Original Research

Oxidized Regenerated Cellulose/ Collagen Dressings With Silver Rebalance Protease Activity in Venous Ulcer Fibroblasts: Translational Implications for Clinical Practice

Citation: Teixeira Nicolosi J, Cereijido Altran S, Scorzoni L, Oliveira Paggiaro A, Fernandes de Carvalho V, Levy D, Gemperli R, Isaac C. "Oxidized Regenerated Cellulose/ Collagen Dressings With Silver Rebalance Protease Activity in Venous Ulcer Fibroblasts: Translational Implications for Clinical Practice" (2026) *International Journal of Wound Research* 2(2): 141-152. DOI: https://doi.org/10.64115/ijwr-2026-341

Received: February 25, 2026

Revised: April 21, 2026

Just accepted: June 29, 2026

Published: June 29, 2026

Correspondence: Júlia Teixeira Nicolosi, Nursing Postgraduate Program, Guarulhos University, Guarulhos, São Paulo, Brazil. Email: juliatnicolosi@yahoo.com.br

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Conflict of Interest: The author(s) declare(s) no conflict of interest.

Funding: This study was supported by the Fundação de Amparo à Pesquisa Do Estado De São Paulo (FAPESP, process #2014/21443-0).

Júlia Teixeira Nicolosi[1], Silvana Cereijido Altran[2], Liliana Scorzoni[1], André Oliveira Paggiaro[3], Viviane Fernandes de Carvalho[1], Debora Levy[4], Rolf Gemperli[5], Cesar Isaac[5]

[1] Nursing Postgraduate Program, Guarulhos University, Guarulhos, São Paulo, Brazil
[2] Cell Culture and Wound Healing Research Laboratory (LIM 04), Division of Plastic Surgery, Hospital das Clínicas, University of São Paulo, São Paulo, Brazil
[3] Tissue Bank, Hospital das Clínicas, University of São Paulo, São Paulo, Brazil
[4] Laboratory of Immunology and Histocompatibility (LIM-19), Faculty of Medicine, University of São Paulo, São Paulo, Brazil
[5] Plastic Surgery Department, Faculty of Medicine, Hospital das Clínicas, University of São Paulo, São Paulo, Brazil

Abstract

Introduction. Chronic venous leg ulcers disproportionately affect older adults and are characterized by delayed healing, high recurrence rates, and substantial impacts on quality of life and healthcare costs. Persistent inflammation and an imbalance between matrix metalloproteinases and their tissue inhibitors contribute to healing failure, maintaining the wound in an inflammatory state. Oxidized regenerated cellulose/ collagen dressings, with and without silver, accelerate healing in venous ulcers, but the cellular mechanisms linking these outcomes to changes in the wound microenvironment are not fully understood. This study evaluated how oxidized regenerated cellulose/collagen and oxidized regenerated cellulose/collagen/silver modulate venous ulcer fibroblasts, focusing on proteases, their endogenous inhibitors and key growth factors, to connect *in vitro* findings to potential clinical applications.

Methods. Fibroblasts were isolated from chronic venous

leg ulcers and cultured for 72 hours in control medium, oxidized regenerated cellulose/collagen (2.8 mg/mL) or oxidized regenerated cellulose/collagen/silver (4.2 mg/mL). Cell metabolic activity was assessed by MTT assay. MMP 1, MMP 2, MMP 3, MMP 10, MMP 13, TIMP 1, TIMP 2, TIMP 3 and TGF β1, TGF β2, TGF β3 were quantified in conditioned media using bead based multiplex immunoassays. Vimentin, collagen type I and collagen type III expression were evaluated by immunofluorescence. Statistical analysis used the paired Wilcoxon signed rank test ($p< 0.05$).

Results: Both dressings maintained venous leg ulcer fibroblast metabolic activity at levels comparable to, or slightly higher than, control medium, without evidence of overt cytotoxicity. Oxidized regenerated cellulose/collagen alone did not significantly alter MMPs, TIMPs or TGF β isoforms. Oxidized regenerated cellulose/collagen/silver significantly increased MMP 1 ($p = 0.0002$) and reduced MMP 13 ($p = 0.0155$) versus control, while decreasing TIMP 2 and TIMP 3 and preserving TIMP 1. TGF β1, TGF β2, TGF β3 and collagen type I and III expression remained unchanged across groups, and vimentin expression was preserved.

Discussion. In this *in vitro* model, oxidized regenerated cellulose/collagen and oxidized regenerated cellulose/collagen/silver preserved fibroblast viability and did not overtly interfere with TGF β signaling or collagen type I and III expression. The silver free matrix did not modify the analyzed protease inhibitor levels, supporting a predominantly extracellular protease sequestering mechanism. The silver containing matrix induced a shift in the fibroblast derived protease inhibitor profile, characterized by increased MMP 1, decreased MMP 13 and reduced TIMP 2 and TIMP 3 with preserved TIMP 1, a pattern that may rebalance protease activity toward matrix remodeling under non cytotoxic conditions. These findings offer a mechanistic, hypothesis generating framework with translational implications for the clinical use of silver-containing oxidized regenerated cellulose/collagen dressings in venous leg ulcers.

Keywords: Oxidized Cellulose, Silver, Venous Ulcer, Wound Healing, Metalloproteinases

Introduction

Chronic venous ulcers are a major clinical and public health problem, affecting approximately 1% of the population and becoming frequent with advancing age. They account for up to 80% of all chronic ulcers and are characterized by prolonged healing times, frequent episodes of recurrence and a tendency to become long-standing lesions. The recurrence rate is high, ranging from 40% to 70% within five years of initial healing, creating a cycle of chronic morbidity that profoundly impacts patient quality of life.[1-3]

Beyond the physical burden, venous ulcers impose substantial psychological distress, social isolation and economic hardship on affected individuals. Healthcare systems bear

considerable financial costs, with treatment expenses reaching billions annually in developed countries, encompassing direct medical costs, lost productivity, and long-term disability management.[1-3]

Despite advances in wound care, venous ulcer management remains challenging. Standard compression therapy, while effective for venous hypertension control, does not directly address the molecular imbalances within the wound bed.[2,4] Persistent inflammation and protease dysregulation are central features of venous ulcer pathophysiology. Excessive activity of matrix metalloproteinases, not adequately counterbalanced by tissue inhibitors, leads to degradation of the newly formed extracellular matrix and key growth factors, perpetuating the inflammatory phase and preventing transition to effective tissue repair. A better understanding of these molecular mechanisms has supported the development of targeted strategies to modulate the wound microenvironment.[4-6]

Among these strategies, bioactive dressings that interact with components of wound exudate have gained prominence in recent years.[6] Dressings composed of oxidized regenerated cellulose and collagen, with or without silver addition, have demonstrated clinical benefits in accelerating healing.[5,7-9] These bioactive materials have been proposed to interact directly with proteases and other signaling molecules present in wound exudate, which may reduce local inflammatory burden and help create a more favorable environment for tissue repair. The oxidized regenerated cellulose component has been proposed, based on *ex vivo* chronic wound fluid studies, to bind and sequester excessive proteases, while the collagen matrix provides a provisional scaffold that supports cell migration and tissue organization.[10,11] When supplemented with silver, these dressings acquire antimicrobial properties that may help prevent or treat wound infection, a common complication in chronic venous ulcers that further delays healing and increases morbidity.[9,12] Despite clinical evidence supporting the efficacy of oxidized regenerated cellulose/collagen dressings with and without silver[10,13-19], important gaps remain in understanding how specific cellular components are modulated in chronic venous ulcers.

Understanding how these dressings influence the expression of matrix metalloproteinases, tissue inhibitors of metalloproteinases, growth factors, and extracellular matrix components in venous ulcer-derived fibroblasts is important for elucidating mechanisms of action and may help identify potential biomarkers associated with treatment response in clinical practice. This knowledge gap represents a critical barrier to optimizing clinical application and to developing more personalized treatment strategies based on individual wound characteristics.

Therefore, this study aimed to evaluate the *in vitro* behavior of venous ulcer fibroblasts in the presence of oxidized regenerated cellulose/collagen and oxidized regenerated cellulose/collagen/silver by monitoring the expression levels of matrix metalloproteinases, tissue inhibitors of metalloproteinases, transforming growth factor beta isoforms and collagen I and III. This translational approach seeks to bridge the gap between clinical observations and cellular mechanisms, providing an evidence based rationale to inform treatment selection and to advance understanding of how bioactive dressings may influence the wound microenvironment in chronic venous ulcers.

Methods

The study protocol was reviewed and approved by the Ethics and Research Commission of the Hospital das Clínicas, Medical School, Universidade de São Paulo (CAAE #21868413.5.0000.0068). This was an *in vitro*, prospective, controlled, non randomized, non blinded study. Venous ulcer fibroblasts (VUFs) were collected from 12 female patients aged 40–65 years with chronic lower limb ulcers (> 6 months) due to venous insufficiency (CEAP C6), without arterial insufficiency. No patient identifiable data or sensitive medical record information were used in this study, and all analyses were performed on anonymized cell cultures derived from donated tissue samples.

Optimal concentrations of oxidized regenerated cellulose/collagen (2.8 mg/mL) and oxidized regenerated cellulose/collagen/silver (4.2 mg/mL) were defined in preliminary MTT assays as those maintaining fibroblast metabolic activity without evidence of marked cytotoxicity. For main experiments, VUFs were seeded at 4×10^3 cells per well in 24 well plates and cultured for 48 hours to allow adhesion. Cells were then exposed for 72 hours to control medium (D10), oxidized regenerated cellulose/collagen (2.8

mg/mL) or oxidized regenerated cellulose/collagen/silver (4.2 mg/mL). After this period, conditioned media were collected, centrifuged (378 × g, for 5 minutes) and analyzed by bead-based multiplex immunoassay (Millipore, St. Louis, MO) for MMP-1, MMP-2, MMP-3, MMP-10, MMP-13, TIMP1, TIMP2, TIMP3, and TGF-β isoforms, according to the manufacturer's protocol. For MTT and multiplex immunoassays, each experimental condition was performed in technical triplicates for each of the 12 patient derived fibroblast cultures.

For immunofluorescence analyses, VUFs from three independent patients (biological replicates) were seeded in black, flat bottom 96 well plates at 4×10^3 cells per well (technical triplicates per condition), allowed to adhere for 48 hours and then exposed for 72 hours to control medium (D10), oxidized regenerated cellulose/collagen (2.8 mg/mL) or oxidized regenerated cellulose/collagen/silver (4.2 mg/mL). Cells were fixed with 4% paraformaldehyde, permeabilized with 0.1% Triton X 100 and blocked with 5% bovine serum albumin in PBS. Primary antibodies against collagen type I (rabbit polyclonal, Rockland, 1:100, #600.401.103-0.5), collagen type III (rabbit polyclonal, Rockland, 1:100, #600-401-105-0.1) and vimentin (mouse monoclonal, Dako Flex clone 9, ready to use, #IR630) were applied overnight at 4°C. Detection was performed using Alexa Fluor 488 goat anti rabbit IgG (H+L) secondary antibody (1:1000, Invitrogen/Thermo Fisher Scientific, #A11008) for collagen types I and III and R phycoerythrin labeled anti mouse secondary antibody (1:1000, Invitrogen, #P852) for vimentin. Fluorescence was quantified using the ImageXpress system (Molecular Devices), and the number of positive cells and their fluorescence intensity were determined with MetaXpress software (Molecular Devices). Results are expressed as fluorescence intensity.

Statistical analyses were performed using IBM SPSS Statistics, version 24.0 (IBM, USA). Numerical variables are expressed as mean and standard deviation (SD). For all outcomes (MTT absorbance, MMPs, TIMPs, TGF β isoforms and immunofluorescence intensity), paired comparisons were conducted using the Wilcoxon signed rank test, considering each patient derived fibroblast culture as its own control. The following contrasts were assessed: control medium versus oxidized regenerated cellulose/collagen, control medium versus oxidized regenerated cellulose/collagen/silver, and oxidized regenerated cellulose/collagen versus oxidized regenerated cellulose/collagen/silver. Z values are reported, and statistical significance was set at $p < 0.05$.

Results

After 72 hours, venous ulcer fibroblasts exposed to ORC/Collagen (mean = 1.141; SD = 0.058) or ORC/Collagen/Ag (mean = 1.119; SD = 0.066) showed higher MTT absorbance values compared with cells cultured in control medium (mean = 1.005; SD = 0.067). These differences were statistically significant for both formulations when compared with control ($p < 0.05$, Wilcoxon signed rank test), indicating preserved mitochondrial activity and absence of overt cytotoxicity under the tested conditions (Figure 1).

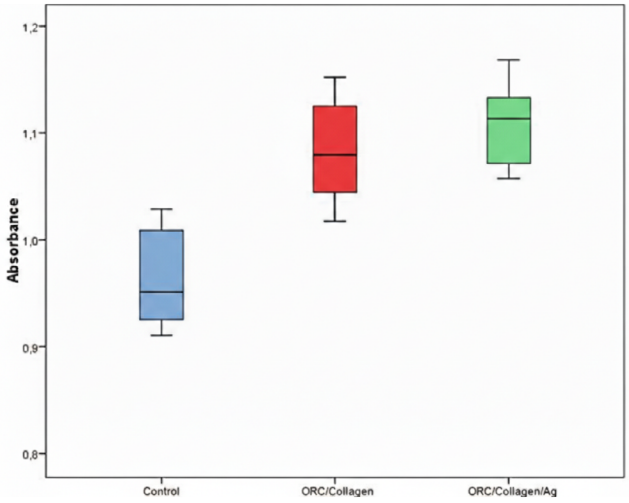

Figure 1. MTT assay of venous ulcer fibroblasts (VUFs) after 72 hours in control medium (blue), ORC/Collagen (red) and ORC/Collagen/Ag (green). Bars represent mean ± SD of 12 patient derived cultures. Statistical comparisons were performed using the paired Wilcoxon signed rank test; $p < 0.05$.

VUF-Specific Expression of Metalloproteinases, TIMPs, and Growth Factors

The secretion of MMP 1, MMP 2, MMP 3, MMP 10 and MMP 13 by venous leg ulcer fibroblasts after 72 hours of exposure is shown in Figure 2. When compared with the control medium, ORC/Collagen did not induce significant changes in MMP 1, MMP 2, MMP 3, MMP 10 or MMP 13 concentrations ($p > 0.05$, Wilcoxon signed rank test). In cultures treated with ORC/Collagen/Ag, MMP 1 levels were significantly increased,

showing an approximately 1.9 fold upregulation relative to control medium (p = 0.0002), whereas MMP 13 levels were significantly reduced, with an approximately 1.62 fold downregulation (p = 0.0155). No significant differences were observed for MMP 2, MMP 3 or MMP 10 in the ORC/Collagen/Ag group compared with control medium (p > 0.05).

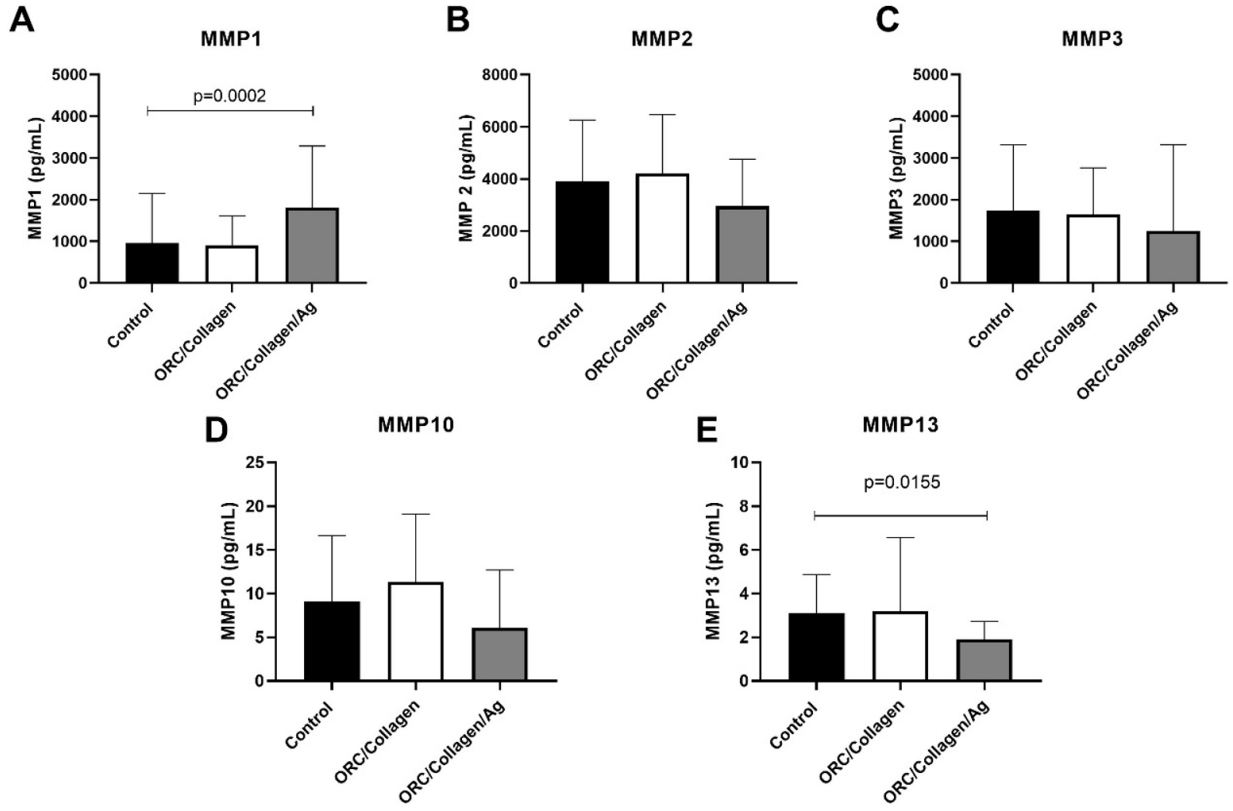

Figure 2. Secretion of MMP 1 (A), MMP 2 (B), MMP 3 (C), MMP 10 (D) and MMP 13 (E) by venous ulcer fibroblasts after 72 hours in control medium, ORC/Collagen and ORC/Collagen/Ag. Protein concentrations in conditioned media were measured by bead based multiplex immunoassay. Bars represent mean ± SD. Statistical comparisons were performed using the paired Wilcoxon signed rank test; p < 0.05 versus control medium.

The treatment with ORC/Collagen and ORC/Collagen/Ag did not significantly change TGF β1, TGF β2 or TGF β3 levels compared with control medium, as shown in Figure 3.

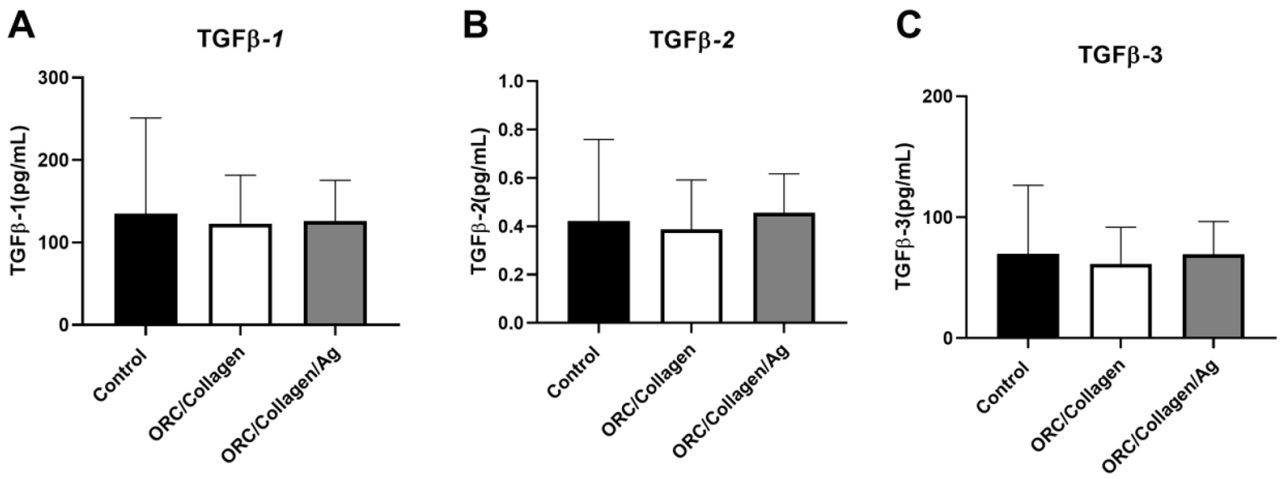

Figure 3. TGF β1, TGF β2 and TGF β3 secretion by venous ulcer fibroblasts after 72 hours in control medium, ORC/Collagen and ORC/Collagen/Ag. Protein concentrations in conditioned media were measured by bead based multiplex immunoassay. Bars represent mean ± SD. Statistical comparisons were performed using the paired Wilcoxon signed rank test; no significant differences were detected between treatments and control medium (p > 0.05).

TIMP 1, TIMP 2 and TIMP 3 levels in venous ulcer fibroblast conditioned media after 72 hours are shown in Figure 4. Compared with control medium, ORC/Collagen did not induce significant changes in TIMP 1, TIMP 2 or TIMP 3 concentrations ($p > 0.05$, Wilcoxon signed rank test). In contrast, VUFs exposed to ORC/Collagen/Ag showed reduced TIMP 2 (≈ 1.65 fold decrease; $p = 0.0048$) and TIMP 3 (≈ 6 fold decrease; $p < 0.0001$) levels relative to control medium, while TIMP 1 secretion remained unchanged.

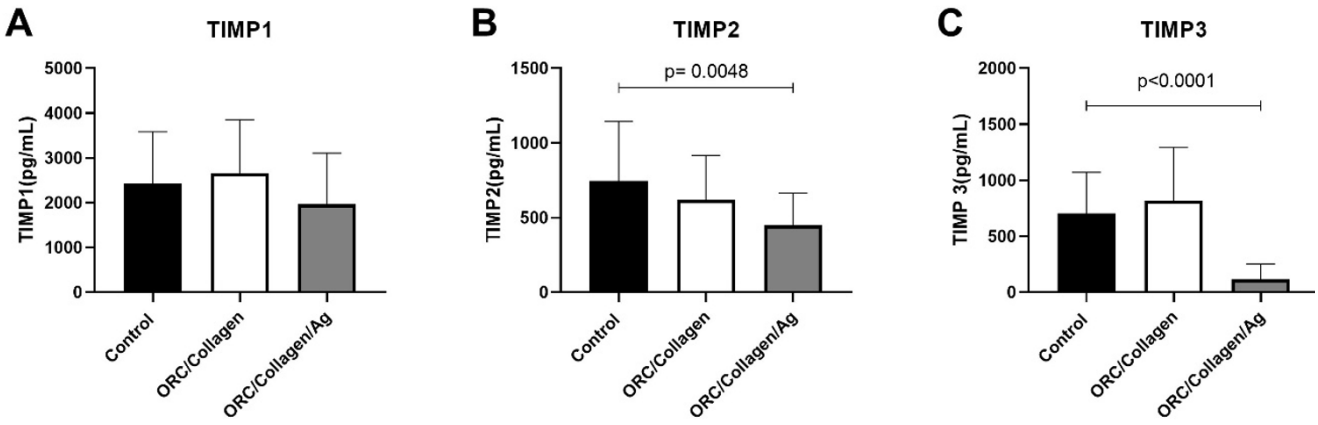

Figure 4. Secretion of TIMP 1, TIMP 2 and TIMP 3 by venous ulcer fibroblasts after 72 hours in control medium, ORC/Collagen and ORC/Collagen/Ag. Protein concentrations in conditioned media were measured by bead based multiplex immunoassay. Bars represent mean ± SD. Statistical comparisons were performed using the paired Wilcoxon signed rank test; $p < 0.05$ versus control medium.

VUF-specific quantification of vimentin, type I and III collagen by Immunofluorescence

Immunofluorescence analysis showed that vimentin, collagen type I and collagen type III expression in venous ulcer fibroblasts exposed for 72 hours to ORC/Collagen or ORC/Collagen/Ag did not differ significantly from cells cultured in control medium ($p > 0.05$ for all comparisons, Wilcoxon signed rank test), as illustrated in Figures 5–8. Vimentin expression remained stable under all conditions, confirming preservation of the fibroblast phenotype.

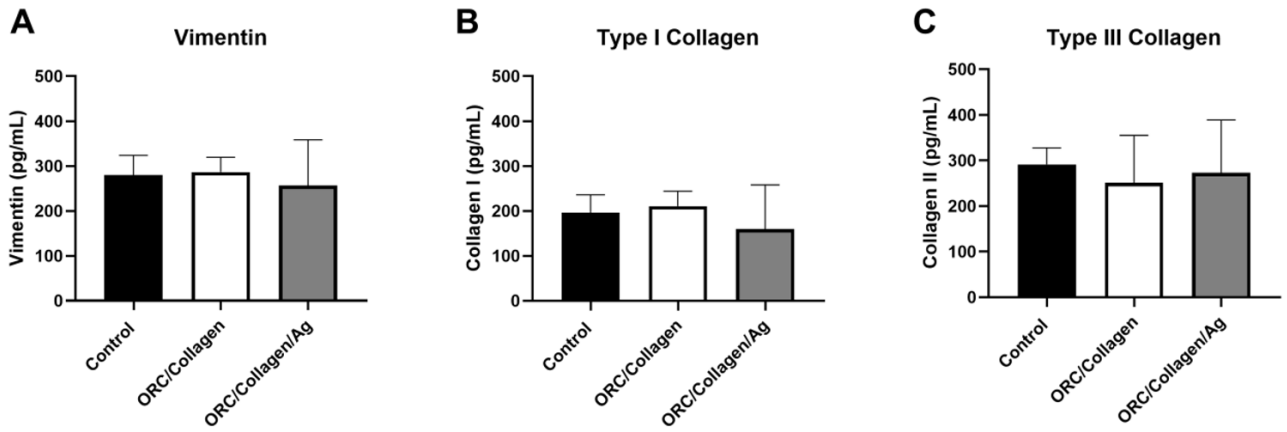

Figure 5. Quantification of vimentin, collagen type I and collagen type III expression in venous ulcer fibroblasts by immunofluorescence. Cells were cultured for 72 hours in control medium, ORC/Collagen or ORC/Collagen/Ag, and fluorescence intensity for vimentin, collagen type I and collagen type III was measured. Bars represent mean ± SD. Statistical comparisons were performed using the paired Wilcoxon signed rank test; no significant differences were detected between treatments and control medium ($p > 0.05$).

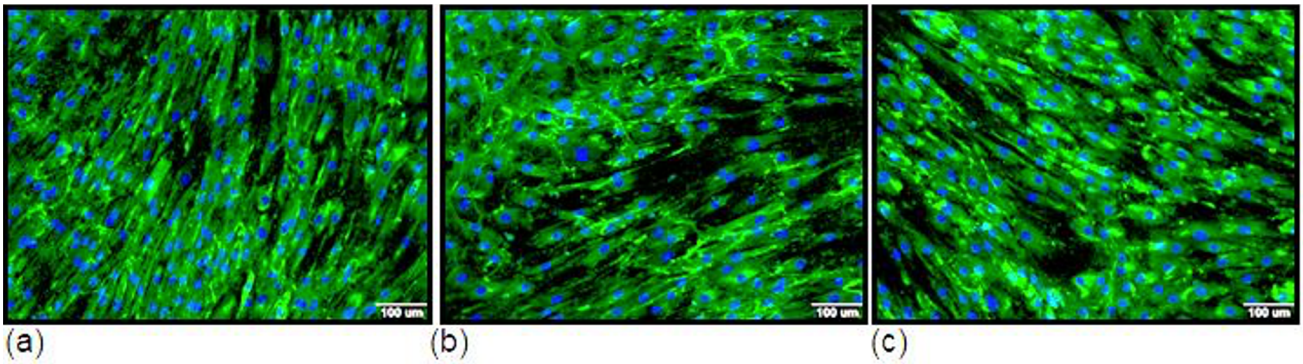

Figure 6. Representative images of collagen type I immunofluorescence in venous ulcer fibroblasts cultured for 72 hours in (a) control medium, (b) ORC/Collagen and (c) ORC/Collagen/Ag. Scale bar = 100 µm.

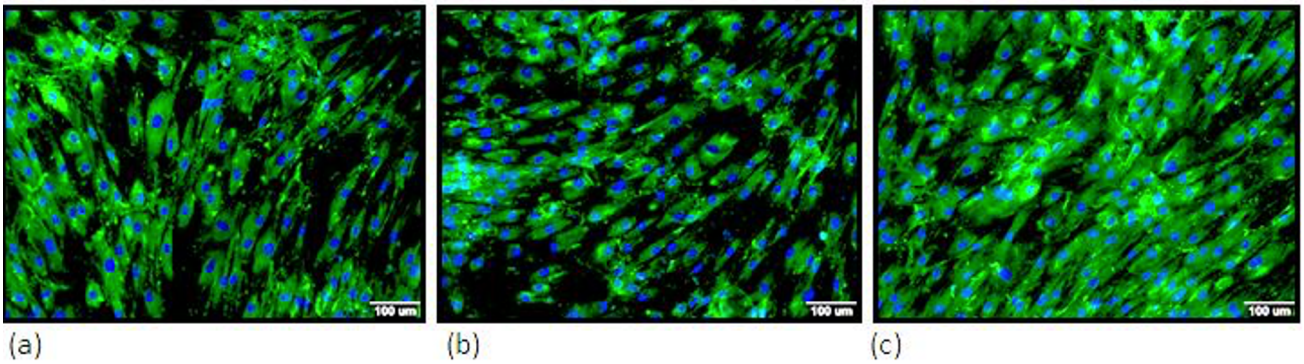

Figure 7. Representative images of collagen type III immunofluorescence in venous ulcer fibroblasts cultured for 72 hours in (a) control medium, (b) ORC/Collagen and (c) ORC/Collagen/Ag. Scale bar = 100 µm.

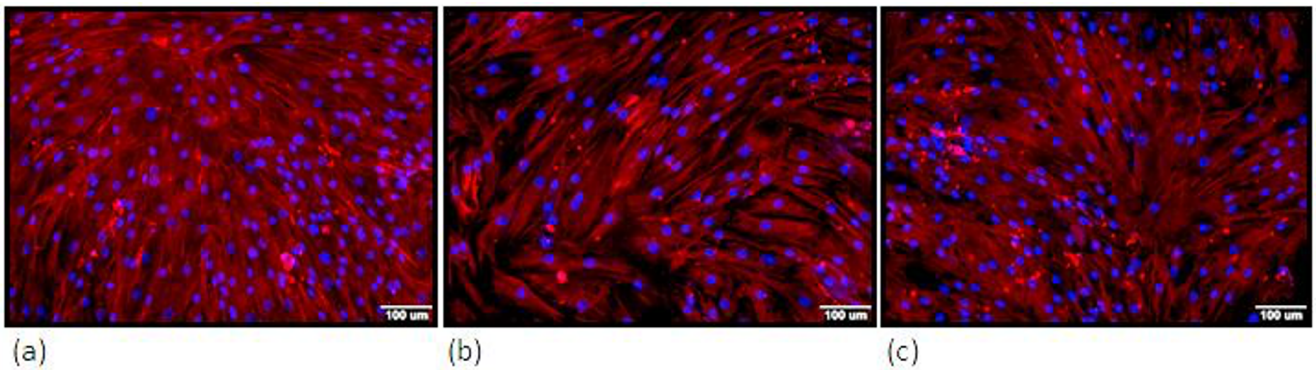

Figure 8. Representative images of vimentin immunofluorescence in venous ulcer fibroblasts cultured for 72 hours in (a) control medium, (b) ORC/Collagen and (c) ORC/Collagen/Ag. Scale bar = 100 µm.

Discussion

In this study, the effects of oxidized regenerated cellulose/collagen dressings, with and without silver, on proteases, their inhibitors and key growth factors were evaluated in fibroblasts derived from chronic venous leg ulcers. Both formulations preserved venous ulcer fibroblast viability *in vitro*, without evidence of overt cytotoxicity at the concentrations tested, which supports their use in subsequent molecular analyses. Importantly, the silver containing dressing induced specific changes in the fibroblast protease profile, whereas the formulation without silver produced minimal direct modulation of fibroblast secretory behavior, findings that are consistent with the concept of predominantly extracellular protease sequestration for the silver free matrix.

The MTT assays showed that oxidized regenerated cellulose/collagen at 2.8 mg/mL and oxidized regenerated cellulose/collagen/silver at

4.2 mg/mL maintained venous ulcer fibroblast metabolic activity at levels comparable to, or slightly higher than, control conditions, with MTT indices of 1.141 ± 0.058 and 1.119 ± 0.066 respectively, versus 1.005 ± 0.067 in control medium. These findings indicate the absence of an acute reduction in cell metabolic activity under the tested conditions and support the use of these concentrations for subsequent molecular analyses.[20] This pattern is in line with previous investigations reporting preserved fibroblast viability in culture systems exposed to oxidized regenerated cellulose/collagen based dressings, without evidence of overt toxicity at therapeutic concentrations.[21] Although the MTT assay does not distinguish between increased cell number and enhanced mitochondrial activity, the maintenance of metabolic activity and fibroblast phenotype in our model suggests preservation of fundamental cellular functions required for tissue repair, while highlighting the need for additional assays to fully characterize long term proliferative and biocompatibility responses.

In the skin, matrix metalloproteinases degrade elements of the extracellular matrix, including collagen, elastin, proteoglycans and other structural glycoproteins, and play essential roles in the activation of growth factors such as transforming growth factor beta and vascular endothelial growth factor. These proteolytic enzymes are produced by multiple cell types, including keratinocytes, fibroblasts, macrophages, endothelial cells, mast cells, eosinophils and neutrophils. Matrix metalloproteinase mediated proteolytic degradation of the extracellular matrix is an essential component of tissue remodeling and repair during normal wound healing.[22]

Matrix metalloproteinases are classified based on substrate specificity and structural organization into collagenases, gelatinases, stromelysins, matrilysins, metalloelastases, membrane type MMPs and other subgroups. In this study, venous ulcer fibroblasts exposed to oxidized regenerated cellulose/collagen/silver showed a significant increase in MMP 1 secretion compared with control medium, whereas the silver free formulation did not modify MMP 1 levels. MMP 1 is a key interstitial collagenase involved in the initial cleavage of fibrillar collagens, which enables subsequent degradation and replacement by newly synthesized matrix. In chronic venous ulcers, persistent inflammation and disorganized collagen deposition contribute to a rigid, fibrotic wound bed that impairs cell migration and re epithelialization. A moderate upregulation of MMP 1 under non cytotoxic conditions, as observed in our model, may therefore facilitate removal of disorganized collagen fibers and support the transition toward a more structured matrix, provided that this occurs within a controlled proteolytic environment.[23] The preservation of venous ulcer fibroblast viability and the maintenance of type I and III collagen expression in our experiments suggest that this increase in MMP 1 is not associated with overt degradation of newly formed matrix.

In contrast, the reduction in MMP 13 levels observed in venous ulcer fibroblasts exposed to the silver containing dressing may represent a protective effect on nascent granulation tissue. MMP 13 has been reported to be upregulated in chronic wounds and is associated with extensive degradation of extracellular matrix components. Excessive MMP 13 activity can compromise granulation tissue stability and contribute to a persistent non healing state. In our model, the combination of increased MMP 1 and decreased MMP 13 in response to oxidized regenerated cellulose/collagen/silver is compatible with a shift from a more destructive proteolytic profile toward a pattern that may favor matrix remodeling. This interpretation remains hypothetical but provides a biologically plausible framework to reconcile the need for collagen degradation to remove damaged tissue with the preservation of structural support required for cell migration and proliferation.

The changes observed in tissue inhibitors of metalloproteinases further suggest that the action of the silver-containing dressing on the protease system is more nuanced than a simple suppression of proteolytic activity. In our experiments, TIMP 2 and TIMP 3 levels were reduced in venous ulcer fibroblasts exposed to oxidized regenerated cellulose/collagen/silver, whereas TIMP 1 secretion remained relatively stable across conditions. TIMP 1 is a major inhibitor of several MMPs and is often decreased in chronic venous ulcers, contributing to the predominance of proteolytic activity.[23] The preservation of TIMP 1 in our model may help maintain a basal level of control over matrix degradation, while the reduction in TIMP 2 and TIMP 3 may permit a degree of proteolytic activity that could be necessary for matrix

remodeling. Taken together, these observations indicate that the silver-containing dressing does not induce a generalized blockade of proteases but rather promotes a reconfiguration of the protease inhibitor balance that is compatible with controlled matrix turnover, although functional assays of protease activity would be required to confirm this hypothesis.

Neither formulation significantly altered the concentrations of TGF β1, TGF β2 or TGF β3 in culture supernatants, nor the expression of type I and III collagen by venous ulcer fibroblasts. TGF β signaling is central to fibroblast activation, myofibroblast differentiation and extracellular matrix synthesis, and therapies that markedly suppress TGF β or collagen production could impair granulation tissue formation and long term wound strength. In this context, the maintenance of TGF β isoforms and collagen type I and III expression in our experiments suggests that the oxidized regenerated cellulose/collagen matrix, with or without silver, does not overtly interfere with these core reparative pathways in this *in vitro* model. From a translational perspective, this is consistent with the notion that protease modulating effects of the silver containing dressing can occur without obvious detrimental inhibition of fibroblast synthetic capacity, although *in vivo* studies are required to confirm this balance.

The absence of significant changes in MMPs, TIMPs and TGF β isoforms in cultures exposed to oxidized regenerated cellulose/collagen without silver is also informative. These results are consistent with the concept that this formulation acts primarily at the extracellular level, by binding and sequestering proteases and other soluble mediators present in the wound exudate, rather than by directly reprogramming fibroblast secretion. Experimental work with oxidized regenerated cellulose/collagen has shown that this biomaterial can rapidly adsorb proteases and other reactive species from wound fluid, thereby limiting proteolytic and oxidative damage to the extracellular matrix and growth factors.[10,11] In this context, the lack of major changes in the secretory profile of fibroblasts exposed to the non silver formulation *in vitro* is compatible with its proposed mechanism of action as a protease absorbing scaffold, particularly in wounds with a moderate protease burden.

By contrast, the addition of silver appears to confer a more active influence on venous ulcer fibroblast protease profiles, beyond its well known antimicrobial effect. Silver containing dressings are widely used in chronic wounds with heavy bioburden or clinical signs of local infection, and clinical studies have reported improved healing trajectories when they are used in appropriately selected venous ulcers.[9,12] In our *in vitro* model, oxidized regenerated cellulose/collagen/silver increased MMP 1 and decreased MMP 13, while reducing TIMP 2 and TIMP 3, without affecting TGF β isoforms or collagen type I and III expression. This pattern is compatible with a reorganization of fibroblast derived protease and inhibitor levels toward a profile that may favor matrix remodeling under non-cytotoxic conditions. Although the precise molecular pathways linking silver exposure to these protease changes were not investigated in this study, the data are compatible with a broader concept in which antimicrobial activity and modulation of the inflammatory proteolytic milieu act in synergy to support restoration of a healing trajectory.

These *in vitro* results need to be interpreted in the context of important limitations. First, the study used isolated fibroblasts derived from chronic venous leg ulcers, cultured in a controlled environment. Chronic wounds *in vivo* comprise a complex interplay of keratinocytes, endothelial cells, immune cells, microbial communities and a dynamic exudate rich in cytokines, proteases and degradation products. The dressing concentrations and exposure time used here were chosen to avoid acute toxicity *in vitro* and may not fully replicate the kinetics of interaction between the materials and the wound bed in clinical practice. Second, the sample consisted only of female patients within a restricted age range and with a specific etiology (venous insufficiency, CEAP C6), which may limit generalizability to other patient groups or wound types. Third, the analysis focused on a selected panel of MMPs, TIMPs and TGF β isoforms; other proteases, cytokines and signaling pathways that are relevant to chronic wound pathophysiology were not assessed.

The absence of bacterial contamination or biofilm in the culture system represents another relevant limitation, particularly given the role of silver containing dressings in managing wounds with high bioburden. Infection and biofilm profoundly influence the wound microenvironment and can modulate both protease activity and fibroblast behavior. The interaction between antimicrobial action and

protease modulation in the presence of biofilm therefore requires further investigation through co culture models incorporating bacteria and fibroblasts.[21]

Despite these limitations, the study offers insights that may be relevant for the translational use of oxidized regenerated cellulose/collagen based dressings. The observation that oxidized regenerated cellulose/collagen/silver modulated the balance between MMP 1 and MMP 13, while preserving TGF β isoforms and collagen type I and III expression, provides a plausible biological rationale that is consistent with reports of improved granulation quality and accelerated closure in venous leg ulcers treated with this dressing.[9,12] Conversely, the essentially extracellular mode of action suggested for oxidized regenerated cellulose/collagen without silver is in line with its use as a protease absorbing matrix in wounds with elevated proteolytic activity and without clear signs of critical colonization or infection.

Future translational research should correlate the molecular markers identified in this study with clinical healing outcomes through prospective trials incorporating serial measurements of MMP 1, MMP 13, TIMP 2 and TIMP 3 alongside standardized wound assessment. The development of point of care biomarker assays enabling real time protease status determination could guide more personalized dressing selection. Investigation of optimal silver concentrations, formulation characteristics, application frequencies and combination therapies with other advanced modalities warrants systematic study to maximize therapeutic benefits while minimizing potential adverse effects. Although combination therapies incorporating oxidized regenerated cellulose/collagen/silver with negative pressure wound therapy have demonstrated promising results in complex lower extremity wounds with exposed critical structures[24], further investigation is required to elucidate the molecular mechanisms underlying these synergistic effects and to establish optimal protocols for combined use with other advanced modalities such as growth factor supplementation or cellular therapies.

Conclusion

In this *in vitro* model using fibroblasts derived from chronic venous leg ulcers, oxidized regenerated cellulose/collagen and oxidized regenerated cellulose/collagen/silver preserved cell viability at the tested concentrations and did not overtly interfere with TGF β isoforms or collagen type I and III expression. The silver free formulation did not induce significant changes in the secretion of the analyzed MMPs, TIMPs or TGF β isoforms, which is compatible with a predominantly extracellular protease sequestering mechanism. By contrast, the silver containing matrix increased MMP 1 and decreased MMP 13, while reducing TIMP 2 and TIMP 3 without affecting TIMP 1, suggesting a shift in the fibroblast derived protease–inhibitor profile toward a pattern that may favor matrix remodeling under non cytotoxic conditions. These findings provide a mechanistic hypothesis that is consistent with clinical observations of improved granulation and healing in venous leg ulcers treated with oxidized regenerated cellulose/collagen/silver dressings, but they should be interpreted as preliminary and hypothesis generating, requiring confirmation in more complex *in vitro* models and prospective clinical studies that integrate molecular markers with wound healing outcomes.

References

1. Rochon PJ, Reghunathan A, Kapoor BS, Kalva SP, Fidelman N, Majdalany BS, et al. ACR Appropriateness Criteria® Lower Extremity Chronic Venous Disease. *J Am Coll Radiol.* 2023;20(11s):S481-s500. https://doi.org/10.1016/j.jacr.2023.08.011
2. Meaume S, Senet P, Thomé B, Aragno VA, Bohbot S, Fortin S, et al. Aetiological treatment of venous leg ulcers with compression therapy: real-life outcomes with two different procedures. *J Wound Care.* 2023;32(10):615-23. https://doi.org/10.12968/jowc.2023.32.10.615
3. Probst S, Saini C, Gschwind G, Stefanelli A, Bobbink P, Pugliese MT, et al. Prevalence and incidence of venous leg ulcers-A systematic review and meta-analysis. *Int Wound J.* 2023;20(9):3906-21. https://doi.org/10.1111/iwj.14272
4. Ligi D, Mosti G, Croce L, Raffetto JD, Mannello F. Chronic venous disease – Part I: Inflammatory biomarkers in wound healing. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease.* 2016;1862(10):1964-74. https://doi.org/10.1016/j.bbadis.2016.07.018
5. Westby MJ, Norman G, Dumville JC, Stubbs N, Cullum N. Protease-modulating matrix treatments for healing venous leg ulcers. *The Cochrane database of systematic reviews.* 2016;12:Cd011918. https://doi.org/10.1002/14651858.CD011918.pub2
6. Cullen B, Gefen A. The biological and physiological impact of the performance of wound dressings. *Int Wound J.* 2023;20(4):1292-303. https://doi.org/10.1111/iwj.13960
7. Wollina U, Schmidt WD, Kronert C, Nelskamp C, Scheibe A, Fassler D. Some effects of a topical collagen-based matrix on the microcirculation and wound healing in patients with chronic venous leg ulcers: preliminary observations. *Int J Low Extrem Wounds.* 2005;4(4):214-24. https://doi.org/10.1177/1534734605283001
8. Vin F, Teot L, Meaume S. The healing properties of Promogran in venous leg ulcers. *J Wound Care.* 2002;11(9):335-41. https://doi.org/10.12968/jowc.2002.11.9.26438
9. Wu S, Applewhite AJ, Niezgoda J, Snyder R, Shah J, Cullen B, et al. Oxidized Regenerated Cellulose/Collagen Dressings: Review of Evidence and Recommendations. *Advances in Skin & Wound Care.* 2017;30(11 Suppl 1):S1-S18. https://doi.org/10.1097/01.ASW.0000525951.20270.6c
10. Cullen B, Smith R, McCulloch E, Silcock D, Morrison L. Mechanism of action of PROMOGRAN, a protease modulating matrix, for the treatment of diabetic foot ulcers. *Wound Repair and Regeneration.* 2002;10(1):16-25. https://doi.org/10.1046/j.1524-475x.2002.10703.x
11. Cullen B, Watt PW, Lundqvist C, Silcock D, Schmidt RJ, Bogan D, et al. The role of oxidised regenerated cellulose/collagen in chronic wound repair and its potential mechanism of action. *Int J Biochem Cell Biol.* 2002;34(12):1544-56. https://doi.org/10.1016/s1357-2725(02)00054-7
12. Gottrup F, Cullen BM, Karlsmark T, Bischoff-Mikkelsen M, Nisbet L, Gibson MC. Randomized controlled trial on collagen/oxidized regenerated cellulose/silver treatment. *Wound Repair Regen.* 2013;21(2):216-25. https://doi.org/10.1111/wrr.12020
13. Omugha N, Jones AM. The management of hard-to-heal necrobiosis with PROMOGRAN. *Br J Nurs.* 2003;12(15 Suppl):S14-20.
14. Franceschini G, Visconti G, Terribile D, Fabbri C, Magno S, Di Leone A, et al. The role of oxidized regenerate cellulose to prevent cosmetic defects in oncoplastic breast surgery. *Eur Rev Med Pharmacol Sci.* 2012;16(7):966-71.
15. Temiz A, Ozturk C, Bakunov A, Kara K, Kaleli T. A new material for prevention of peritendinous fibrotic adhesions after tendon repair: oxidised regenerated cellulose (Interceed), an absorbable adhesion barrier. *Int Orthop.* 2008;32(3):389-94. https://doi.org/10.1007/s00264-007-0335-8
16. Tausche A-K, Sebastian G. Wound conditioning of a deep tissue defect including exposed bone after tumour excision using PROMOGRAN* Matrix, a protease-modulating matrix. *International Wound Journal.* 2005;2(3):253-7. https://doi.org/10.1111/j.1742-4801.2005.00120.x
17. Romanelli M, Mulder G, Paggi B, Macchia M, Panduri S, Dini V. The use of a collagen matrix in hard-to-heal venous leg ulcers. *J Wound Care.* 2015;24(11):543-4, 6-7. https://doi.org/10.12968/jowc.2015.24.11.543
18. Kakagia DD, Kazakos KJ, Xarchas KC, Karanikas M, Georgiadis GS, Tripsiannis G, et al. Synergistic action of protease-modulating matrix and autologous growth factors in healing of diabetic foot ulcers. A prospective randomized trial. *Journal of diabetes and its complications.* 2007;21(6):387-91. https://doi.org/10.1016/j.jdiacomp.2007.03.006
19. Veves A SPPHT. A randomized, controlled trial of promogran (a collagen/oxidized regenerated cellulose dressing) vs standard treatment in the management of diabetic foot ulcers. *Archives of Surgery.* 2002;137(7):822-7. https://doi.org/10.1001/archsurg.137.7.822
20. Hart J, Silcock D, Gunnigle S, Cullen B, Light ND, Watt PW. The role of oxidised regenerated cellulose/collagen in wound repair: effects *in vitro* on fibroblast biology and *in vivo* in a model of compromised healing. *Int J Biochem Cell Biol.* 2002;34(12):1557-70. https://doi.org/10.1016/s1357-2725(02)00062-6

21. Bourdillon KA, Delury CP, Cullen BM. Biofilms and delayed healing - an *in vitro* evaluation of silver- and iodine-containing dressings and their effect on bacterial and human cells. *Int Wound J.* 2017. https://doi.org/10.1111/iwj.12761
22. Ligi D, Mosti G, Croce L, Raffetto JD, Mannello F. Chronic venous disease – Part II: Proteolytic biomarkers in wound healing. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease.* 2016;1862(10):1900-8. https://doi.org/10.1016/j.bbadis.2016.07.011
23. Krishnaswamy VR, Mintz D, Sagi I. Matrix metalloproteinases: The sculptors of chronic cutaneous wounds. *Biochimica et biophysica acta Molecular cell research.* 2017;1864(11 Pt B):2220-7. https://doi.org/10.1016/j.bbamcr.2017.08.003
24. Loh ML, Goh BKL, Kong Y, Varughese G, Ng JL, Lo ZJ, et al. Combination therapy of oxidised regenerated cellulose/collagen/silver dressings with negative pressure wound therapy for coverage of exposed critical structures in complex lower-extremity wounds. *Int Wound J.* 2020;17(5):1356-65. https://doi.org/10.1111/iwj.13406