Evaluation of the mode of action of a new gel wound dressing

KEY WORDS
- Protease inhibition
- MMPs
- Leg ulcer
- Wound pain

This study aimed to evaluate the mode of action of a new dressing, KerraLite Cool®. KerraLite Cool was shown to inhibit the pro- and activated forms of the matrix metalloproteinase MMP-2 and MMP-9 *in vitro*. In the corresponding clinical investigations involving 11 patients with venous or mixed aetiology leg ulcers, visible signs of improvement were apparent after 14 days treatment in all subjects. KerraLite Cool exerts a marked protease inhibitory activity that may be effective under physiological conditions. The mechanism of inhibition appears to be a combination of modifying the ionic environment and selective protease depletion. Clinical work with KerraLite Cool consistently points to a significant reduction in wound pain and the stimulation of stalled wounds.

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Wound healing is a dynamic and complex biological process involving highly organised overlapping phases, including coagulation, inflammation, epithelialisation, matrix deposition, angiogenesis, proliferation, cellular remodelling and wound contraction (Clark, 1985; Mast and Schultz, 1996). For completion of each stage of wound healing, complex interactions must occur between various biological factors, e.g. growth factors and cell types (Nwomeh et al, 1998). Together these processes result in the restoration of tissue integrity and functional healing (Hackam and Ford, 2002). A variety of factors influence wound healing adversely, including the presence of infection, necrotic tissue, impaired tissue perfusion, and clinical conditions such as venous and arterial insufficiency, advanced age, steroid administration and diabetes (Hackam and Ford, 2002).

The majority of cells playing a role in wound healing require matrix metalloproteinases (MMPs) to perform their normal physiological role. As part of the inflammatory response, macrophages and neutrophils produce and utilise MMPs for debridement of necrotic tissue, while all cells require expression of surface MMPs to allow them to dissect a migratory pathway through extracellular matrix (ECM). MMPs are produced by fibroblasts to remodel ECM, which strengthens scar tissue following wound closure (Gibson et al, 2009). MMPs thus play a role in inflammation, granulation tissue formation, re-epithelialisation and remodelling during normal healing, although their levels decrease rapidly as healing proceeds (Tarlton et al, 1997).

In acute wounds, a burst of protease activity is seen at the start of wound healing, which peaks at about day 3 and starts to reduce by day 5 (Gibson et al, 2009). In chronic wounds, however, an imbalance of MMPs is associated with impaired wound healing (Schultz and Wysocki, 2009). In these non-healing wounds, MMPs reach higher levels and persist for longer, which may lead to the degradation of the ECM, prevention of cellular migration and cause tissue destruction (Trengove et al, 1999). A prolonged high protease activity may be stimulated by the presence of damaged tissue, foreign material, bacteria and biofilms (International Consensus, 2011).

The gelatinases MMP-2 and MMP-9 digest a number of ECM molecules, including type IV, V and XI collagens, laminin, aggrecan core protein, while MMP-2, but not MMP-9, digests collagens I, II and III (Nagase et al, 2006). High levels of activated MMPs, including MMP-2 and MMP-9, are seen in non-healing wounds and this may play a major role in chronic wounds failing to close (Wysocki et al, 1993). Elevated levels of MMP-9

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appear to directly delay wound healing, possibly through interference with re-epithelialisation (Reiss et al, 2010).

New local treatments for chronic wounds need to interact or modify these local conditions, and so move the wound to a stage where healing occurs. KerraLite Cool® (Crawford Healthcare) consists of a patented pro-ionic® copolymer matrix with a high Moisture Vapour Transmission Rate (MVTR) polyurethane (PU) film barrier. It has been formulated and designed specifically to encourage wound-bed preparation, granulation and subsequent epithelialisation of chronic wounds, while minimising pain levels and the risk of infection. The matrix's absorbency comes from a super-absorbent polymer that is partially pre-hydrated, to help moisturise and rehydrate dry skin. Unlike fibrous dressings, the matrix maintains its gel-like consistency even if the wound exudate level decreases, meaning that the dressing will not adhere to or dry out the wound bed.

The aim of this study was to:

- Evaluate protease inhibition *in vivo* and *in vitro* by KerraLite Cool
- Evaluate the wound environment during treatment with KerraLite Cool, including the change in number and species of bacteria present before and after treatment
- Relate the ability of the wound dressing to both debride the leg ulcer and to promote healing of the wound.

MATERIALS AND METHODS

Wound healing products

The topical wound healing products used in this study were KerraLite Cool and a non-adherent hydrogel sheet dressing.

Pre-clinical study: protease inhibition *in vitro*

MMP inhibition by KerraLite Cool was tested *in vitro* using gelatin zymography. Gelatin zymography was performed as previously described (Tarlton et al, 1999), using MMP-2 and MMP-9 (Calbiochem) as standards. Proteolysis buffers (6.25 mM NaCl/0.625 mM $CaCl_2$ and 3.125 mM NaCl/0.3125 mM $CaCl_2$ initial buffer concentrations) were pre-treated, for 3 hours at room temperature with agitation, with KerraLite Cool and the non-adherent hydrogel sheet control dressing. Zymograms incorporating MMP-2 and MMP-9 standards were incubated in 10 ml of the pre-absorbed buffers, in duplicate, and in untreated buffer as control for 18 hours at 37˚C. Each gel was washed, stained and scanned, while each assay was repeated five times.

CLINICAL PILOT STUDY

This evaluation was a single-centre, prospective, non-randomised, non-blinded pilot observational clinical trial involving individuals who have venous or mixed aetiology leg ulcers. The trial was conducted by research nurses from the Wound Healing Research Unit (WHRU) in Cardiff. The research nurses performed all assessments.

Eleven patients attending the WHRU were recruited. They had venous or mixed aetiology leg ulcers with a surface area of 2–25 cm^2 that had persisted for more than 3 months. Each subject's leg ulcer was dressed with KerraLite Cool for 14 days, combined with the use of appropriate multilayered compression bandages. Dressing changes were made on days 1, 3, 7, 10 and 14.

Collection and analysis of wound fluid

Chronic wound fluid samples were collected from all patients using filter paper to sample fluid at the surface of the wound. Wound fluid samples were collected immediately prior to the first application of KerraLite Cool, then after 24 hours, 3 and 14 days. The filter paper remained in place until soaked with wound fluid. Following removal of cellular debris, samples were stored at –80°C until analysis. The wound fluid collected at days 0, 1, 3 and 14 was analysed to identify protease activity (colorimetric method), sodium, potassium, calcium and chloride levels (ion-specific electrode method) with the pH of the fluid also recorded.

Microbiological swab and analysis

The microbiological flora was sampled using wound surface swabs taken before and after each treatment session on days 0, 1, 3 and 14. The bacteria in the saline solution were quantified using standard microbiological techniques; colony counts were performed and viable cells in the original sample were calculated using standard enumerative methods. The average colony forming units (CFU) per ml was determined (for

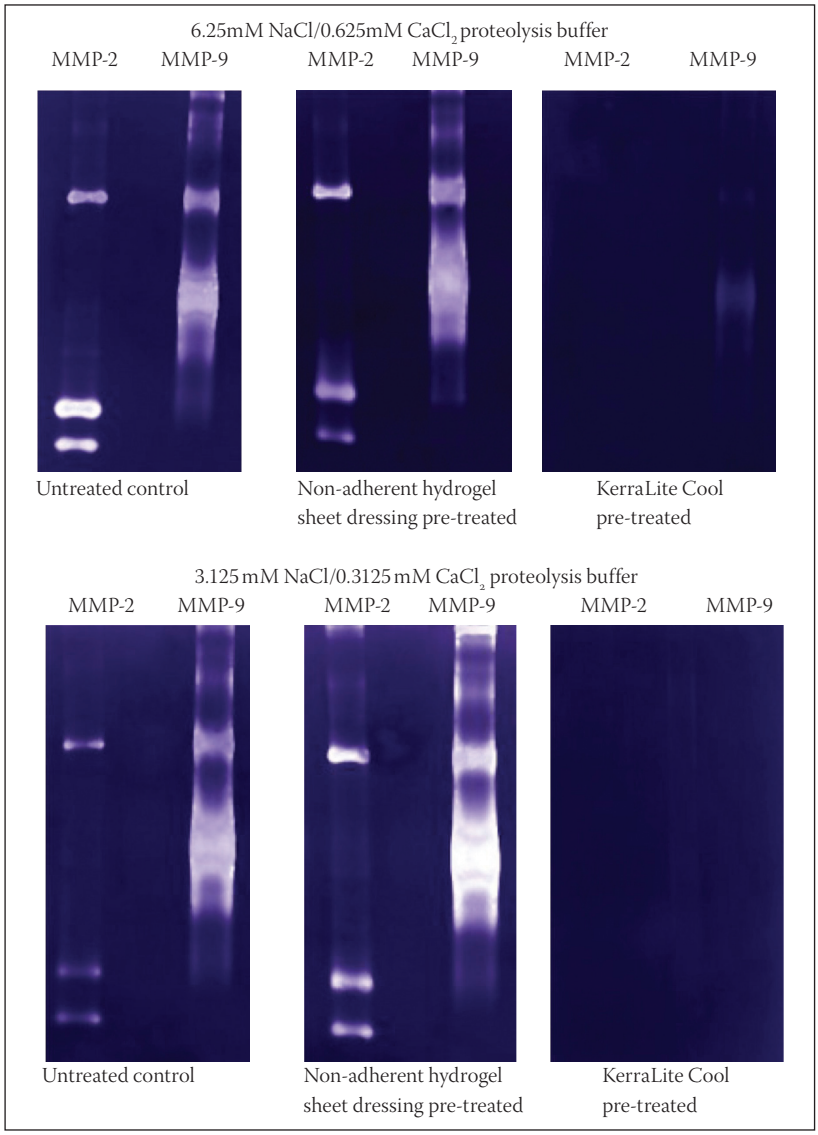

Figure 1. Example zymogram showing the effect of dressing pre-treatment of low ionic strength proteolysis buffer.

aerobic and anaerobic organisms) and recorded for each test area. The first and last swabs from each subject were characterised and the bacterial species present recorded on the laboratory case report form.

Wound healing measurements

Digital images of the wounds taken using standardised lighting and background at each clinic visit were used to investigate the ability of KerraLite Cool to promote healing of the wound. The surface area of each wound was then traced using Visitrak (Smith & Nephew), an electronic system that quantifies the area and depth of wounds. Skin blood flow and surface temperature were recorded on days 0, 1, 3, 7, 10 and 14. Blood flow and skin temperature were measured within 2 cm around the peri-ulcer skin, using a single-point laser Doppler flowmetry probe and infrared non-contact thermometer (Exergen) respectively.

Pain scale

Any pain or discomfort associated with the treatment were recorded daily using a 10 cm Likert scale anchored by descriptors 'very painful' to 'no pain' (leg ulcer) and 'no discomfort' to 'very uncomfortable' (treatment process). Each subject was asked to mark their perceptions of leg ulcer pain and the treatment process on the scales.

Statistical analysis

The analysis of the collected data was performed by the Wound Healing Research Unit using SPSS (SPSS Inc). Given that this was a pilot study to elucidate whether the KerraLite Cool dressing played any role in affecting wound fluid components or in the control of bacterial numbers (and promotion of wound healing), no formal sample size calculation was performed and there was no control arm. Consequently, most data are presented as descriptive statistics. All analyses were based on the data collected using the Clinical Report Forms.

Ethics

This study was conducted in accordance with the principles that have their origin in the Declaration of Helsinki and all appropriate local laws and regulations controlling investigations using patients as research subjects. Research Ethics Committee approval was obtained by First Water Ltd prior to study commencing.

RESULTS

Protease inhibition

Pre-treatment of proteolysis buffer with KerraLite Cool inhibited the pro- and activated forms of both MMP-2 and MMP-9. In contrast, pre-treatment with the non-adherent hydrogel sheet dressing enhanced the observed enzyme activities. These effects are clearly demonstrated by the example zymogram shown in *Figure 1*.

Clinical pilot study

A total of 11 patients were successfully recruited to the study. One subject was withdrawn from the 14-day evaluation period after developing a wound infection on day 10. This was not considered to be related to the primary dressing. No patient was excluded from all the analyses. For two patients there is an incomplete data set, with the appearance of the wound bed not recorded on day 0 or day 14 respectively, but these have been included in the analyses for those time points where data were present.

The subjects ranged in age from 56 to 89 years (mean age +/- 1 standard deviation (SD); 71.0 +/- 10.6 years). The most commonly reported related medical conditions reported among the subjects were circulatory disorders (*n*=6), diabetes (*n*=4), and connective tissue disorders (*n*=4). Of the 10 subjects, eight received analgesia during the study, however the dosages were not recorded as this fell outside the study protocol.

All subjects bar one had a history of previous leg ulcers at the current site of open ulceration with the current ulcer located at the medial malleolus (*n*=8), calf (*n*=1) or gaiter area (*n*=1). At the start of treatment with KerraLite Cool, the mean surface area of the leg ulcers was 6.9 cm^2 (SD 4.6 cm^2) and ranged from 2.6 to 15.8 cm^2. Only two wounds had an initial surface area greater than 10 cm^2. Nine patients reported their leg ulcer to be painful before the start of treatment, with eight reporting moderate pain and one experiencing mild pain. No subject reported severe pain associated with their leg ulcer. All of the subjects had light levels of exudate discharge from their wound prior to treatment.

Visual assessment of the principal types of tissue visible within the wound bed was undertaken prior to the start of treatment, with most of the wound bed covered with slough (mode response 60%) or granulation tissue (mode response 30%). Other tissue types (e.g. islands of epithelium or fibrous tissue) were observed rarely within the wounds, and in no case did these other tissue types cover more than 10% of the wound surface area. The skin surrounding the ulcer prior to treatment was dry and exhibited local erythema across all subjects, with localised oedema reported in three wounds and eczema seen in five people. Other surrounding skin conditions were observed, such as lipodermatosclerosis (*n*=2) and atrophy blanche (*n*=3), however these tended to be relatively rare within the patient group.

Effect of KerraLite Cool on wound fluid composition

Problems were encountered during the analysis of the collected wound fluid. Primarily these issues were:

- pH was only measured to whole pH units.
- Potassium and chloride data was invalid, with a non-linear response to dilution of the samples reported by the test laboratory after the analyses had been performed.
- The protease analysis was discontinued given uncertainty regarding the validity of the data at the wound fluid volumes available for testing.

Given these issues that generally reduced confidence in the quality of the data reported by the test laboratory, limited inferences have been drawn from the available data. Little change in pH was noted over the 14-day study. The fluid in three subjects was slightly acidic at baseline (pH range from three measurements at each time point 6.3 to 6.7). The fluid from three was neutral, and four showed slightly alkaline wound fluid (pH 7.3–8). After 14 days, five were pH neutral with the remaining four slightly alkaline (pH 7.3–8); one subject's wound fluid pH was unreported at 14 days). The total protein to sodium ratio increased between day 0 and day 1 and remained elevated during the study period, whereas the total protein-to-calcium ratio remained relatively constant over the study period.

Effect of KerraLite Cool on bacteria

A key outcome measure of this pilot evaluation was whether covering the wound with the KerraLite Cool dressing reduced the number of bacteria located on the surface of the leg ulcers. Prior to treatment, the mean (SD) number of colony-forming aerobic and anaerobic bacteria were 11.7 (14.5) and 17.2 (45.4) respectively. On recruitment to the study, aerobic bacteria were identified from six subjects and anaerobic bacteria were isolated from only three subjects. Given the abnormal distribution of the number of bacteria identified in the leg ulcers on recruitment to

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the study, the median number of CFU is shown in *Table 1*. The mean and median number of CFU aerobic and anaerobic bacteria showed no consistent trends over the 14 days of dressing the wound with the KerraLite Cool dressing. Twelve species of aerobic bacteria and five anaerobic species were identified from swabs taken on day 0 and day 14, with the most common species being *Staphylococcus aureus* identified from six subjects (day 0) and from seven subjects on day 14.

Changes in wound size

Over the course of the 14-day treatment of the subjects' leg ulcers with the KerraLite Cool dressing, it was not anticipated that any of the wounds would fully heal (defined as a restoration of an intact epithelial layer over the surface of the wound). While no wound healed during treatment, the surface area of five wounds decreased (mean decrease [SD]; 18.0% [18.4]) with the other five wounds becoming slightly larger (mean increase [SD]; 17.6% [8.7]). In absolute terms, these changes in wound surface area were small, ranging from a maximum reduction of 1.5 cm^2 to a maximum increase of 1.2 cm^2. Increase in wound size may be due to the KerraLite Cool dressing debriding the wound bed and edges.

Relationship between number of bacteria and wound surface area

On recruitment to the study there was an apparent trend for large wounds (surface area greater than 5 cm^2) to have more aerobic or anaerobic CFU compared with smaller wounds (aerobic CFU mean [SD]; small wounds 8.7 [10.7], large wounds 13.7 [17.2]; anaerobic CFU mean [SD]; small wounds 1.5 [3.0], large wounds 27.7 [58.1]). However, given the wide variability in CFU counts, this apparent trend did not achieve statistical significance.

Table 1. Median number of CFU aerobic and anaerobic bacteria present on the surface of the leg ulcers dressed with KerraLite Cool.

Day of treatment	Median number of colony forming units (mean* and SD in brackets)	
	Aerobic	Anaerobic
0	5.5 (11.7, 14.5)	0 (17.2, 45.4)
1	1.5 (9.7, 15.3)	0 (1.6, 4.1)
3	5.5 (12.1, 17.7)	0 (3.4, 6.5)
14	1.5 (9.1, 15.1)	0 (10.4, 23.8)

* Mean (SD) is shown to highlight the skewed distribution of this data.

Experience of the dressing

Subjects reported pain associated with their leg ulcer while dressed with the KerraLite Cool dressing – on a visual analogue scale scored from 0 (no pain) to 10 (severe pain) the mean (SD) pain scores were 2.5 (3.0) on the day of recruitment to the study (day 0); on day 1 1.7 (2.7) day 3, 2.1 (2.9) and 1.5 (2.9) on the final day of treatment with the KerraLite Cool dressing. The reduced pain reported between day 0 and day 14 approached statistical significance ($t=2.24$, $df=9$, $P=0.052$).

Changes in wound appearance

Exudate levels remained relatively constant throughout the 14 days use of KerraLite Cool with all subjects reported light exudate on days 0 and 1 with moderate exudate levels reported from three and two patients on days 3 and 14, respectively. Other aspects of the wound's appearance improved during the study period.

The wound edge was reported to show epithelial tissue in six subjects on recruitment, with three showing a static wound edge — after 14 days all subjects were reported to have visible epithelial tissue at the wound edge. The tissues visible within the wound bed also changed during the 14 days — on recruitment to the study, 40% of the wound bed contained granulation tissue with 60% covered by slough.

At the end of the pilot study, 80% of the wound bed was covered by granulation tissue with the amount of slough reduced to 20% of the wound bed. There were relatively few changes in the appearance of the skin surrounding the wound over the 14 days — all patients had areas of dry skin at the time of recruitment and 14 days later three had areas of macerated skin following use of the KerraLite Cool dressing.

Blood flow, skin temperature and use of the dressing

The temperature of the clinical area where subjects were assessed ranged from a mean temperature of 24.76˚C (SD 0.85, day 1) to a

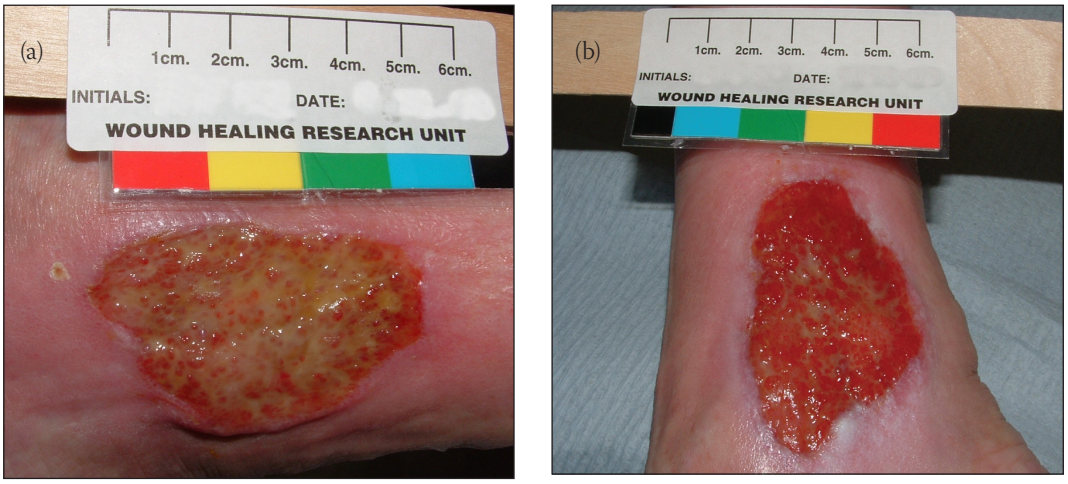

Figure 2. (a) Patient with ulcer of 4 months duration which measured 15.8 cm^2 at day 0. (b) After 10 days, slough decreased and granulation tissue increased.

maximum of 25.32˚C (SD 0.94, day 14). The surface temperature at the wound site increased over the course of treatment from a mean temperature of 29.03˚C (SD 1.35, day 0) to 29.76˚C (SD 1.06, day 14), this increase was statistically non-significant (t=1.61, df=9, *P*=0.14). The elevated surface temperature was not mirrored by increased skin blood flow around the wound site with mean blood flow reducing over the 14 days from a mean flux of 28.30 arbitrary units (SD 35.11) to a mean of 20.05 (SD 11.45, on day 14).

Anecdotal feedback from clinicians

Although not collected in any systematic manner, the clinicians involved in this study offered several relevant observations about the KerraLite Cool wound dressing. The dressing was easy to apply, with no apparent discomfort or stinging on application, and was easy to remove with irrigation. While in use the dressing was conformable and well-tolerated beneath compression garments. It was noted that in comparison to other hydrogel products there was no odour associated with the KerraLite Cool dressing and less maceration to the surrounding skin. A reduction in the intake of analgesics was noted among several patients in the study.

DISCUSSION

Reducing excessive protease activity in the wound is thought to convert a non-healing wound to a healing state. Products designed to reduce excessive proteolytic activity and rebalance the wound environment ideally need to inactivate both host- and bacteria-derived MMPs and other proteases (Gibson et al, 2009).

MMPs are clearly important in many biological processes in wound healing, hence they are critical to consider when developing improved therapies to enhance both wound healing times and wound healing outcomes.

This initial study has demonstrated that KerraLite Cool may act *in vitro* to inactivate the proteolytic activity of MMP-2 and MMP-9. Protease inhibitory activity was restricted to KerraLite Cool with the non-adherent hydrogel sheet dressing demonstrating little or no inhibition.

The clinical pilot study aimed to explore the potential use of the KerraLite Cool dressing within the treatment of hard-to-heal venous leg ulcers. The intervention was used in the treatment of 11 patients with a leg ulcer. No subject withdrew for a device-related event.

The problems encountered during the analysis of the wound fluid collected from the study leg ulcers reduced confidence in the quality of the electrolyte data. However, there did appear to be an increase in the ratio of total protein to sodium within the first day of exposure to the wound dressing. The significance of this observation requires further exploration.

There were no evident trends between changes in the number of aerobic and anaerobic bacteria

while using the KerraLite Cool dressing, which may simply reflect the contamination of the wound surface during each dressing change combined with the exposure of the wound during the assessment visits to the WHRU clinic.

No wound healed during the short evaluation period. Only minor changes in wound surface area were reported, as would be expected given that the study period was 14 days. An increase in wound size during the study may be due to the KerraLite Cool dressing debriding the wound bed and edges. Both the wound edge and the wound bed appeared to improve over the course of treatment with the KerraLite Cool dressing.

KerraLite Cool facilitates the painless debridement of the wound bed through a combination of autolytic and osmotic debridement. The strong osmotic nature of the gel created a chemical pull that enabled the necrotic tissue to be lifted from the wound bed. Further, this strong osmotic pull helped to draw fluid into the wound bed from the underlying tissues.

Maceration of the surrounding skin was observed in a minority of subjects following use of the KerraLite Cool dressing (n=3). There were apparent increases in skin temperature at the wound site, but reductions in local blood flow over the same period. These observations appear contradictory and may well be false trends generated through the wide variability between the measured skin blood flows across a small sample of subjects.

In recent years there has been an increase in the emphasis of minimising pain experienced during dressing changes. Chronic wound pain forces many people into a life of isolation. Reductions in self-reported pain associated with the leg ulcer were reported following use of the KerraLite Cool dressing and this observation may be useful to follow up in a larger study focused on pain measurement and the use of primary wound dressings, including KerraLite Cool.

Given that positive signs of wound improvement were seen during this short duration evaluation, there would be benefit from formal longer-term observation of wound healing under the KerraLite Cool dressing given that rapid changes were seen in a population of wounds that had not previously responded to treatment.

The non-comparative nature of this pilot study does not allow inferences to be drawn regarding the effectiveness of the intervention — in this evaluation, self-reported pain reduced while the wound edge and bed appeared to show positive improvements following use of the KerraLite Cool dressing, although these trends need to be confirmed in an appropriately powered controlled study.

CONCLUSION

KerraLite Cool exerts a marked protease inhibitory activity that may be effective under physiological conditions. Clinical work with KerraLite Cool points consistently to a significant reduction in wound pain in painful wounds and the stimulation of stalled wounds.

Suggested mechanisms for this include the dressing's enhanced ability to maintain appropriate moisture levels, modulate the activity of proteases in wound fluid and the modulation of inflammation. WUK

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