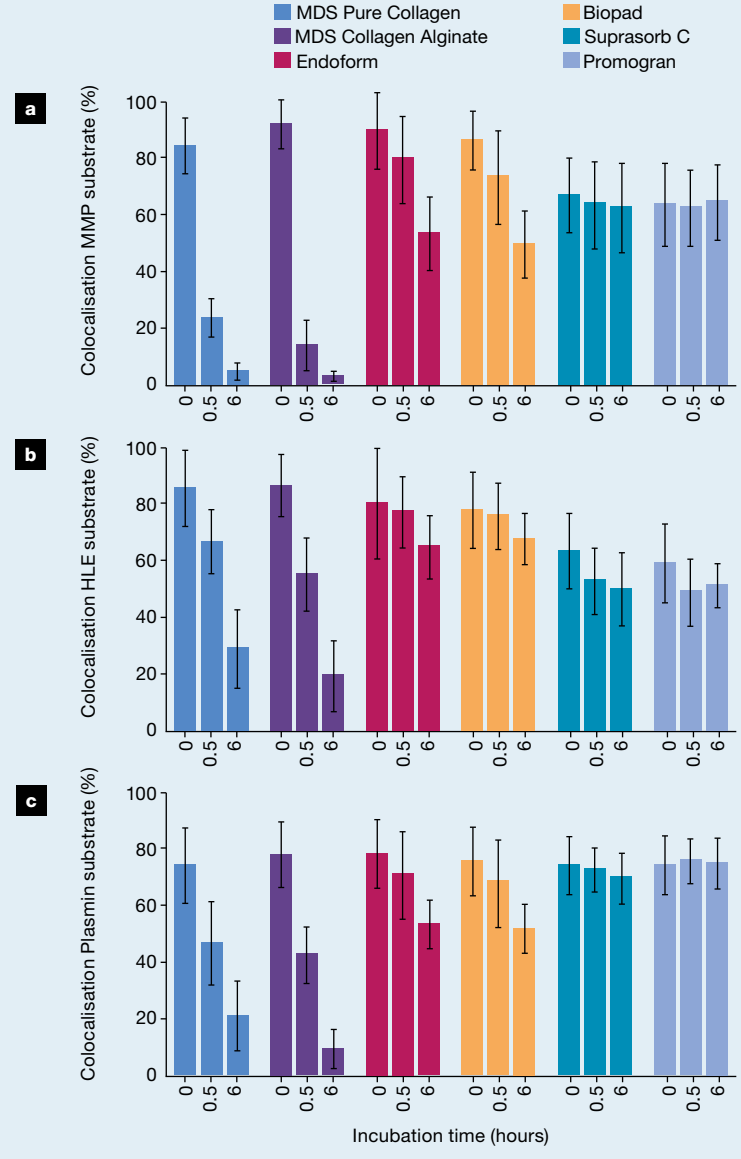

Fig 6. Quantitative evaluation of protease binding kinetics to collagen-based dressings, assessed by transmission immunoelectron microscopy. Dressing samples were incubated with proteases and identified on ultrathin sections. Protease activity was assayed by adding gold-labelled specific substrates as shown in Fig 6. In each case the location of about 500 gold particles in 50 different sample profiles were evaluated. Mean±standard deviation of three different experiments

MMP—matrix metalloprotineases; HLE—human neutrophil elastase

Immunoelectron microscopy

The binding and activity of the different proteases on thin sections of the collagen-based dressings was visualised by transmission immunoelectron microscopy as described previously.[19,20] Briefly, sections mounted on nickel grids were subjected to antigen retrieval with sodium metaperiodate, washed and blocked with 50mM glycine. They were then incubated for 15 minutes in 5% goat serum in 0.2% BSA-c in PBS (pH7.6), and incubated overnight at 4°C with polyclonal antibodies against the respective protease (50–500mg/ml). Next, grids were washed, incubated (two hours, 4°C) with 10µg/ml various species specific gold-labeled IgG conjugates (BBI Solutions, UK), washed, and postfixed in 2% glutaraldehyde. Finally, sections were washed with water and poststained with 2% uranyl acetate and lead citrate. In some experiments, specimens were examined by immunolabeling and TEM. Samples were adsorbed to 400-mesh carbon-coated copper grids and stained with 0.75% (w/v) uranyl formate as recently described in detail.[23] Specimens were examined in a Philips/FEI CM 100 TWIN transmission electron microscope.

Assessment of protease activity in chronic wound exudate

A pooled wound fluid samples,obtained from 12 human chronic foot ulcers, was analysed for levels of MMP-1, -2, -8, and -9 and HLE/plasmin activity upon exposure to the collagen-based dressings. Residual protease levels and activity over time were directly monitored by electron microscopy at t=0, after 30 and

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360 minutes incubation with MDSC and MDSCA, BP, EF, SC and PG at 37ºC.

Statistical analysis

Results were expressed as means ± standard deviation on the figures.

Results

Ultrastructural features of different collagen-based wound dressings

Prompted by our recent findings that MedSkin Solutions collagen products consist of native collagen networks ,which are similar in structural appearance to native dermis collagen, we investigated these properties in further detail. Specimens were examined by TEM. Human skin biopsies and samples of the different collgen-based dressings were subjected to ultrathin sectioning (Fig 1).

Analysis of MDSC (Fig 1c) and MDSCA (Fig 1d) showed similar ultrastructural features to native collagen fibre bundles in dermis (Fig 1a–b). In contrast to skin biopsies (Fig 1a–b), the cellular structures and elastin fibre bundles were not present. Instead, they consisted of tightly packed collagen patches with many regions of different fibre orientations. In BP (Fig 1e) and EF (Fig 1f) structures of varying diameters, resembling collagen fibres of varying thickness, were observed embedded in a more amorphous matrix. SC and PG appeared amorphous throughout the whole specimens with no discernible fibrillar structural features (Fig 1g–h).

Collagen-based dressings adsorb and retain tissue proteases at the host/biomaterial interface

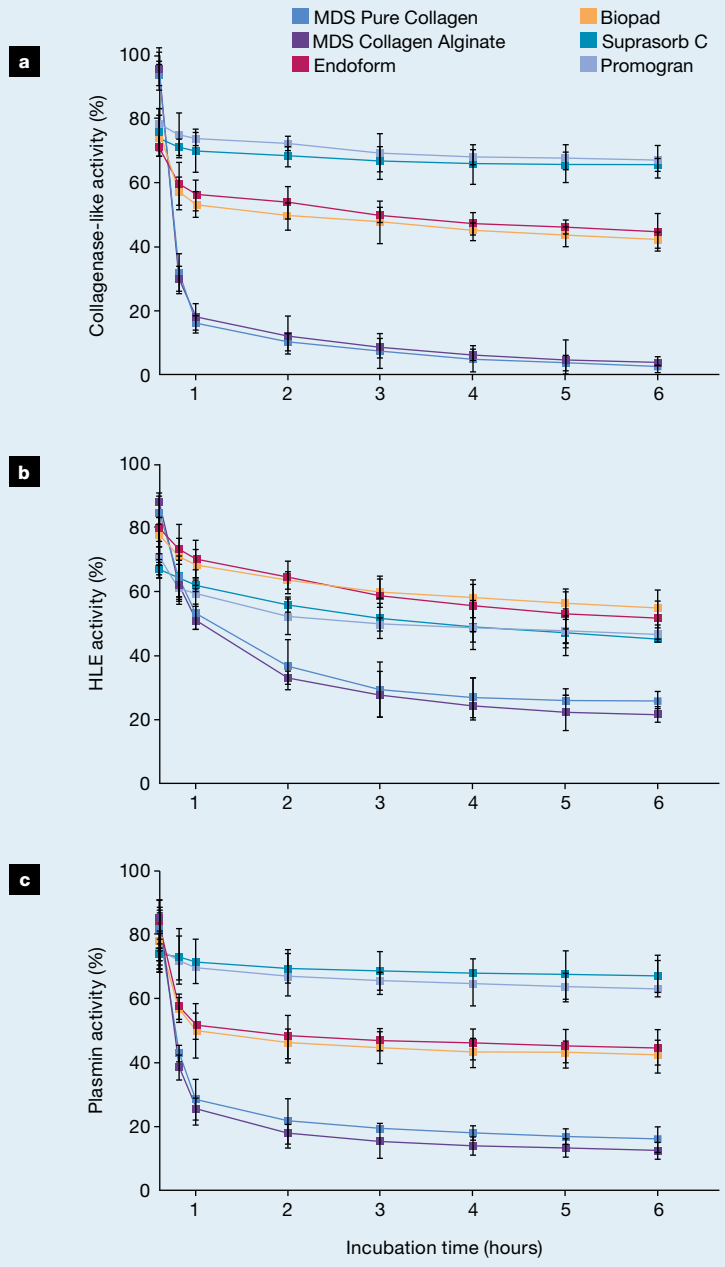
As a next step, we set out to correlate the ability of these biomaterials to adsorb inflammatory proteases with the potential to modulate protease activity on the host/ biologic dressing interface. Proteases adhered to the different dressing surfaces at varying amounts and with different kinetics. Interestingly, the affinity of MDSC and MDSCA surfaces for MMPs and HLE/plasmin was comparatively high and did not decline much over time (Fig 2a–f, Fig 3). Protease binding to BP and EF appeared lower. After 360 minutes incubation, about half of the original immunogold signal was still present (Fig 2g–l, Fig 3). The comparatively lowest protease adherence and steepest decline of anti-protease immunogold signal was observed for SC and PG (Fig 2m–r, Fig 3). The kinetics of protease adherence to the different collagen-based biomaterials was also assessed by determining levels of radiolabelled proteases in a given specimen. The results are in general accordance with the observations obtained by immunoelectron microscopy (Fig 4).

Collagen-based dressings inactivate tissue proteases at the host/biomaterial interface

To correlate the ability of the collagen-based dressing material to adsorb inflammatory proteases with the potential to modulate protease activity on the host/ dressing interface, bound proteases were then visualised by immunolocalisation with antibody-gold conjugates and colocalised with their gold-labelled substrates by TEM (Fig 5–6).

Notably, the ability of MDSC and MDSCA surfaces to inactivate MMPs and HLE/plasmin was high when compared with the other collagen-based dressings. On the MDSC and MDSCA surfaces, in contrast to the

Fig 7. *In vitro* protease inactivation kinetics on different collagen-based dressings. Residual protease activity of MMPs (**a**), HLE (**b**) and plasmin (**c**) after 1–6 hours exposure to different dressing samples with varying amounts of native collagen fibrils. Mean±standard deviation of three different experiments



MMP—matrix metalloprotineases; HLE—human neutrophil elastase

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other collagen-based dressings examined, the ability of all proteases to bind substrate molecules declined rapidly over time, indicating protease inactivation (Fig 5a–f, Fig 6). After 360 minutes incubation, less than 10% of the different MMPs, about 30% of HLE and some 20% of plasmin molecules, were colocalised with their substrates. Protease inactivation by BP and EF appeared to be lower, and about two thirds of the substrate-gold signal was still found colocalised with individual protease molecules after 360 minutes (Fig 5g–l, Fig 6). The comparatively lowest inactivation, and thus least decline in colocalisation of proteases and substrate-gold signal were observed for SC and PG (Fig 5m–r, Fig 6). In a parallel experimental setup, protease inhibition was assessed by a fluorometric assay.[14] The residual activities of all tested proteases on the different biomaterials were reduced in a time-dependent way similar to the observations made by immunoelectron microscopy (Fig 7).

Biological collagen-based dressings inactivate inflammatory proteases in chronic wound fluid

Wound fluid samples from chronic foot ulcers were analysed for levels of MMP-1, -2, -8, and -9 and HLE/plasmin activity upon exposure to the collagen-based dressings. A marked reduction in activity was observed within the first 30 minutes, an effect that was sustained throughout the subsequent 360 minutes (Fig 8). Reduction in MMP activity was observed to rapidly decline over time than HLE/plasmin activity in all samples (Fig 9). MDSC and MDSCA had the most pronounced effect on both depletion and inactivation of the different proteases (Fig 9).

Discussion

Here, for the first time we describe how biological dressings expose native collagen networks on their surface, and display a pronounced potential to inhibit tissue proteases directly on the host/biomaterial interface. Our results show that the different collagen-based dressings tested in this study possess the ability to adsorb, retain and inactivate different inflammatory proteases at the host/dressing interface, to a varying extent. The dressing biomaterial with higher amounts of intact collagen fibre networks appear to exhibit a more pronounced potential to bind and inactivate tissue proteases on their surface.

In normal wound repair, the healing process is a carefully orchestrated series of cellular and extracellular events. A defect in one or more of these mechanisms may result in an increase in proteolytic activity. When proteolysis is excessive, the balance is shifted towards an overall catabolic state in the wound. This may lead to general tissue degradation, which is regarded as a key factor in chronic wound pathophysiology.

It is commonly believed that a decrease in proteolytic activity in the chronic wound environment and subsequent reduction of tissue damage is concomitant with chronic wound healing.[24,25] This is supported by observations of investigators who have examined the biochemical and molecular profiles of the chronic wound bed in human.[26,27] A common observation is that most types of chronic wounds contain elevated levels of proteases, including cathepsin G, urokinase, neutrophil elastase, plasmin[28–32] and MMP-1, -2, -3, -8 and -9.[26,33–39]

It has been postulated that the overall catabolic state in chronic wounds precludes *de novo* tissue synthesis, which is not beneficial for the wound healing process. The proteolytic imbalance leads to degradation of ECM components[25] and damage of key regulators of tissue repair, such as peptide growth factors, epidermal

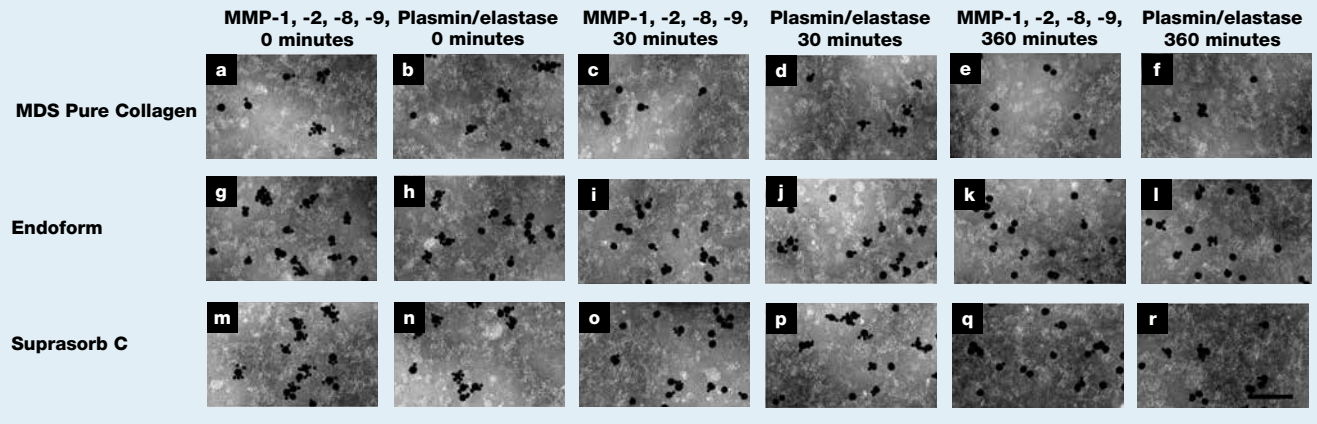

Fig 8. Proteinase inactivation kinetics in chronic wound exudate by different collagen-based dressings as visualised by immunoelectron microscopy. MDS pure Collagen (**a–f**), Endoform (**g-l**) and Suprasorb C (**m–r**). Wound exudates from the ulcers of patients with diabetes were incubated with dressing samples for 0, 30 and 360 minutes. Protease activity was then detected by adding gold-labelled enzyme-specific substrates (5nm gold particles). Proteases were identified with antibody mixtures against total MMP-1, -2, -8, and -9 or total elastase/plasmin, conjugated with 10nm gold. Specimens were examined by transmission electron microscopy. Similar observations to a–f were made with MDS Collagen Alginate, g–l with Biopad, and m–r with Promogran. The scale bar=100nm

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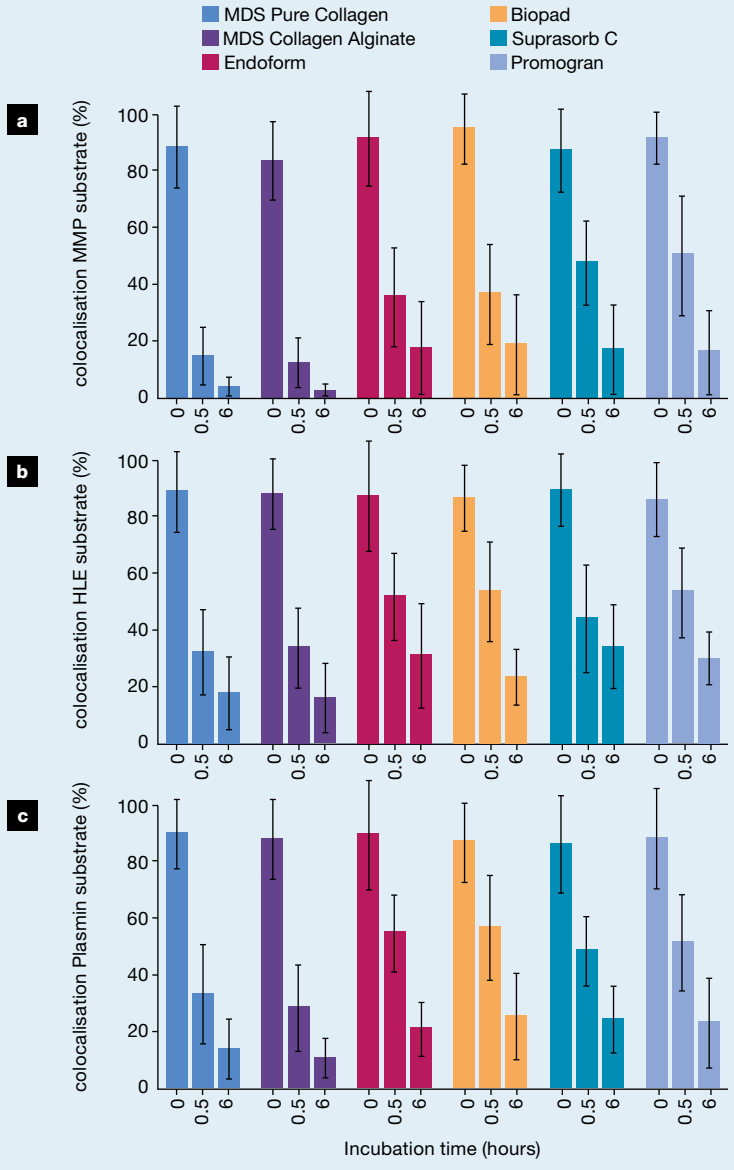
growth factor (EGF), platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF),[40–42] and endogenous protease inhibitors tissue inhibitor of metalloproteinase (TIMP)-1 and α-1-antitrypsin.[26,37,42–43] This may be an underlying factor for the limitations in the clinical use of growth factors in the treatment of chronic wounds.[44,45]

In this study, we set out to investigate how biological wound dressings can control the proteolytic imbalance in the wound bed. Biomaterials with the potential to bind and inactivate tissue proteases in chronic wounds would decrease tissue destruction and prevent growth factor degradation, leading to an overall increase in granulation tissue formation and faster wound repair. Here, we observed by immunoelectron microscopy that of all tested collagen-based dressings, only MDSC and MDSCA, consisted of intact collagen fibrillar networks resembling native dermis structures. Interestingly, these dressings exhibited the most pronounced ability to bind and inactivate proteases on their surface, both with purified enzymes and in chronic wound fluid from diabetic foot ulcers, analysed by immunoelectron microscopy, radiolabeling and a fluorometric protease activity assay.

The effect was more rapid and sustained compared with other biomaterials containing lesser amounts of native collagen networks. Adsorption of MMP-1, -2, -8, -9, neutrophil elastase and plasmin as well as reduction in activity was observed within the first 30 minutes and throughout the subsequent test period. This suggests that MDSC and MDSCA, when applied topically to a chronic wound, can adsorb and inactivate excessive proteases efficiently from the wound bed. By this means, these collagen-based dressings would provide a less hostile wound environment with reduced net tissue destruction and thus stimulate *de novo* tissue synthesis. Due to the particular high content of native collagen fibrils, MDS matrices are more effective products compared with the other collagen-based dressings, with respect to proteinase adsorption, immobilisation and inactivation in the wound bed demostrated by the results of this study. Hence, they might have a beneficial effect on the imbalance between tissue deposition stimulated by growth factors and tissue destruction mediated by tissue proteases. Consequently, such dressings might be first choice for clinicians for chronic wounds.

The exact molecular mechanisms through which the biomaterials modulate protease activity are, at present, beyond the scope of this work and remain to be explored in more detail. The native collagen fibres and/or perifibrillar glycosaminoglycans might act as alternate or competitive enzyme substrates. The perifibrillar component, which is negatively charged under physiological conditions, will bind positively charged molecules, such as metal ions essential for MMP activity. Inactivation of a given protease by conformational change of the active site upon binding to native collagen fibres might be an alternative explanation for the rapid decline in activity.

Fig 9. Quantitative evaluation of protease inactivation kinetics on different collagen-based dressings as visualised by transmission electron microscopy. Wound exudates from the ulcers of patients with diabetes were incubated with dressing samples. Protease activity was assayed by adding gold-labelled specific substrates as shown in Fig. 8. In each case the location of about 500 gold particles in 50 different sample profiles were evaluated. Mean±standard deviation of three different experiments



MMP—matrix metalloprotineases; HLE—human neutrophil elastase

Importantly, in a follow-up study, patient cohorts containing larger numbers of individuals will be carefully investigated by a similar integrated approach.

Limitations

Our study looked at the efficacy of native, collagen-based wound dressings as a new wound treatment in patients with diabetes and a DFU. The results were very positive; however, it should be remembered we only

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looked at:

- A limited number of different wound dressings
- A limited number of different proteinases
- Wound fluid collected from a limited number of Swedish patients.

In order to verify that these findings translate to a wider application, larger patient cohorts as well as a broader spectrum of wound dressings and relevant proteinases from the wound bed are to be included in a follow-up study

Conclusion

Taken together, the data presented in this study suggest that native dermis-like collagen fibrillar networks found in biological wound dressings like MDSC and MDSCA can efficiently reduce proteolytic activity by adsorption and inactivation on the host/biomaterial interface. This observation was possible by direct measurement of enzyme concentration and activity by immunoelectron microscopy and an *in vitro* fluorometric MMP activity assay. The results imply that wound dressings, consisting of native collagen networks, are particularly effective in modulating the proteolytic imbalance in the chronic wound bed and therefore may be beneficial for the treatment of such wounds. **JWC**

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