Meeting report

Biofilms and the role of debridement in chronic wounds

This meeting report focuses on the final plenary session of the opening day of the Wounds UK Harrogate 2009 Conference presented by Professor Rose Cooper, Professor Keith Cutting and Professor Marco Romanelli.

The role of biofilms in chronic wound management has been an issue of intense debate among clinicians and scientists in recent years. Biofilms have been recognised as a problem in engineering and industrial processes for around 120 years. However, their potential influence on wound healing has only recently come under the spotlight. The focus of this session was to identify what biofilms are, how their unique structure and action can influence wound management interventions, how clinicians might recognise them in practice, and how it may be possible to modulate their negative impact on wound healing by the use of an appropriate wound healing technology.

Biofilm in chronic wounds

The event was chaired by Professor Rose Cooper, University of Wales Institute Cardiff, who opened the session with an overview of biofilms; what they are, how they form and what impact they may have on wound healing. She defined biofilms as organisms attached to a substrate and covered with an extracellular polymeric substance (EPS), which is produced collectively. This 'slime city' is a key characteristic of biofilms. In the laboratory it has been possible to cultivate single-species biofilms. However in nature biofilms are found as multi-species colonies.

Professor Cooper explained that mature biofilms act differently to their planktonic, or free-floating counterparts. The bacteria within biofilms change their appearance, shedding flagella if present, and slow their metabolic activity. This change in metabolic status reduces the effectiveness of antimicrobials, making mature biofilms 500–1,000 times less susceptible to their action. The ability of bacteria to communicate is known as quorum sensing. This influences gene expression and contributes to biofilm maturation. Biofilms are not static communities, they will change their characteristics and mobility in response to changes in their environment.

The link between chronic infections and biofilms was identified through research on a number of diseases, such as cystic fibrosis and has shown that possibly 80% of chronic infections can be attributable to biofilm activity (Costerton and Stewart, 1999). In wounds, chronicity is characterised by chronic inflammation, raised pro-inflammatory cytokines and proteases. Damaged growth factors and dysfunctional neutrophils ultimately lead to cell senescence and wound stagnation. However, a link needed to be made between the presence of biofilms and wound chronicity.

Akiyama et al (2003) and Serralta et al (2001) were able to identify the presence of glycocalyx in contaminated animal wounds, however it was the landmark work of James et al (2008) that has led to firm evidence of a biofilm-chronic wound relationship. Here, 30 out of 50 patients with chronic wounds were found to have biofilms present; confirmed by electron and confocal microscopy, and DNA technology. This compared with only 1:16 acute wounds ($p<0.001$). However, Professor Cooper warned clinicians that the presence of wound chronicity in itself does not guarantee the presence of a biofilm; clinicians have to be aware that other potential causes, rather than biofilms, may be a factor in chronicity. Identification of biofilms is not easy. As with planktonic bacteria, the naked eye cannot determine the presence of a biofilm or its causative organisms. Conventional swabbing is often inconclusive and characterising DNA can lead to problems with contamination from transient bacterial carriage. Scanning electron microscopy and confocal laser scanning microscopy have both been of benefit in research, however a much simpler technique needs to be adopted for practical widespread use. If we cannot easily swab and culture bacterium, then the detection of quorum sensing molecules may offer opportunities.

So, how then do biofilms interrupt normal wound healing? Many wounds such as diabetic foot ulcers and pressure ulcers develop into a chronic state, in part due to failure to eradicate infecting, opportunistic pathogens such as *Pseudomonas aeruginosa*. Bjarnsholt et al (2008) developed a theory based on their experimental work in staining bacterial colonies, the precursors to biofilms. They found that *Pseudomonas* produces rhamnolipid, a substance that interrupts neutrophil function ultimately causing their destruction. This chemical, combined with the previously recognised exotoxin A and the blue-green pigment, pyocyanin, produced by *Pseudomonas* produces a toxic wound environment; one in which mammalian cells are broken down and protease and pro-inflammatory cytokine levels rise. The findings of Wolcott et al (2008) suggest that this chronic inflammatory status is further potentiated by the release of planktonic bacteria from the biofilm, which acts as a lure to the host immune system and repeatedly stimulates an inflammatory response; the bacterial products deactivating or interfering with both innate and adaptive immune responses.

The challenge for clinicians is how to manage biofilms. There appears to be five key factors:

- Interfere with matrix formation
- Digest EPS

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- Prevent cell-to-cell communication
- Remove already formed biofilm
- Stop bacteria attaching to host cells.

Some progress towards developing these tools has been made with the early development of products that bind iron in the wound (disrupting bacterial metabolism and attachment to the substrate); dissolve polysaccharides (breaking down EPS); and confuse cell-to-cell communication. In a study carried out by Wolcott and Rhoads (2008), we see that a combined anti-biofilm approach can have exceptional results. Here, in a group of 190 patients with critical limb ischaemia, 77% achieved complete healing with a multifaceted approach, including wound debridement. Of those that failed to heal, 75% had osteomyelitis and 77% had diabetes. When patients with these conditions were excluded, 91% achieved complete wound closure.

In conclusion, Professor Cooper summarised where our knowledge extends to; biofilms are to be found in wounds and they are linked to chronicity. At present there is no simple way to detect their presence and although available, treatment options are currently limited.

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Biofilm and slough

Keith Cutting, Visiting Professor at Buckinghamshire New University, continued the session to highlight the role of slough and challenge our conception of it as merely devitalised tissue.

Professor Cutting began by reminding the audience of the relationship between the organisms that make up a biofilm and the substrate they are attached to. He argued that it is the wound surface that predetermines which organisms attach and grow and remain components of early biofilms. These species change as the biofilm matures, and as the action of bacteria modify the wound environment, making subsequent species more likely to settle.

As has been pointed out by various individuals, it is impossible to say definitively that a biofilm is present in a wound just by its appearance; however, some features can raise suspicion. The presence of slime within the wound may lead the clinician to suspect a biofilm is present. A number of statements on the recognition of wound infection may apply here. Some authors describe translucent film, glazed appearance, increased exudation or increased odour as an indicator of infection. It might also be fair to assume that if a wound is chronic, biofilm will be present. However, the South Western Wound Care Center in Texas makes a very bold statement, stating that a sloughy wound is a biofilm wound (www.woundcarecenter.net).

This raises a number of issues. If we look at the literature we find slough described as, 'moist, dead, fibrinous material (protein) that tends to recur especially in poorly perfused chronic wounds accompanied by proteinaceous exudate, and is tethered to the wound bed'. If it is easily removed the substance is unlikely to be slough. Many regard slough as a major inhibitory factor in wound repair. Nylen and Carlsson (1980) investigated 180 acute surgical hand wounds. They found that the presence of slough significantly increased the frequency of subsequent infection. There has been a general impression that slough is little more than a nuisance, something which needs to be removed but which in itself is not pathological. However, as the earlier definition eludes, it quickly reappears despite its regular removal. The clinician is left to wonder what is going on. If a wound is debrided every week to remove this devitalised tissue, why does the wound not appear to get deeper week on week? Is it that slough is merely dead tissue or could it be something else?

Professor Cutting argues that slough is not innocuous but contributes directly to delaying wound healing. It behaves like a living organism, a well differentiated polymicrobial-multicellular organism growing on the surface of the wound. This view is supported by Costerton et al (1999).

From clinical images we see that in 'healthy' wounds, i.e. those actively progressing to healing, the wound bed appears pink-red with a distinct margin between the surrounding skin and advancing epithelium and the granulation tissue. There is an absence of slough and no signs of infection despite the theoretical presence of bacterial colonies; the host defence mechanisms are keeping the bacteria in check. However, in images of some chronic wounds we see the wound bed covered with slough which extends onto the surrounding periwound margin. This 'feathered' appearance is indicative of poor host defences and from experience we know that healing outcomes will be poor unless radical action is undertaken. As an extrapolation of this thought, we need to consider the use of simple dressing

Table 1
Factors indicative of early infection

Wound type	Indication
Arterial ulcers	Dry necrosis turns wet
Burns	Black/dark brown focal areas of discolouration
Diabetic foot ulcer	Ulcer base changes from pink to yellow/grey
Pressure ulcers	Viable tissue become sloughy
Venous leg ulcers	Sudden appearance or increase in amount of slough

materials such as gauze. Rhoads et al (2008) determined that biofilm maturation is potentiated by the presence of gauze in the wound bed. It acts as an effective substrate for attachment and may increase the release of biofilm virulence factors, such as homoserine lactones into the wound while also seeding the wound with planktonic bacteria, thereby contributing to the maintenance of a chronic inflammatory state. This provides us with another reason why the use of gauze in wound care should be discouraged.

Professor Cutting suggests that the presence of slime (EPS) or slough on dressings may be sufficient to establish the presence of biofilms — therefore, the heavy fouling of dressings could suggest heavy biofilm presence.

The European Wound Management Association's *Position Document: Identifying criteria for wound infection* (EWMA, 2005) was developed following a Delphi process among experts in the field. It identified six key wound types:

1. Acute/surgical wounds (primary and secondary)
2. Arterial ulcers
3. Burns (partial and full thickness)
4. Diabetic foot ulcers
5. Pressure ulcers
6. Venous leg ulcers.

Professor Cutting has reviewed the data from this work to ascertain how the key clinical features identified might relate to slough and biofilm activity. Clinicians identified that a number of indicators were considered indicative of early infection (*Table 1*). Could these actually be indicators of biofilm development?

There is a polarity in the literature on the subject of debridement. Sinha (2007) suggested there is no published controlled trials to provide evidence that debridement accelerates the wound healing process and yet from 1990 (Thomas, 1990) to the present day, writers have identified that debridement of devitalised tissue is necessary to promote granulation and epithelialisation (Tong, 1999; Payne et al, 2008). The consensus is that despite the lack of controlled studies, regular, aggressive debridement should be undertaken as part of a management strategy (including the use of topical antimicrobials and where appropriate systemic antibiotics) to remove slough, devitalised tissue and biofilm and optimise the healing potential.

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Slough and soft debridement

The final presentation of the symposium was undertaken by Professor Marco Romanelli, Director of the Wound Healing Research Unit, University of Pisa. His presentation outlined his experiences of Sorbion Sachet S, the recent trial he undertook with the product, and its potential to facilitate what he referred to as 'soft debridement'.

Professor Romanelli explained to the audience that the study was undertaken within the Wound Healing Research Unit. The aim of the study was to investigate the influence of Sorbion Sachet S on the exudate management properties of debridement and wound edge protection, by means of non-invasive dermatological methods on lower leg venous ulcers.

The trial population was made up of patients with moderate to highly exuding venous leg ulceration. The study incorporated clinical observation and objective scientific assessment. During the study, assessments were made of wound size, wound bed status and periwound skin condition. Patients were included in the study if they were over 18 years of age, had a venous ulcer to the lower limb, could give informed consent and were concordant with the treatment regimen.

Patients were excluded from the study if they had clinical signs of infection, were pregnant, had a known allergy to any of the wound care products being investigated, were undergoing

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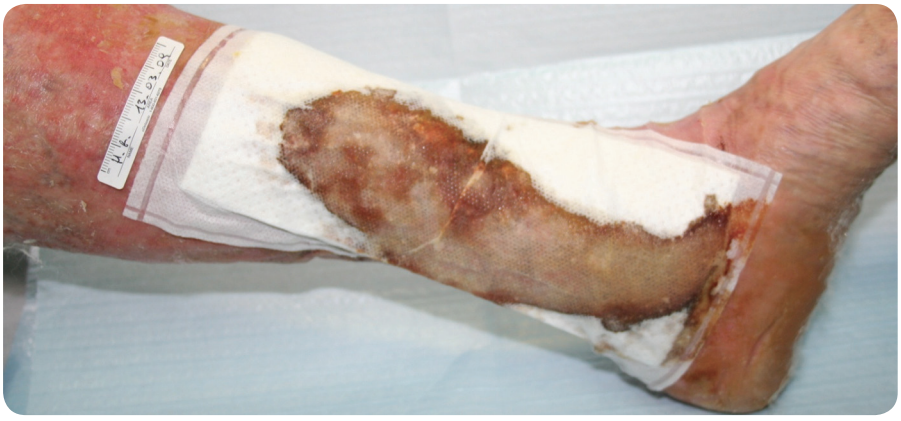
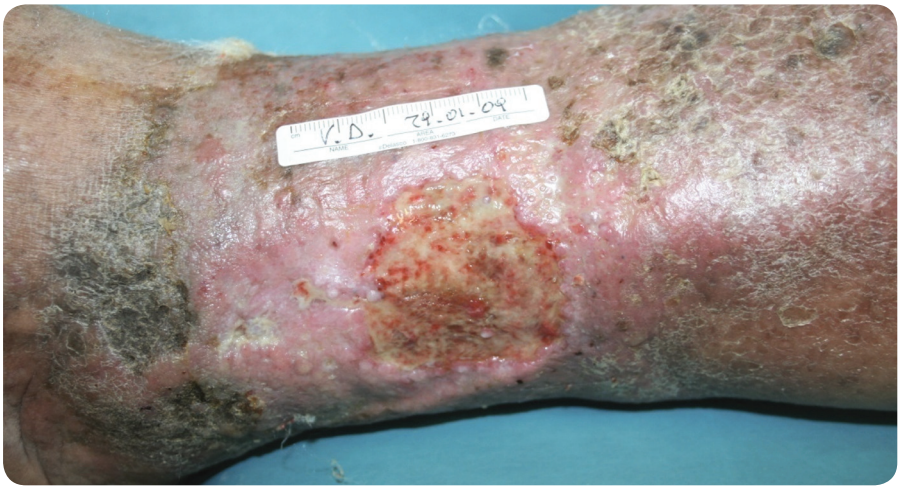


Figure 1



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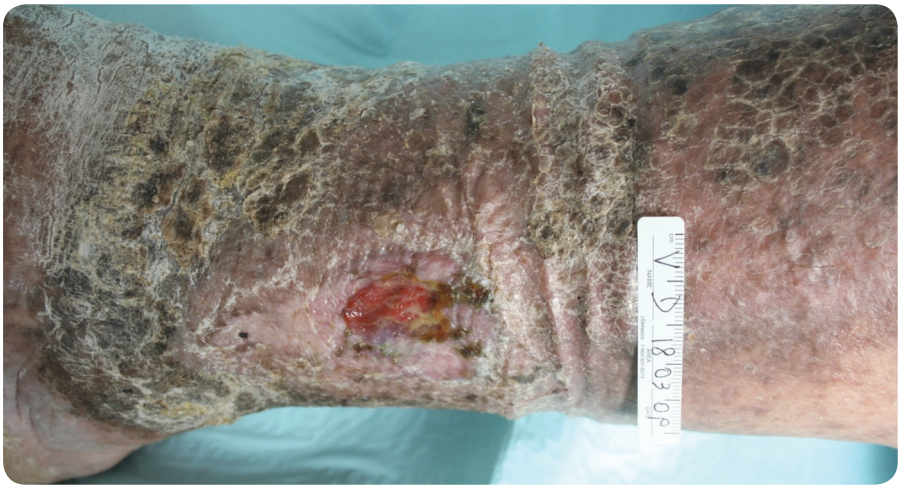


Figure 3

immunosuppressive therapies, had renal or cardiac disease, or any other underlying medical condition which would influence wound repair.

Wound assessment was undertaken using a Silhouette® portable 2D and 3D laser scanning device (ARANZ Medical). Trans-epidermal water loss was used to estimate periwound skin health, particularly the presence of maceration. This was estimated with a portable unit, the Vapometer (Delfin Technologies Ltd). Numerous recent studies, including some by Professor Romanelli, have shown that wound pH may have a correlation to bacterial burden within the wound. Within the study this was estimated with a handheld pH metre (HANNA Instruments™) and used within subsequent data analysis.

Twenty subjects were recruited and divided equally into the two study arms. One group received alginate dressings and compression bandages (control) and the other received Sorbion Sachet S dressings and compression. Compression bandaging was standardised between the two groups and consisted of a two-layer system, which was changed twice-weekly. The evaluations were carried out over four weeks. Records were made at four time points; baseline, week 1, week 2, and week 4.

Of the 20 patients recruited, eight were male and 12 female. Their mean age was 63 years. Mean wound size at baseline assessment was 136.4cm^2 and the ulcers had been present for an average of two years.

Sorbion Sachet S was found to be easy to use in conjunction with the compression regimen. The product absorbed exudate well. Exudate was taken up vertically and locked into the dressing material without pooling and with very little lateral spread (*Figure 1*). In fact, on dressing removal a mirror image of the wound was observed marked in exudate on the contact surface of the material. The product was easy to remove and patients reported it was comfortable to wear. One notable feature was the quality of the wound bed after the use of Sorbion. At dressing removal, debris lifted off the wound surface easily. This 'soft debridement' revealed a wound surface covered in fine, highly vascular granulation tissue similar to that seen following the use of topical negative pressure therapy.

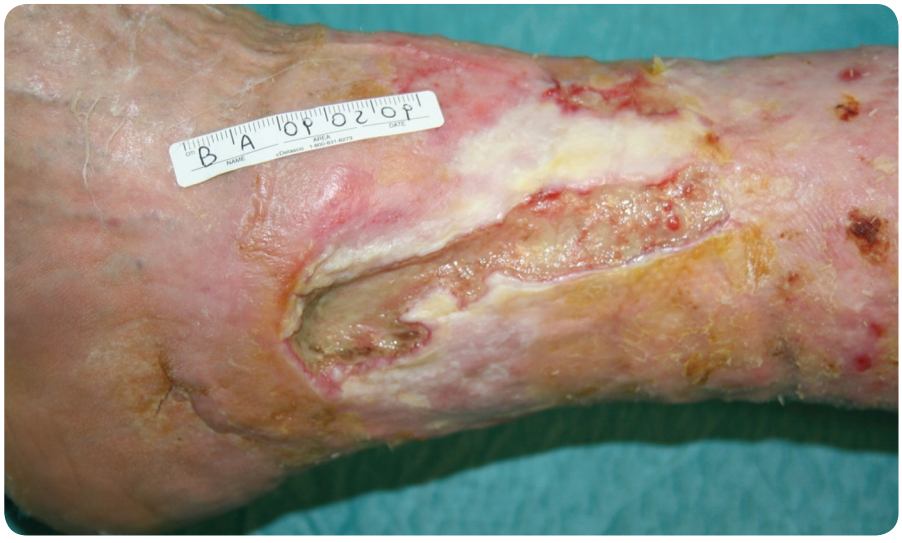
Clinicians felt the removal of exudate, debris and slough was significant in the wound's subsequent progress.

To illustrate the findings, Professor Romanelli presented case studies from the Sorbion group.

Case studies

Figure 2 features a 70-year-old man with a leg ulcer to the outer aspect of the gaiter region. At baseline assessment, the ulcer measured 18.25cm^2. He had moderate exudate with a moderate wound odour. The wound had a pH of 8.1 and the skin immediately surrounding the wound had a transepidermal water loss reading of 45.3g/h/m^2. If we look at the wound after four weeks of treatment (*Figure 3*), we see the wound has reduced in size to 3.2cm^2. Exudate has now reduced significantly and was assessed as 'none'. This is supported by the transepidermal water loss measurement, which was now 40.6 g/h/m^2 and indicated an improvement in periwound maceration. Wound odour is no longer present and the wound pH is 7.2.

Figure 4 features a 72-year-old man with a chronic ulcer and maceration to the periwound skin. At baseline the ulcer measured 22.7cm^2. His exudate was assessed as moderate and he had a transepidermal water loss of 30.3 g/h/m^2. Maceration can clearly be seen on the anterior and posterior aspects of the wound margins. The wound bed is covered by a fine but adherent layer of slough. Wound pH was 7.81 and there was moderate odour present. After four weeks of treatment with Sorbion Sachet S (*Figure 5*), soft debridement of the wound bed has taken place. There was some minor bleeding noted on dressing removals. No pain was felt and this was easily managed with local wound compression. On completion of the trial the wound measured 10.1cm^2 (over 50% reduction in four weeks). Exudate was no longer a problem and the trans-epidermal water loss of the periwound skin was 22.3g/h/m^2. Wound pH had also reduced to 7.06.



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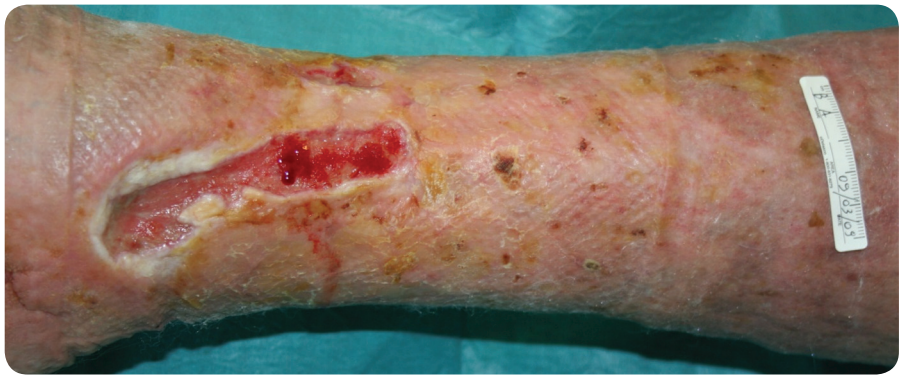

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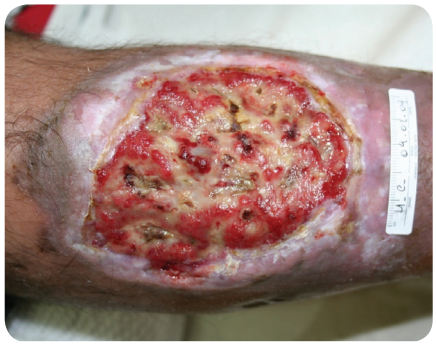

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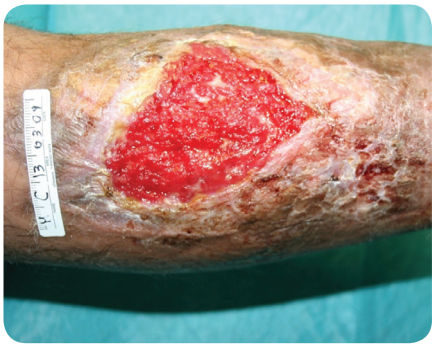

Figure 7

Figure 6 features a 45-year-old man who presented with a large (104.4cm^2) ulcer to his leg. He had moderate to high exudation from the wound and maceration is evident on the periwound skin (TEWL 64.1g/h/m^2). As can be seen in the photo, there are significant areas of slough and debris present on the wound bed. The wound pH was 7.5. Four weeks of Sorbion Sachet S dressings produced dramatic improvements (*Figure 7*); the surrounding skin was much improved with no signs of maceration. Soft debridement has been very successful and there is no longer slough in the wound bed; instead very good quality granulation tissue can be seen across the wound

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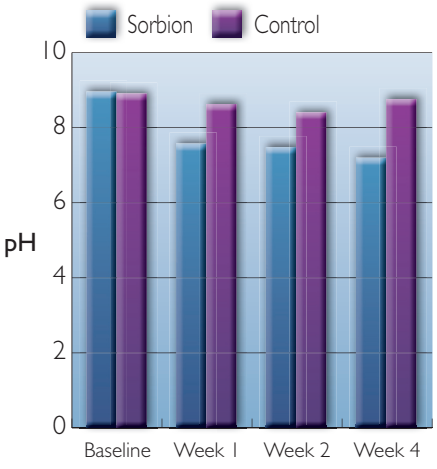

Figure 8

surface. Wound exudate has now dropped and transepidermal water loss in the surrounding skin is now only 34.6 g/h/m^2; nearly half its previous value. The wound pH has also reduced to 6.02.

Overall trial results

Professor Romanelli explained that analysis of the results gave some interesting findings. The Sorbion Sachet S group achieved a mean pH reduction at each dressing change. Comparing mean pH values at baseline to week 4 showed statistical significance ($p<0.05$) in favour of the Sorbion Sachet S regimen (*Figure 8*).

The measurement of transepidermal water loss is an indicator of periwound tissue health; a reduction towards normal values indicates improvement in skin moisture levels and a quantitative reduction in maceration. On assessment of the readings from the Sorbion treated group, there is an overall reduction in values over the four-week trial period. This represents an overall improvement in maceration within the treatment group. This has been seen clinically in the case studies presented, however, statistical analysis supports this and demonstrates that this reduction is highly statistically significant ($p<0.001$).

Within the study site, dressing and compression changes are normally carried out weekly for patients with low to moderately exuding wounds. Those with higher levels of exudate require more frequent dressings. In the trial, the patients who were selected demonstrated moderate to high exudate levels and therefore it was decided to change dressings and bandages twice a week. Many of these patients might normally require more frequent changes.

The team found that Sorbion Sachet S was immediately able to manage very high levels of exudate and that following 'soft debridement, exudate levels dropped considerably for most patients. This has major clinical significance as it reduces the frequency of dressing changes required, and so reduces direct (product) cost and reduces clinicians' intervention (indirect costs), while achieving the desired clinical outcomes.

In conclusion, the team like the concept of 'soft debridement' as, with the right product, it is easy to achieve in the clinical setting and brings about good healing outcomes. There was improvement in the periwound skin condition of all the patients within the Sorbion group. Patients found Sorbion Sachet S easy to tolerate (which indicates the product is able to achieve this without discomfort), and compliance was excellent. The team was able to see a reduction in the number of dressings required over alternative products and, although the trial was only over four weeks, there was good reduction in wound surface area; even in patients with past histories of chronicity extending over two years.

Questions

After the presentations, Professor Cooper opened the session to questions from the delegates.

Question 1: 'Did Professor Romanelli think that the reduction in wound pH could have been attributable to just the action of the compression?'

Professor Romanelli: 'Prior to the trial the product was tested with and without compression. There was no difference in the results obtained; surface pH reduction appears attributable to Sorbion Sachet S. The product works well under compression; we did not see the periwound skin changes we normally associate with absorbent dressings.'

Question 2: 'Are there any silver products which could actually encourage biofilm formation?'

Professor Cooper: 'There appears to be conflicting evidence on silver's efficacy in biofilm management; although concentration seems important. In my *in vitro* work on honey, I've found it is the concentration and exposure time which are crucial. Remember, sugar is a potential substrate itself; you have to make sure concentrations are high enough (higher than when treating planktonic bacteria) and are available for long enough to inhibit biofilm.

Question 3: 'Professor Cutting, are you suggesting that if slough develops on a previously un-sloughy wound we need to consider using an anti-biofilm agent?'

Professor Cutting: 'Yes!'

Question 4: 'If we don't get the classic signs of infection with an inhibitory biofilm, when do we use antimicrobials, particularly as we're advised to only use them in overt infections?'

Professor Cutting: 'This is one problem we face when there are no universally accepted definitions available. This has to be the starting point for all of us. I have postulated that one form of infection, "the biofilm phenotype" can be present in the presence of slough. Others may also be present. My message is we need to regard slough as a major inhibitory factor, rather than innocuous devitalised tissue.

Professor Cooper: 'As a microbiologist I would always advise careful, judicious use of antimicrobial agents due to the risk of bacterial resistance.' WUK

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