The use of an antioxidant dressing on hard-to-heal wounds: a multicentre, prospective case series

Objective: Oxidative stress can contribute to impaired wound healing and chronic wounds. Our objective was to test the results of a new antioxidant dressing that could help stop the oxidative stress of cells in the wound bed.
Method: A multicentre, prospective case study series was conducted in three Spanish hospitals. The RESVECH 2.0 index was used for healing assessment. Data from each patient was collected by the attending clinical researchers. Data analysis was performed using the statistical concept intention-to-treat (ITT). Descriptive results were presented as frequency and percentages for qualitative variables and mean, standard deviation (SD), range and median for quantitative variables. For analytical-inferential analyses, incidence of healing was calculated for chronic and acute wounds. Relative risk (RR) was used to establish the differences of healing between both types of wounds. Healing was represented by Kaplan-Meier survival curves, and these were compared using the log-rank test.
Results: A total of 31 patients with hard-to-heal wounds were recruited. During the 8-week follow-up period, nine wounds (29%) completely healed, of which seven (77.8%) were acute and two (22.2%) chronic. The remaining wounds (22) showed a significant improvement after treatment with the antioxidant dressing. RESVECH 2.0 scores decreased an average of 10.16 points over the 8-week period.
Conclusion: The antioxidant dressing could represent an alternative in the dressing landscape for many types of acute and chronic wounds.
Declaration of interest: B. Castro holds the patent that protects the technology under the antioxidant dressing. B. Castro and F.D. Bastida work in the research and development department of Histocell, the company that has developed the dressing.

antioxidant dressing • wound healing • hard-to-heal wounds • oxidative stress • moist wound care

Wound treatment products and guidelines have significantly improved in the past decades,[1–3] and most facilities and health professionals have completely abandoned the old dry wound treatment.[1,4,5] However, wound closure is not always achieved by just using moist wound-healing techniques.

The excessive production of reactive oxygen species (ROS) has been associated with oxidative stress of tissues, wound chronification and delayed healing.[6] An excess of ROS causes a pro-inflammatory environment in the wound bed,[7,8] drawing more inflammatory cells into the wound and generating a negative feedback loop that could delay or prevent wound closure.[9,10] The potent oxidising capabilities of ROS can therefore damage many of the cell's molecules and structures.[6] However, the use of a dressing that can exert the antioxidant effect on wound exudates could restore the appropriate ROS balance.[10,11]

Although the association between oxidative stress in the wound and hard-to-heal wounds has been around for many years,[12] few dressings that address this point are available.[10,13] Honey and honey dressings have antioxidants that include phenols, flavonoids and vitamins.[14] However, honey also contains glucose oxidase that continuously synthesises the potent ROS, H_2O_2 at levels sometimes up to 10 times higher than those present in the exudate,[9,15,16] complicating honey's role in oxidative stress in wounds. A new antioxidant dressing (company code HR006; commercial name REOXCARE; developed by Histocell) combines an absorbent matrix obtained from Locust Bean Gum (LBG) galactomannan and a hydration solution with curcumin and N-acetylcysteine (NAC). Curcumin, the natural phenol from the rhizome of the plant Curcuma longa, has been used for over 2000 years as an antioxidant, anti-inflammatory and also specifically in wounds to improve healing.[17–20] NAC is widely applied as an antioxidant molecule, and more recently also successfully for the treatment of wounds.[21,22] These three components act as free radicals

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Table 1. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Venous leg ulcers	Local infection in wound area*
Neuropathic diabetic foot ulcers	Patient under negative pressure wound therapy
Traumatic ulcers	Intolerance to any of the components of HR006
Surgical wound dehiscence	Other ulcer types not included in the inclusion criteria (ischaemic ulcers)
	Oncological patients

*The following clinical signs could appear in case of local infection: increased drainage; purulent or malodorous drainage; continuous or increased pain; redness and swelling around the wound; warmth around the affected area; cellulitis; and delayed healing not previously anticipated

scavengers, and two of them have also a synergistic antioxidant effect.[23]

The antioxidant dressing had previously been tested in an acute wound model in pigs with good results;[24] however, chronic wounds would be the ideal target for this type of dressing, since these wounds have a problem with oxidative stress that arrests the wound in the inflammatory phase, preventing its progression to other phases of healing.[9] In the current study, we tested the antioxidant dressing in humans in both types of wounds, acute and chronic.

Methods

Study design and procedures

This multicentre, prospective case study series aimed to report the results of the new antioxidant dressing on patients with acute and chronic wounds in wound clinic facilities at three hospitals in Spain.

The study was conducted between September 2013 and July 2014. Data collected from each patient by the attending clinical researchers included:

- Demographic characteristics (age and sex)
- Patient's clinical background (concurrent diseases, other medications)
- Initial description of the wound (aetiology, duration, size, diameter, location, exudate and state of periwound skin).

A maximum cut-off point of 8 weeks (or healing, if occurred before) was established. If wound closure was not achieved during the 8 weeks, researchers were given the option to continue using the antioxidant dressing as appropriate. Wounds were assessed every week to determine their state. The RESVECH 2.0 score was used to assess wound healing.[25–27] This scale takes into account six parameters:

- Size of the lesion
- Depth and involved tissues
- Wound margins
- Type of tissue in the wound bed
- Exudate
- Infection/inflammation.

The scale is scored numerically, with values ranging from 0 to 35 points. Both ends of the scale correspond to a healed or to the worst possible wound, respectively. Data collection sheets for RESVECH 2.0 had clear operational definitions for each item and the numerical value that should be assigned to each variable/condition. The attending professional also recorded their opinion regarding the antioxidant dressing's characteristics and usability.

The sample size was estimated using the programme G*Power.[28] The patients' data, such us wound duration and previous treatments applied on the wound, before incorporation into the study were used as treatment controls. Data analysis was performed using intention-to-treat (ITT),[29] where all wounds, even those requiring longer than 8 weeks to heal, were included in the analysis. Data from patients withdrawn from the study due to causes not related to the antioxidant dressing treatment were also included, in which case the last registered RESVECH 2.0 value was extended until the end of the study at week 8.

The clinical study protocol was drafted in collaboration with GNEAUPP, the Spanish wound healing society. The study adhered to all ethical considerations required for this type of studies with medical devices and conformed with the ethical guidelines of the 1975 Declaration of Helsinki. The Spanish Agency of Medicines and Medical Devices (AEMPS) authorised the study (439/13/EC) to take place in three institutions: Hospital Universitario Puerta de Hierro Majadahonda (Madrid), Hospital General Universitario de Elche (Alicante) and Health Department of Alcoy (Alicante). Ethics committees at all three centres reviewed and approved the study protocol. Before entering the clinical trial and following study protocol guidelines, all patients read the study information, had the opportunity to ask questions to the attending nurse or clinician, and signed consent forms.

Subjects

Patients over 18 years were recruited in the selected centres. The inclusion and exclusion criteria are shown in Table 1. The criteria established for withdrawal included any medical condition or lack of concordance to the study protocol as determined by the clinical investigator.

Statistical analysis

Descriptive results were presented as frequency and percentages for qualitative variables and mean, standard deviation (SD), range and median for quantitative variables. For analytical-inferential analyses, incidence of healing was calculated for chronic and acute wounds. Relative risk (RR) was used to establish the differences of healing between both types of wounds. Also, healing

Table 2. Patient demographics

Mean age± standard deviation years	71.7 ± 10.10
Range, (median) years	44–86, (73)
Female:male number (%)	20 (64.5%):11 (35.5%)

was represented by Kaplan-Meier survival curves, and these were compared using the log-rank test.

To test differences and compare evolution over time, data were analysed using repeated measures ANOVA (Analysis of Variance) and non-parametric Friedman test. Significance was set at an alpha value of 0.05. All analyses were performed using IBM SPSS Statistics V.21 package.

Results

A total of 31 patients were recruited. Of these, 11 (35.5%) were men and 20 (64.5%) were women. All patients were Caucasian and had a mean age of 71.7±10.10 years (range: 44–86; median: 73 years) (Table 2).

Most patients suffered from comorbidities, such as diabetes and lower-limb arteriopathy, and took two or more drugs to treat these (mainly anticoagulants and antihypertensives). The majority of patients suffered from hard-to-heal wounds. All wounds were located on lower limbs, as per the study's inclusion criteria. The wounds' characteristics are listed in Table 3.

Overall, 31 wounds were treated with a mean dressing change every 3 days. During the 8-week follow-up, nine wounds (29%) completely healed, of which seven (77.8%) were acute and two (22.2%) chronic. The incidence of healing was 77.8% in acute wounds (seven out of nine acute wounds healed) and 9.1% in chronic wounds (two out of 22 chronic wounds healed) resulting in an RR of 8.56 of healing for acute versus chronic wounds [95% confidence interval (CI) 2.18 – 33.56] (Fisher's exact test, p≤0.001). This RR means that acute wounds have 8.56 times higher likelihood of healing than chronic wounds. The remaining wounds (22) showed a significant improvement after treatment with the antioxidant dressing.

Only 16 (51.6%) of the 31 patients completed the 8-week treatment with the antioxidant dressing. Complications unrelated to the antioxidant dressing treatment was the only reason cited for patients withdrawn from the study before 8 weeks (hospital admission due to the appearance of other diseases (e.g. pneumonia), infectious agent isolated in the wound that required specific antimicrobial treatment).

The evolution and healing likelihood of acute versus chronic wounds are plotted in Fig 1. The log-rank test was used to establish whether there were any statistically significant differences between the curves. The curves clearly show that the performance and activity of the antioxidant dressing is very similar over the first 4 weeks of treatment for both types of wounds. However, after that time, while acute wounds progressed quickly to closure, chronic wounds, as expected, lagged behind and only 22% of them achieved healing at 8 weeks.

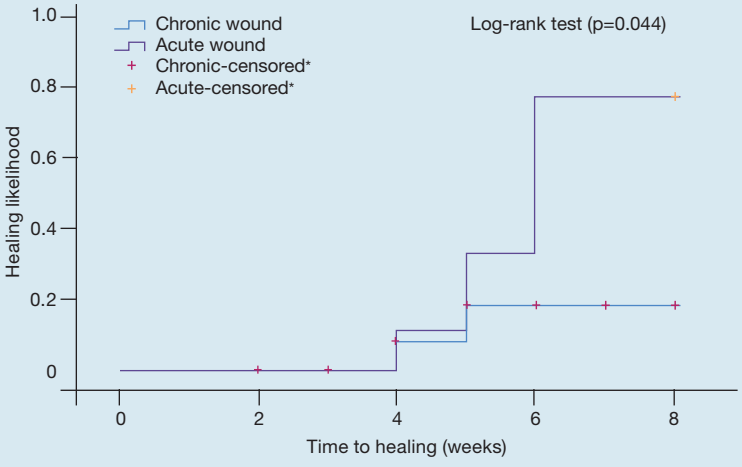
The results on evolution of wound healing during the study, as assessed by RESVECH 2.0, are shown in Fig 2. The data showed statistically significant differences (p≤0.001) in wound evolution along time, as determined by the Friedman test. Data from all wounds, including those of patients withdrawn from the study before the 8-week period, were included in the analysis (ITT).

Table 3. Wounds' characteristics

	Duration (months), mean ± standard deviation (range; median)
Acute (n=9, 29.1%)	3.47±3.07 (0.2–8; 2)
Chronic (n=22, 70.9%)	65.61±105.31 (1–360; 16)
Recurrent (n=13, 41.9%)	100.36±128.54 (2–360; 32)
Acute (n=0, 0.0%)	
Chronic (n=13, 41.9%)	
Non-recurrent	10.22 ± 15.42 (0.2–60; 6)

RESVECH 2.0 scores decreased an average of 10.16 points over the 8 weeks. Surprisingly, by week 4, the mean score had fallen 8.32 points from the start of the study, indicating the important role of the antioxidant dressing in wound activation,

Fig 1. Evolution and healing likelihood of acute versus chronic wounds. Kaplan–Meier curves show the evolution of healing over time. Censored* cases are represented by marks on the curves



Censored—treatment was stopped due to causes not related to the antioxidant dresing

Fig 2. Evolution of wound healing during the study, as assessed by the standard scoring system RESVECH 2.0 (error bar represent 95% confidence interval)

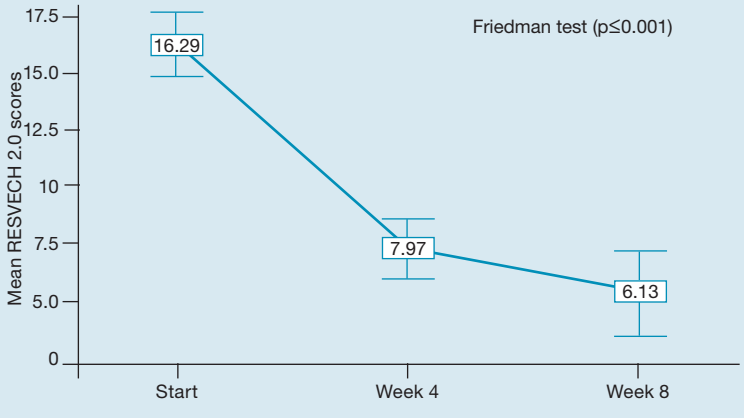
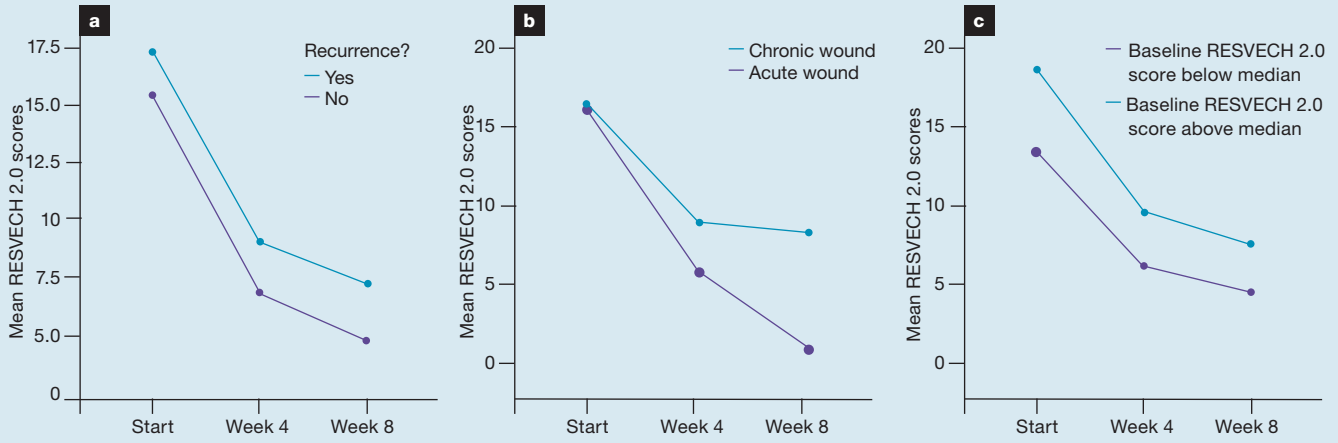


Fig 3. Mean RESVECH 2.0 scores based on the level of recurrence (**a**). Scores obtained when patients were grouped as either having an acute or chronic wound (**b**) and score evolution obtained when patients were grouped following wound severity at baseline (**c**)



exudate control, slough and necrotic tissue elimination, in addition to showing a relevant pain and inflammation reduction.

On the other hand, when RESVECH 2.0 data were analysed based on the recurrence of wounds (Fig 3a), the results showed no differences between recurrent and non-recurrent wounds, since both wounds appeared to respond to treatment in the same manner. In addition, when RESVECH 2.0 scores of acute wounds were plotted against chronic wounds (Fig 3b), a significant reduction in scores in all types of wounds was observed ($p \leq 0.001$). Also, a statistically significant difference between both types of wounds (acute versus chronic) was observed ($p \leq 0.001$) and especially noticeable after week 4, probably

Fig 4. Case 1, a 67-year-old patient treated with the antioxidant dressing for a traumatic wound measuring 62cm^2 and had a volume of approximately 23ml. Complete wound closure was achieved. Before treatment (**a**), at weeks 1 (**b**), 3 (**c**), 5 (**d**), 8 (**e**) and 9 (**f**)

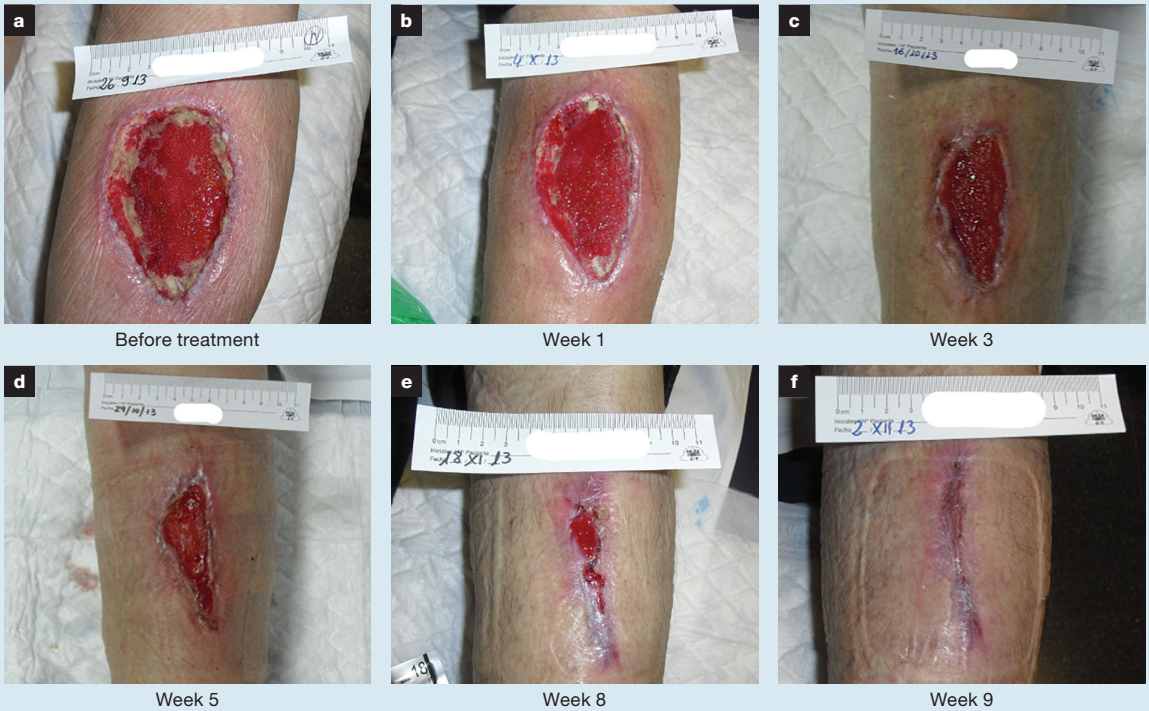
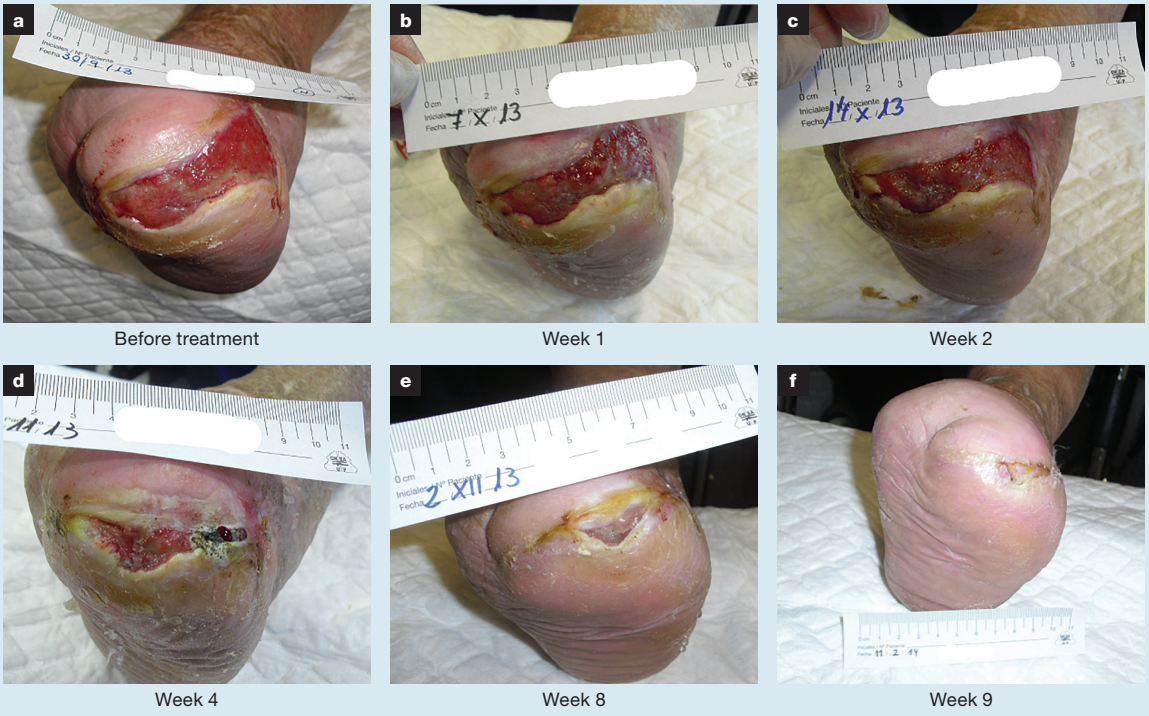


Fig 5. Case 2, a 56-year-old patient with a 6-month dehisced wound measuring around 30cm^2. Wound closure was achieved at week 8. Before treatment (**a**), at weeks 1 (**b**), 2 (**c**), 4 (**d**), 8 (**e**) and 9 (**f**)



due to the high number of wounds healed in the acute group before 8 weeks. When taking into account the severity of wounds according to baseline RESVECH 2.0 scores below or above the median value (Fig 3c), there were no differences between curves and both types of wounds appeared to respond in the same manner.

The following case studies demonstrated good results in the treatment of traumatic wounds (5 of 31 patients), even though all patients with this type of wound presented other important comorbidities, such as diabetes, hypertension or vascular disease.

Case 1

Fig 4 shows a 67-year-old patient with ischaemic cardiomyopathy, chronic obstructive pulmonary disease (COPD), saphenectomy and coronary revascularisation, who was treated with the antioxidant dressing for a traumatic wound (62cm^2 and approximately 23ml). The promotion of granulation tissue formation at the beginning of the treatment reduced the wound's depth by 80% within 1 week, from 23ml to 4.8ml (Fig 4b). The antioxidant dressing also allowed proper epithelialisation of the wound and

Fig 6. Case 3: an 80-year-old patient with a calciphylaxis ulcer. Complete wound closure was achieved. Before treatment (**a**), at weeks 1 (**b**), 3(**c**), 4 (**d**), and 6 (**e**)

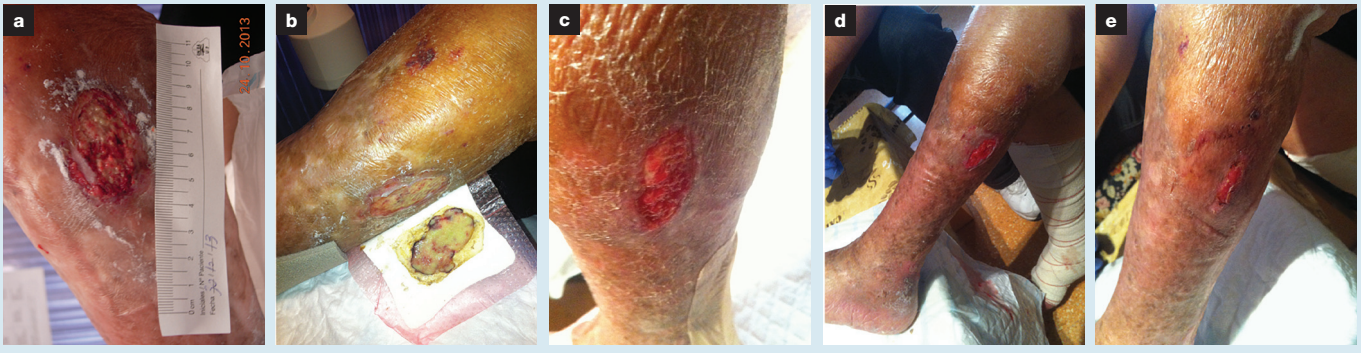
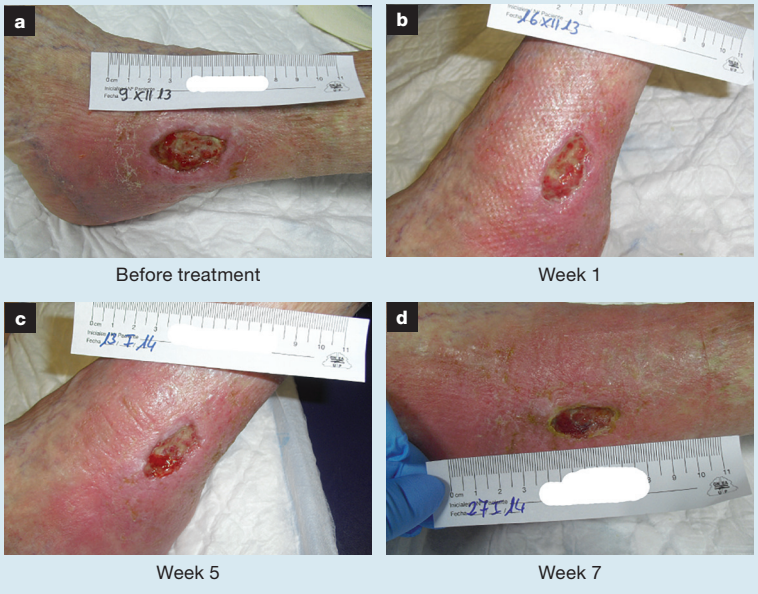


Fig 7. Case 4: a 73-year-old patient with a deep, 16-month chronic venous leg ulcer (VLU) located on the outer ankle. Good evolution towards closure were seen at week 7. Before treatment (**a**), at weeks 1 (**b**), 5 (**c**), and 7 (**d**)



preservation of periwound tissue. Complete wound closure was achieved.

Case 2

Similar results were observed in a 56-year-old patient with multiple comorbidities (diabetes, hypertension, nephropathy, chronic renal failure and obesity) and a 6-month dehisced wound (Fig 5). The wound was surgically debrided before applying the antioxidant dressing. The use of the dressing avoided slough formation and no maceration was observed. A decrease in oedema and exudate was also observed. The patient expressed pain relief after the antioxidant dressing was first applied and throughout the wound healing progress. Despite its large size (around 30cm^2), wound closure was achieved at week 8 (Fig 5e).

Case 3

An interesting case of calciphylaxis ulcer in an 80-year-old patient with chronic kidney disease undergoing dialysis treatment was also observed (Fig 6). After treatment with the antioxidant dressing, a rapid evolution of the wound from the first dressing change was observed. The potent debridement effect of the antioxidant dressing eliminated all necrotic tissue from the wound. Healthy wound bed tissue and granulation tissue was observed. Complete wound closure was achieved.

Case 4

A 73-year-old patient with a deep, 16-month chronic venous leg ulcer (VLU) located on the outer ankle was treated with the antioxidant wound dressing (Fig 7). At week 1, a reduction in oedema, erythema and exudate was observed (Fig 7b). Good evolution towards closure were seen at week 7 (Fig 7d). However, healing in this and similar cases progressed, in general, slower than in other cases. It is worthwhile noting that most patients in the VLU group presented very old wounds (up to 360 months) with previous recurrent infections and important concomitant diseases, and that 30% of these wounds were located on the outer ankle (7 of a total of 20 ulcers).

Overall, the antioxidant dressing helped achieve complete healing or significant improvement at the end of the study in all wounds, regardless of their aetiology, stage or duration. In addition, the dressing demonstrated, in both acute and chronic cases, a marked autolytic debridement action that removed slough and necrotic tissue, leaving a clean and healthy wound bed. Levels of exudate were well controlled, so that neither maceration signs nor drying effects were observed. The assessment of the product by patients and professionals was very good in all aspects evaluated, and no adverse events or side effects were reported.

Discussion

We opted for a multicentre, prospective, single-group, repeated measures study, using patients' previous lack of response to treatment as baseline control. We wanted to evaluate safety and performance, and how well the dressing worked in wounds of different aetiologies. The selected study design has the advantage that the same patient serve as its control; however, the patient's situation before recruitment must be stable and no changes in evolution can occur. This was achieved by reviewing the patients' clinical history and assuring that wounds were hard to heal. We thought that hard-to-heal wounds frequently present in elderly patients or individuals with many other serious comorbidities could benefit most from the treatment with the antioxidant wound dressing; therefore, most patients recruited for the study fit this profile.

As a consequence of the selected population of patients, a large number of them were withdrawn from the study before the 8-week cut-off point, but for reasons not related to the antioxidant dressing treatment. A drawback of limiting the study time to 8 weeks with this type of wounds is that a lower number of patients will achieve wound closure during the study. As the antioxidant dressing was designed to control the excess of ROS present mainly in the first inflammatory phase of the wound healing process, we considered that 8-week period was a suitable timeframe to observe the effects of the dressing in non-healing wounds. In fact, approximately 29% of the wounds healed before 8-weeks.

Only ulcers from any aetiology located on lower limbs but excluding ischaemic conditions were included in the study. The study's exclusion criteria intentionally left out patients with pressure ulcers, because in this type of wounds, many other factors that depend on the quality of care of the patient, such as appropriate repositioning, support surfaces and

nutrition, are key to wound progression and healing. It would be harder to isolate the benefits of the wound dressing from the rest of the factors in a study with a small number of patients and a large number of different types of wounds.

The use of the scale RESVECH 2.0 allowed us to estimate simultaneously different parameters that indicated the progression towards healing of wounds treated with he antioxidant dressing. This wound healing index was specifically developed for chronic wounds after a systematic analysis of most available scoring systems.[25] The results showed that RESVECH scores fell rapidly especially at the beginning of the treatment, a result in good agreement with the expected mechanisms of action of the antioxidant dressing. Despite the fact that RESVECH 2.0 was originally developed for chronic wounds,[25] we applied it also to assess the acute wounds included in the study. The scale proved to be an excellent tool for comparison of the data grouped according to the type of wound (acute or chronic), recurrence or severity.

One of the main problems in wound care is the presence of bacterial biofilms that delay healing and reduce the effectiveness of antibiotics and antiseptics.[30] In the current study, we followed Metcalf et al's clinical diagnosis of bacterial biofilms recommendations, even though they are not yet validated.[31] We observed that the antioxidant dressing worked well when removing biofilm and preventing new biofilm formation. This could be due to a physical effect resulting in the interaction of the galactomannan-based porous matrix of the dressing with the bacteria and the biofilm's polysaccharides. In addition, although the concentration of NAC in the hydration solution is low (5mm) to prevent cytotoxic effects on the wound bed, this molecule may partially be responsible for the anti-biofilm activity observed. It has previously been described that NAC can inhibit both bacterial and fungal biofilm formation.[32–34] Also, the other antioxidant component present in the hydration solution, curcumin, has been described to have antibiofilm activity.[35,36] Finally, the presence of the stabiliser EDTA in the hydration solution could similarly have partially been responsible for the antibiofilm activity.[37,38]

The detoxification effects of the antioxidant dressing probably had an important impact on the reduction of inflammation, which translated into pain relief and dermal and epidermal tissue regeneration. In most cases, once the investigators saw the dramatic change in wound health and progression out of the inflammatory phase into other phases of wound healing, they changed the treatment procedures to other types of dressings. This was due to the fact that a chronic wound, especially the types included in this study, is a more complex and different disease,[39] where factors related to patient's comorbidities, life conditions and a different wound environment make it necessary to apply other treatment options that induce specifically wound re-epithelialisation. These additional treatments, such as collagen or other components that provide new extracellular matrix and protect newly formed tissue, are used due to the poor capacity of their fibroblasts and keratinocytes to proliferate and finally close the wound.

It is worth bringing up the case of the patient with calciphylaxis. The yearly incidence of this disease is estimated at 1% in patients undergoing dialysis, and the mortality rate is up to 80%, often within several months of onset.[40] The primary cause of death from this pathology is due to secondary infection of the ulcers and sepsis.[41] Although we treated only one case with this type of ulcer, our results indicate that the antioxidant dressing could be considered a first choice of treatment for calciphylaxis ulcers. The results obtained in this case are probably due to the fact that the dressing is capable of liberating the wound bed from necrotic tissue and controlling the pro-inflammatory environment.

Limitations

Most of the patients included in the study presented very chronic wounds and also important comorbidities. These facts caused that a per centage of patients could not finish the entire 8-week treatment period with the antioxidant dressings. Furthermore, the number of recruited patients was less than initially expected, due to causes beyond our control (one of the investigators changed his working hospital).

Conclusion

The results obtained in this case study series indicate that treatment with the antioxidant dressing was more marked in the first 4 weeks, that the dressing works well both with acute and chronic wounds, and that it can be applied to wounds independently of their level of recurrence or severity, effectively eliminating the biofilm and facilitating the progression of the wound out of the inflammatory phase. These findings suggest that the antioxidant dressing could represent a new and advanced alternative in the dressing landscape for many types of acute and chronic wounds. JWC

Acknowledgments

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Reflective questions

- Describe the role of oxidative stress in delayed wound healing?
- Is the antioxidant dressing indicated for both acute and chronic wounds?
- Which wound healing phase benefit most from the use of the antioxidant dressing? Explain your answer

References

1 Günter CI, Machens HG. New strategies in clinical care of skin wound healing. Eur Surg Res 2012; 49(1):16–23. http://doi.org/10.1159/000339860

2 Tsala DE, Amadou D, Habtemariam S. Natural wound healing and bioactive natural products. Phytopharmacology 2013; 4(3):532–560

3 Jull AB, Walker N, Deshpande S. Honey as a topical treatment for wounds. Cochrane Database Syst Rev 2013; 6(2): CD005083

4 Murphy PS, Evans GR. Advances in wound healing: a review of current wound healing products. Plast Surg Int 2012; 2012:1–8. http://doi.org/10.1155/2012/190436

5 Saha S, Smith MB, Totten A et al. Pressure Ulcer Treatment Strategies: Comparative Effectiveness. Rockville (MD): Agency for Healthcare Research and Quality (US); 2013

6 Schäfer M, Werner S. Oxidative stress in normal and impaired wound repair. Pharmacol Res 2008; 58(2):165–171. http://doi.org/10.1016/j.phrs.2008.06.004

7 Vermeij WP, Backendorf C. Skin cornification proteins provide global link between ROS detoxification and cell migration during wound healing. PLoS ONE 2010; 5(8):e11957. http://doi.org/10.1371/journal.pone.0011957

8 Wagener F, Carels C, Lundvig D. Targeting the redox balance in inflammatory skin conditions. Int J Mol Sci 2013; 14(5):9126–9167. http://doi.org/10.3390/ijms14059126

9 Roy S, Khanna S, Nallu K et al. Dermal wound healing is subject to redox control. Mol Ther 2006; 13(1):211–220. http://doi.org/10.1016/j.ymthe.2005.07.684

10 Fitzmaurice SD, Sivamani RK, Isseroff RR. Antioxidant therapies for wound healing: a clinical guide to currently commercially available products. Skin Pharmacol Physiol 2011; 24(3):113–126. http://doi.org/10.1159/000322643

11 Sen CK. The general case for redox control of wound repair. Wound Repair Regen 2003; 11(6):431–438. http://doi.org/10.1046/j.1524-475X.2003.11607.x

12 Moseley R, Walker M, Waddington RJ, Chen WY. Comparison of the antioxidant properties of wound dressing materials–carboxymethylcellulose, hyaluronan benzyl ester and hyaluronan, towards polymorphonuclear leukocyte-derived reactive oxygen species. Biomaterials 2003; 24(9):1549–1557. http://doi.org/10.1016/S0142-9612(02)00540-9

13 Hemagel. Wound healing with the aid of free radical binding technology. http://www.hemagel.co.uk (accessed 24 November 2017)

14 Chua LS, Rahaman NLA, Adnan NA et al. Antioxidant activity of three honey samples in relation with their biochemical components. J Anal Methods Chem 2013; e313798. http://doi.org/10.1155/2013/313798

15 Brudzynski K, Abubaker K, Laurent M, Castle A. Re-examining the role of hydrogen peroxide in bacteriostatic and bactericidal activities of honey. Frontiers in Microbiology 2011; 2:213. http://doi.org/10.3389/fmicb.2011.00213

16 Kwakman PH, Zaat SA. Antibacterial components of honey. IUBMB Life 2012; 64(1):48–55. http://doi.org/10.1002/iub.578

17 Gupta SC, Patchva S, Koh W, Aggarwal BB. Discovery of curcumin, a component of golden spice, and its miraculous biological activities. Clin Exp Pharmacol Physiol 2012; 39(3):283–299. http://doi.org/10.1111/j.1440-1681.2011.05648.x

18 Scharstuhl A, Mutsaers HA, Pennings SW et al. Curcumin-induced fibroblast apoptosis and in vitro wound contraction are regulated by antioxidants and heme oxygenase: implications for scar formation. J Cell Mol Med 2009; 13(4):712–725. http://doi.org/10.1111/j.1582-4934.2008.00339.x

19 Sidhu GS, Singh AK, Thaloor D et al. Enhancement of wound healing by curcumin in animals. Wound Repair Regen 1998; 6(2):167–177. http://doi.org/10.1046/j.1524-475X.1998.60211.x

20 Jagetia GC, Rajanikant GK. Effect of curcumin on radiation-impaired healing of excisional wounds in mice. J Wound Care 2004; 13(3):107–109. http://doi.org/10.12968/jowc.2004.13.3.26589

21 Ozkaya H, Bahat G, Tufan A et al. Successful treatment of non-healing pressure ulcers with topical n-acetyl cysteine. J Wound Care 2015; 24(12):606–611. http://doi.org/10.12968/jowc.2015.24.12.606

22 Deniz M, Borman H, Seyhan T, Haberal M. An effective antioxidant drug on prevention of the necrosis of zone of stasis: N-acetylcysteine. Burns 2013; 39(2):320–325. http://doi.org/10.1016/j.burns.2012.06.015

23 Kheradpezhouh E, Panjehshahin MR, Miri R, Javidnia K, Noorafshan A, Monabati A et al. Curcumin protects rats against acetaminophen-induced hepatorenal damages and shows synergistic activity with N-acetyl cysteine. Eur J Pharmacol 2010; 628(1-3):274–281. http://doi.org/10.1016/j.ejphar.2009.11.027

24 Castro B, Palomares T, Azcoitia I et al. Development and preclinical evaluation of a new galactomannan-based dressing with antioxidant properties for wound healing. Histol Histopathol 2015; 30(12):1499–1512. http://doi.org/10.14670/HH-11-646

25 Restrepo-Medrano JC, Verdú Soriano J. Development of a wound healing index for chronic wounds. EWMA J 2012; 12(2):39–46.

26 Avilés MJ, Sánchez JJ. [In Spanish] Guía de práctica clínica para el cuidado de personas con úlceras por presión o riesgo de padecerlas. https://tinyurl.com/y828ymlh (accessed 26 October 2017)

27 González de la Torre H, Quintana-Lorenzo ML, Perdomo-Pérez E et al. Correlation between health-related quality of life and venous leg ulcer's severity and characteristics: a cross-sectional study. Int Wound J 2017; 14(2):360–368. http://doi.org/10.1111/iwj.12610

28 Faul F, Erdfelder E, Buchner A, Lang AG. Statistical power analyses using G*Power 3.1: Tests for correlation and regression analyses. Behav Res Methods 2009; 41(4):1149–1160. http://doi.org/10.3758/BRM.41.4.1149

29 Gupta S. Intention-to-treat concept: A review. Perspectives in Clinical Research 2011; 2(3):109–112. http://doi.org/10.4103/2229-3485.83221

30 James GA, Swogger E, Wolcott R et al. Biofilms in chronic wounds. Wound Repair Regen 2008; 16(1):37–44. http://doi.org/10.1111/j.1524-475X.2007.00321.x

31 Metcalf DG, Bowler PG, Hurlow J. A clinical algorithm for wound biofilm identification. J Wound Care 2014; 23(3):137–142. http://doi.org/10.12968/jowc.2014.23.3.137

32 Zhao T, Liu Y. N-acetylcysteine inhibit biofilms produced by Pseudomonas aeruginosa. BMC Microbiol 2010; 10(1):140. http://doi.org/10.1186/1471-2180-10-140

33 Gupta P, Sarkar S, Das B et al. Biofilm, pathogenesis and prevention—a journey to break the wall: a review. Arch Microbiol 2016; 198(1):1–15. http://doi.org/10.1007/s00203-015-1148-6

34 Rasmussen K, Nikrad J, Reilly C et al. N -Acetyl- L -cysteine effects on multi-species oral biofilm formation and bacterial ecology. Lett Appl Microbiol 2016; 62(1):30–38. http://doi.org/10.1111/lam.12513

35 Packiavathy IA, Priya S, Pandian SK, Ravi AV. Inhibition of biofilm development of uropathogens by curcumin – An anti-quorum sensing agent from Curcuma longa. Food Chem 2014; 148:453–460. http://doi.org/10.1016/j.foodchem.2012.08.002

36 Hu P, Huang P, Chen MW. Curcumin reduces Streptococcus mutans biofilm formation by inhibiting sortase A activity. Arch Oral Biol 2013 Oct;58(10):1343–1348. http://doi.org/10.1016/j.archoralbio.2013.05.004

37 Finnegan S, Percival SL. EDTA: An antimicrobial and antibiofilm agent for use in wound care. Adv Wound Care 2015; 4(7):415–421. http://doi.org/10.1089/wound.2014.0577

38 Al-Bakri AG, Othman G, Bustanji Y. The assessment of the antibacterial and antifungal activities of aspirin, EDTA and aspirin-EDTA combination and their effectiveness as antibiofilm agents. J Appl Microbiol 2009; 107(1):280–286. http://doi.org/10.1111/j.1365-2672.2009.04205.x

39 Harding K. Simplifying venous leg ulcer management: consensus recommendations. Wounds UK, 2015. http://bit.ly/2zmGa8R (accessed 24 November 2017)

40 Solanky D, Hwang SM, Stone G et al. Successful surgical treatment of severe calciphylaxis using a bilayer dermal replacement matrix. Wounds 2015; 27(11): 302–307.

41 Nigwekar SU, Kroshinsky D, Nazarian RM et al. Calciphylaxis: risk factors, diagnosis, and treatment. Am J Kidney Dis 2015; 66(1):133–146. http://doi.org/10.1053/j.ajkd.2015.01.034