Consensus document:

PHMB and its potential contribution to wound management

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Background

In spring 2010, a diverse group of clinicians comprising microbiologists, tissue viability specialists, orthopaedic, reconstructive and general surgeons, and academics in wound care met in London to discuss the issue of bacterial management in wound healing. The clinical world is currently facing a range of new challenges; health care is becoming increasingly intensive with ever-higher expectations of positive outcomes from both the healthcare industry and those it serves. Much of the focus has been placed on infection and measures to control it, with intense media interest being placed on the role it has on morbidity and mortality. As our understanding of the intricate balance between wound healing and the bio-community of organisms living within the wound expands, clinicians face new challenges in providing effective strategies to manage wound bioburden without inducing pathogen resistance and negatively influencing the healing process.

Over the last ten years clinicians have witnessed the emergence and acceptance of new methods of bacterial management; notably, the re-emergence of topical antiseptic/antimicrobial agents in wound care. However, as with so many false dawns and prophets, the practical issues of their introduction have been frequently overlooked. The confusion caused over the silver debate and issues over cost to healthcare providers and funding agencies has led to concern over their widespread and frequent inappropriate use. In addition, the emergence of bacterial strains resistant to the effects of silver has led to major concerns that a new threat to wound care is emerging.

This group met to discuss the issues for clinicians in the 21st century; where is the evidence, and how can clinicians ensure that the use of antiseptic/antimicrobial agents is carefully managed to maintain clinical-effectiveness and appropriate use of healthcare resources? The group also discussed what the future holds in antiseptic/antimicrobial therapy. With no new antibiotic therapies emerging on the horizon, the role of topical antiseptic/antimicrobial agents may take on an even greater significance. One possible solution lay with polyhexamethylene biguanide (PHMB), a compound which has been available for many years in a number of formats but which has not, until recently, made any significant impact on the UK wound care market.

At the meeting, two formal presentations were given. Professor Richard White (Professor of Tissue Viability, University of Worcester) gave a presentation on PHMB; its history, chemical composition, action and an overview of the evidence to support its use in wound care. Professor Robert Strohal (University Teaching Hospital Felkirch, Austria) presented on the randomised control study his department has recently undertaken with Suprasorb® X+PHMB (Activa Healthcare, an L&R Company), and spoke of how consensus has been reached in Europe that PHMB is the first choice of topical antiseptic/antimicrobial agent in the management of wound bioburden. Open discussion took place where delegates utilised their experiences in general wound care and specifically in using PHMB across a variety of clinical situations. They identified areas of concern in managing bioburden and what evidence clinicians require in making choices in wound management strategies.

If the use of PHMB is to ascend into the armoury of the UK wound care clinician, there needs to be careful review of its effectiveness, control of its use in clinical practice, and education of clinicians at the forefront of wound care. It is the intention of this document to provide material from the group's meeting and additional information to enable the reader to understand the framework of the discussions. It is not a systematic review, but does provide information on which to base a consensus document. In turn, the consensus document will provide a framework for clinical utilisation of this technology, to educate and inform clinicians and to provide industry with key performance indicators, based on the questions that arose from the meeting (*Box 1*).

What is PHMB?

The discussion started with an overview of PHMB. For some in attendance, this antiseptic/antimicrobial compound was a new concept within the field of wound care, others have had experience with it either in the clinical setting or through the scientific published studies.

The antiseptic/antimicrobial polyhexamethylene biguanide (also known as polyhexanide or PHMB) is a relatively new entrant to the UK wound care market, although it is in common use in Europe and the US. PHMB is a heterodisperse mixture of polymers and is a synthetic compound structurally

similar to naturally occurring antimicrobial peptides (AMPs). The basic molecular chain of PHMB can be repeated from two to 30 times, with increasing polymer chain length correlating with increasing antiseptic/antimicrobial efficacy.

AMPs are important in innate immune response and are produced by the majority of living organisms. They have a broad spectrum of activity against bacteria, viruses and fungi (Moore and Gray, 2007), and have been suggested as therapeutic alternatives to antibiotics (Hancock and Sahl, 2006). AMPs are positively-charged molecules that bind to bacterial cell membranes and induce cell lysis by destroying membrane integrity, in a similar way that penicillin and cephalosporin antibiotics do. AMPs are produced by many cells within the wound, such as keratinocytes and inflammatory neutrophils, where they are thought to play a role in protection against infection (Sorensen et al, 2003).

Molecular structure and mode of action of PHMB

The structural similarities between AMPs and PHMB mean that the latter can insert into bacterial cell membranes and kill bacteria in a similar way to AMPs (Moore and Gray, 2007). While it is unclear what the precise action of PHMB on bacteria is, the primary targets appear to be the outer and cytoplasmic membranes. PHMB is thought to adhere to and disrupt target cell membranes, causing them to leak potassium ions and other cytosolic components (Davies and Field, 1969; Davies et al, 1968; Broxton et al, 1984a; Yasuda et al, 2003), which results in cell death. Studies indicate that PHMB does not form association with the neutral phospholipids that populate animal cell membranes, however, it does strongly interact with a key component of bacterial membranes, the acidic phosphatidylglycerol (PG) (Ikeda et al, 1983; 1984). There is also evidence that some of the compound's antibacterial effects follow its penetration into target cells. In 1984, Broxton et al reported that maximal bactericidal activity occurs under conditions that promote rapid transportation of PHMB to the cytoplasm and cytoplasmic membrane (Broxton et al, 1984b). It has since been demonstrated that PHMB binds to DNA and other nucleic acids and precipitates them from aqueous solution (Allen et al, 2004). This suggests it may damage or inactivate bacterial DNA.

BOX 1

Key questions raised at the consensus meeting

- What is PHMB?
- How does it work/mode of action?
- What are the safe and accepted dosages?
- What is the evidence base?
- What are the clinical indications based on the evidence?
- Are there benefits to patients and clinicians based upon the above?

The effects of PHMB on managing bioburden are not just limited to bacterial colonisation. In testing it has been demonstrated that exposure to PHMB causes viral cells to clump together to form aggregates.

Use of PHMB

PHMB has been in general use for approximately 60 years with no evidence of the development of resistance (Moore and Gray, 2007). It exerts little toxicity and has been found safe and effective in applications as diverse as treatment of eye infections (Larkin et al, 1992) and sanitising swimming pools. Both *in vitro* and *in vivo* in these different applications, PHMB's safety is well documented (Larkin et al, 1992; Motta, 2004; Motta and Trigilia, 2005). Studies in 1998 and 2005 (total of 3,529 patients) have shown that skin sensitising to PHMB is low (approximately 0.5%), even though the tested drug concentrations (2.5% and 5%) were five to ten times the concentration normally used in wound applications (Schnuch et al, 2000; 2007). Comparative tests of PHMB's biocompatibility (measurement of an antiseptic/antimicrobial agent's activity in relation to its cytotoxicity) against other commonly used therapies have demonstrated its superiority to chlorhexidine, povidone-iodine, triclosan, silver and sulfadiazine (Müller and Kramer, 2008).

PHMB has been available as a wound irrigation fluid in Europe for some time. Recently, it has been successfully introduced into wound management

within a range of dressings including non-adherent products, gauze, drains and intravenous sponges (Motta and Trigilia, 2005; Moore and Gray, 2007), and hydrogels. In some cases, the PHMB molecule has been chemically bound to the base material, providing it with antiseptic/antimicrobial properties when in contact with wound moisture. The product therefore protects against the development of wound infection by decreasing the bacterial load in the dressing and bacterial penetration through the dressing. In others products, the active component is free to be delivered into the wound and periwound tissues; the dressing in this case being a carrier for a wider antimicrobial activity by donating PHMB to the wound surface.

Antiseptic/antimicrobial activity in wound care is a central role for any PHMB product. A porcine model was used to measure the effectiveness of a 0.2% PHMB-impregnated gauze dressing to prevent ingress of *Pseudomona aeruginosa* against a plain gauze control (Cazzaniga et al, 2002). In the study, sterile wounds were dressed with the two test products and the areas were challenged with an inoculum of the bacterial culture. The wounds and dressings were analysed after 24, 48 and 72 hours. It was demonstrated that in the PHMB-gauze treated wounds, bacterial ingress was substantially reduced or eliminated (4–5 log reduction). In addition, the dressing was able to reduce the inoculums within the dressing itself at 24 and 48 hours post inoculums, compared to the control ($p=0.05$). Normal bacterial flora persisted in the wound bed. This appears to indicate that the PHMB dressing did not exert a donating effect, instead acting as a potent barrier to external contamination.

In wound care specifically, PHMB has previously been demonstrated to block *Pseudomonas aeruginosa*-induced infection (Cazzaniga et al, 2000) and prevent its degradation of wound fluid and skin proteins *in vitro* (Werthen et al, 2004). It can also kill a diverse range of bacteria and fungi (Lee et al, 2004).

Galitz et al (2009) conducted a controlled, randomised prospective multi-centre comparative study of the use of a PHMB containing biocellulose-based wound dressing (Suprasorb® X+PHMB, Activa Healthcare, an L&R Company, Germany) against best local silver standard of care. The subjects (n=37) were all assessed as having high wound pain and critically colonised or locally infected wounds, and had similar demographic and wound-related presentations. Treatment was continued over 28 days. The results of the study identified that both dressing regimens achieved a positive antimicrobial effect and pain reduction. However, in the case of the PHMB product, pain reduction was consistently greater and more immediate with significant pain reduction occurring after the first day of treatment. The authors concluded that the PHMB product provided an efficacious, patient-friendly option for the management of these types of wounds.

Wright et al (2003) undertook a direct comparison of a PHMB-based gauze dressing (Kerlix™ AMD, Covidien) against a nano-crystalline silver dressing (Acticoat® 7, Smith and Nephew) in a laboratory and porcine model. The PHMB test product is composed of absorbent cotton gauze impregnated with 0.2% PHMB. Measurement was recorded of *in vitro* bactericidal efficacy, and porcine healing rates and bactericidal efficacy. This study demonstrated that both products were effective at reducing bacterial burden *in vitro* and within the porcine model. However, the effectiveness of the PHMB dressing was compromised by two factors:

- Adherence of the dressing to the wound bed, causing trauma and a prolonged inflammatory reaction
- The inability of the dressing to 'donate' PHMB to the wound bed. This resulted in antimicrobial activity only being effective when intimate contact with the wound bed was maintained.

It would appear that one of the key factors in the success of PHMB in eradicating bacterial flora is the availability of the active compound to effect an antiseptic/antimicrobial action. A prospective, randomised study was conducted to directly compare the efficacy of two PHMB products in the eradication of MRSA (Wild et al, 2009). Thirty patients with MRSA-colonised pressure ulcers were randomly assigned to either treatment with a PHMB solution (Prontosan®, B. Braun) and cotton dressings or a PHMB-containing biocellulose dressing (Suprasorb X+PHMB). Wound assessments and microbiological swabs were taken before the start of the study and weekly for two weeks. The results showed that in the PHMB solution group, six out of 15 patients (40%) were MRSA free after one week of therapy, and 10 out of 15 were MRSA free by the end

of week two. In the PHMB biocellulose dressing group, 13 out of 15 were MRSA negative at the end of week one ($p<0.05$), and all were negative by the end of week two ($p<0.05$). In addition to the superior antimicrobial activity of the PHMB cellulose dressing, wounds treated with the product demonstrated faster and more prolific production of granulation tissue.

In vitro and *in vivo* studies into the effectiveness of PHMB in wound care have demonstrated that the product may also have other benefits in wound management. Daeschlein et al (2007) reported that the product may reduce pain and malodour, while Mueller and Krebsbach (2008) found that its use reduced fibrin slough and prevented the build-up of necrotic tissue and so promoted connective tissue regeneration. Wiegand et al (2008) demonstrated that PHMB can have a positive effect on tissue proliferation. In a laboratory study, cultures of normal human keratinocytes, fibroblasts and HaCaT-cells (human adult high calcium low temperature keratinocytes) were exposed to varying concentrations of PHMB and the results observed. It was found that concentrations between 0.2–2µg/ml had a significant proliferative effect on keratatinocytes. In concentrations greater than 2µg/ml, a dose-dependent decrease in cell proliferation was noted, thus there is a critical point at which it stops being beneficial and becomes damaging.

A number of German studies have shown that PHMB demonstrates a positive effect on bacterial biofilms (Seipp et al, 2005; Pietsch and Kraft, 2006; Harbs and Siebert 2007).

Healthcare economics and cost-effectiveness

The consensus group unanimously agreed that health economics is an area in wound care that has to be considered when new therapies are being investigated. Pressure is placed on clinicians to cut overall treatment costs, and the current world-wide financial crisis is having a significant impact on future healthcare funding plans. Even when treatments are proven to be clinically effective, there is a reticence to implement their widespread adoption unless overall cost savings can be realised. The group felt the recent controversy over the findings of the 'Vulcan trial' (Michaels et al, 2009) have further raised the issue of cost-effectiveness, particularly in the use of antiseptic/antimicrobial preparations. Although the study was considered flawed by members of the group, and therefore its conclusions may be misleading, it was felt that the assertion that commonly used antimicrobial preparations (silver) lack sound health economic benefits flies in the face of clinicians' experiences. However, it does force clinicians to reconsider the control placed on therapies and the need to justify expenditure with proven health economic outcome measures.

In an excellent overview of the problems and cost impact of managing antibiotic resistance, Sipahi (2008) identifies that antibiotics make up more than 30% of hospital pharmacy budgets. With the rise in bacterial resistance and the lack of next generation products, the emphasis on non-antibiotic control mechanisms is set to rise. One of the key elements in cutaneous and wound infections is undoubtedly the use of topical antiseptic/antimicrobial preparations. However, as has been previously argued, it is important to control their use to effectively prevent long-term complications. Stewardship is required to ensure their appropriate use and reduce unnecessary cost.

The targeted use of antiseptic/antimicrobial dressings has repeatedly been reported to reduce surgical site infection (SSI) rates, thereby yielding substantial cost-savings. However, it is important to consider what other effects the use of antiseptic/antimicrobial products might have and their impact on overall healthcare expenditure. The emergence of silver-resistant bacterial strains is of concern, and factors such as systemic absorption and skin sensitisation among patients may present problems in the future. PHMB has not shown any of these characteristics and so should be considered within health economic studies.

Gilliver (2009) identifies a number of the potential savings the use of PHMB can have for healthcare providers. He identified four US-based papers which had the health economics aspect of the introduction of PHMB-based products as a key outcome measure. In the first, investigators from Nebraska, USA, found that replacing plain gauze dressings with PHMB-impregnated gauze reduced the overall SSI rate by 24% and the MRSA SSI rate by 47% (Mueller and Krebsbach, 2008). Based on estimates of SSI treatment costs, this delivered a $508,605 net saving during the one-year evaluation period. A second study found that replacing conventional gauze with PHMB-impregnated gauze in the treatment of

patients undergoing vascular surgery resulted in a progressive year-on-year fall in infection rates from 4.6% in 2000 to 0.4% in 2005, with an overall estimated saving of $876,176 (Penn et al, 2006). In the third study, the hospital-wide introduction of the same PHMB-impregnated gauze resulted in a reduction in the incidence of infections from 23 to 11 (both reported in separate six-month observation periods) (Beneke and Doner, 2005). Calculated net savings were $171,537. Finally, a trial conducted in the University of California San Diego Medical Center, USA, concluded that Suprasorb X+PHMB was more cost-effective than other treatment regimens for recalcitrant wounds (Mulder et al, 2007). Calculations were based on material costs, which averaged $5.99–9.01 per patient per day and were as low as $2.14 per day in one patient.

A fine balance needs to be maintained. The increased cost of treatment with antiseptic/antimicrobial agents is minimal when compared to the potential gains achieved in managing infection; however, it is still a real cost to healthcare providers. It is essential that this investment is undertaken in an appropriate way, ensuring that antiseptic/antimicrobial use is targeted at those who need it clinically to treat, manage or prevent infection.

World of wound infection

The group considered that the debate on clinical indications for antiseptic/antimicrobial use and, therefore, the health economic and clinical efficacy of interventions needs to be placed within the scientific framework of bacterial effects on the wound healing process.

The influence of bacteria on wound healing is complex and controversial. It is accepted that all open, chronic wounds are colonised with bacteria, and yet most wounds, even chronic wounds, can and do heal. Wound infection is the result of a complex interaction between the patient's immune system, the wound conditions and the numbers and virulence of the bacteria present (Dowsett et al, 2005). Chronic wounds are often heavily colonised with bacteria, due in part to their remaining open for prolonged time periods, but also because of underlying medical problems such as poor blood supply, hypoxia and metabolic disorders (Hunt and Hopf, 1997). Wound infection has been recognised as a factor involved in prolonged wound healing for a long time. Its effective management has been identified as a central tenet when undertaking Wound Bed Preparation (WBP) (Schultz et al, 2003). It prolongs the inflammatory phase of healing, causes pain and discomfort for the patient and, unless correctly treated, can lead to serious and potentially fatal systemic sepsis. Wound infection is not just costly to the patient; financial costs increase with prolonged treatment of the wound and, on occasions, with hospital admission.

The presence of bacteria in chronic wounds does not necessarily indicate that infection has occurred or that it will lead to impaired wound healing (Kerstein, 1997; Dow et al, 1999), and it has even been suggested that certain low levels of bacteria can facilitate healing (De Hann et al, 1974; Pollock, 1984). Most healthcare professionals believe that if the wound does not display traditional signs of infection, the bacteria are not interfering with the healing process. As new information is being presented, many healthcare professionals are starting to believe that high levels of bacteria may inhibit healing in the absence of traditional signs of infection (Edwards and Harding, 2004; Warriner and Burrell, 2005).

All wounds become contaminated with bacteria at, or soon after inception. In most cases this does not impede healing and clinical intervention is not indicated. However, in some individuals there is a linear progression of wounds from contamination through colonisation to critical colonisation and infection (termed the 'infection continuum'; Kingsley, 2001; White et al, 2001). Infection is apparent when the sum of the bacterial load and the virulence factors the bacteria produce is greater than the host's immune defences, resulting in harm to the host. This is seen as the classic signs of infection. Cutting and Harding (1994) describe these signs as erythema, pain, swelling and localised heat, but also added the following potential signs of infection:

- Discharge,
- Delayed healing,
- Wound breakdown,
- Pocketing at the base of the wound,
- Epithelial bridging,
- Unexpected pain or tenderness,
- Friable granulation tissue,
- Discolouration of the wound bed,
- Abscess formation
- Malodour.

It has been agreed that when the equilibrium in the wound is tipped in favour of the bioburden (i.e. the colonising bacteria have a negative impact on the healing potential of the wound), active intervention is indicated (Gethin, 2009; Best Practice Statement, 2010). There is evidence to suggest that inappropriate use of antibiotics and topical antiseptic/antimicrobial agents can lead to resistance developing, therefore, clinicians should be clear on the status of the wound with regards to infection (Vowden and Cooper, 2006). The presence of spreading infection has potential serious implications for patient well-being and appropriate systemic antibiotic therapy should be considered (European Wound Management Association [EWMA], 2006). However, systemic antibiotics are not recommended for wounds that only show signs of local infection (Bowler et al, 2001). In the sub-infection presentation other interventions are indicated. The use of topical antibiotics are linked to the development of bacterial resistance, therefore these should be avoided. However, in the colonised and critically colonised state, topical antiseptic/antimicrobial agents have been shown to play a significant role in reducing bacterial load (EWMA, 2006).

Topical antiseptic/antimicrobial agents represent first-line treatment in the management of bacterial burden, as they provide a high antiseptic/antimicrobial concentration at the site of infection (White et al, 2001; Cooper, 2004). Some preparations have bactericidal effects even against multiresistant organisms such as meticillin-resistant *Staphylococcus aureus* (MRSA) (Lawrence, 1998; Sibbald et al, 2001), and they have the additional advantage that they do not interfere with the remainder of the protective bacterial flora in other parts of the body, and are also less likely to produce an allergic reaction. However, their use has to be targeted and measured. Lack of a noticeable healing response within two weeks may necessitate the use of other topical or systemic agents (Bowler et al, 2001; Best Practice Statement, 2010). Widespread, inappropriate use increases healthcare costs with no gain in outcome. In addition, a number of bacteria have developed tolerance to several products containing the commonest antiseptic/antimicrobial elements, i.e. iodine and silver. This is a major concern as it severely restricts subsequent treatment options. What is required is sensible, measured use of antiseptic/antimicrobial agents where clinically indicated for the active treatment of infection in line with Best Practice (Best Practice Statement, 2010), and the emergence of new, effective formulations.

Biofilms: a risk?

The issue of wound biofilms and their influence on chronic wound healing attracted intense debate among the consensus group.

The concept of bacteria living within a heterogeneous community rather than simply as autonomous entities is one that has quickly gained acceptance, and it is recognised that single species communities of bacteria are rare in nature (Cooper and Okhiria, 2008). These communities of organisms living within a three-dimensional extracellular polysaccharide (EPS) matrix are known as biofilms. The formation of these bacterial communities is well established in industrial and dental research, where biofilms are routinely studied and engineered. However, in the field of wound care, understanding of biofilms and their effect on wound healing is extremely limited, although they seem to be a key component in resistant bacterial colonisation (Serralta et al, 2001).

Research into the behaviour of bacteria is revealing that bacteria are more commonly found associated with other bacterial species, rather than as isolated organisms or planktonic cells (Dworkin and Shapiro, 1997). In order for a biofilm to form gradually over time, bacteria must be able to attach to a substrate. This attachment is largely based on nutritional signals and a critical number of organisms assembling. Once attached, the bacteria relinquish their planktonic state and begin to recruit other bacteria. These small aggregates proliferate and continue to recruit new members. New members can be of different species of bacteria (both aerobic and anaerobic species), fungi or protozoa. Attached bacteria excrete an EPS matrix, which forms the structure of the biofilm. These biofilm colonies are dynamic, constantly changing and adapting to their environment. This constant adaptation results in a colony unique in its ability to survive.

Biofilm bacteria cooperate in the distribution of nutrients, removal of waste, and in their defence against exogenous threats. Bacteria living in biofilms are also able to cooperate in their beneficial or pathogenic potential to

host organisms. The pathogenic potential of bacteria is related to the ability of the bacteria to cooperatively use toxins, while resisting the effects of antiseptic/antimicrobial agents, other biocides, and host defence mechanisms.

This adaptation requires that bacteria within biofilms communicate. Part of this process is known as quorum sensing. This allows all bacteria to access nutrients and dispose of waste, rather than outgrow their resources or become poisoned by waste. In *Pseudomonas aeruginosa*, it appears that an acylated homoserine lactone (acyl-HSL) is an important player in this type of cell-cell signalling (McLean et al, 1997). In addition to quorum sensing, biofilm communities make use of unique structures that allow for maximum availability of nutrients and minimum exposure to waste.

It appears that the wound environment is capable of supporting the development of bacterial biofilms. However, at present there is limited evidence to support this (Serralta et al, 2001). Wounds display many of the characteristics to suggest the existence of biofilms, and the environment can support the life cycle of a biofilm, i.e. attachment, proliferation and quorum sensing. Many biofilm-associated infections within the body have been shown to be unresponsive to antibiotic therapy and comparisons of planktonic and biofilm *Staphylococcus aureus* has found that *S. aureus* biofilms may be 50 to 1000 times more resistant than planktonic or free-floating bacterial cells (Ceri et al, 1999). It may be postulated that biofilm formation within wounds could be problematic, as conventional antibiotic susceptibilities in planktonic cells may not reflect the reduced susceptibilities that biofilm-living bacteria have.

Although emphasis is placed on the detrimental influences of biofilms, they can also play a protective role by preventing colonisation by exogenous pathogens (Reid et al, 2001). For example, it is believed that hair follicles in the skin support biofilm formation, and the normal cutaneous microflora living within the follicles offer some protection against invading pathogens (Mertz, 2003).

As highlighted in the group, the relative stability of biofilm communities may prevent the proliferation of individual pathogenic species. It would appear, therefore, that further research needs to be undertaken in this field before we can conclusively state whether all wound biofilms are a friend or a threat to the wound healing process. In the meantime, the adoption of strategies which reduce the potential for biofilms to act as a locus for infection, while preventing the predominance of resistant and pathogenic organisms, seems sensible. As such, the use of a topical antimicrobial agent with a low propensity to the development of bacterial resistance appears appropriate.

Testing products

To appreciate the effectiveness, or otherwise of a potential antiseptic/antimicrobial dressing and treatment, it is essential that clinicians understand the standard *in vitro* and *in vivo* methods of testing, and their relevance to the clinical situation. This knowledge should be utilised when assessing the evidence presented in this document and new studies as they become available.

In vitro antimicrobial sensitivity testing has long been used in determining the potential effectiveness of antiseptic/antimicrobial therapies.

One of the most frequently used antiseptic/antimicrobial sensitivity tests is a diffusion assay. During a diffusion assay, an antiseptic/antimicrobial agent is placed on an agar (culture) plate that has been inoculated with a known concentration of bacteria. After incubating the plate for 24 hours, the plate is examined and a note made of any area around the antiseptic/antimicrobial agent in which the bacteria fails to proliferate. This is measured and is known as the 'zone of inhibition'. This test demonstrates the ability of the product to influence bacterial proliferation; the greater the zone, the greater the product's influence on surrounding bacteria.

Challenge testing is carried out when comparing the performance of different antiseptic/antimicrobial wound dressings by adding a standardised portion (e.g. 40x40mm) of the antiseptic/antimicrobial dressing to a standardised solution of a log-phase culture of each microorganism. The inoculated dressings are incubated for two hours, then transferred into 10ml of 0.1% peptone water (Oxoid) and vortexed to remove any remaining viable organisms. Serial dilutions are performed in triplicate on each extract, and the number of viable organisms present determined using a standard surface counting technique. If viable organisms are recovered, the test is repeated as before using

a four-hour contact period, and then again with a 24-hour contact period. If no organisms are detected on the particular dressing after two hours, the dressing is placed in 10ml of tryptone soya broth (TSB) to detect very low levels of residual contamination. This test enables the comparison of speed of bacterial kill between different products and in the presence of different bacterium.

Minimum inhibitory concentration (MIC) is the most common test used to measure the physiological effects of an anti-infective agent on microorganisms, and the relationship between product concentration and effect. By definition, MIC is the lowest concentration that completely inhibits visible growth of the organism, as detected by the unaided eye after an 18–24-hour incubation period, with a standard inoculum of approximately 105 colony forming units per millilitre (CFU/mL) (National Committee on Clinical Laboratory Standards [NCCLS], 1997). Although MIC is a useful predictor of the potency of the interaction between the antiseptic/antimicrobial agent and the bacterium, it has disadvantages. It overlooks tissue distribution and protein binding and the MIC approach does not provide information on the rate of bactericidal activity, and whether increasing antiseptic/antimicrobial concentrations can enhance this rate.

Time-kill curves are another method of assessment. Time-kill curves can follow microbial killing and growth as a function of both time and antibiotic concentration. This method has more meaningful information about the interaction between bacteria and antibiotics. However, it does not reflect an *in vivo* setting (Mueller et al, 2004).

In microbial transmission testing, a strip of dressing forms a bridge between two separate agar blocks in a Petri dish, one of which is sterile and the other inoculated with the test organism. This test determines the bacteria's ability to survive on the dressing surface and migrate along it from the contaminated agar to the sterile agar. A positive result suggests that it is possible that microorganisms could be transported laterally out of a contaminated wound onto the surrounding skin, or potentially move in the opposite direction from the intact skin into the wound itself (Thomas and McCubbin, 2003).

Most biofilm research has been performed *in vitro*, and currently there is a growing interest in establishing *in vivo* models to study biofilm-associated diseases. *In vitro* methods to assess biofilm bacteria have been introduced, and these assays may be more appropriate to study the true efficacy of antiseptic/antimicrobial therapies, as compared to other *in vitro* techniques. Harrison-Balestra et al (2003) have utilised a modified staining method for evaluation of biofilm by light microscopy in *Pseudomonas aeruginosa*. In addition, optical density measurements have been used as a tool to assess biofilm formation. Various animal models exist to study biofilms (Malaisrie et al, 1998; Akiyama et al, 1996; Serralta et al, 2001), although in practical terms their use is only applicable to research applications.

In vitro models are useful for the assessment of antibacterial activity, however, they do not consider the effect of wound fluid, growth factors, proteases, antimicrobial peptides, etc which are found in the skin. In addition, they do not account for fluctuations of drug concentrations within the body. Numerous biological and technical factors can interfere with the performance of the various assays and make the interpretation of the results of *in vitro* studies quite difficult. The clinical relevance of *in vitro* studies will ultimately need to be confirmed by *in vivo* studies.

Why the need to revisit our understanding

Within health care, there is a continued need to review and revise the advice given to clinicians. Situations change, as does our understanding and the pressures placed on us as healthcare providers. The situation in the management of bacterial burden in wound care is no different. Wound infection has a significant impact on morbidity and mortality within the patient population and clinicians constantly seek new approaches to manage this issue. However, the widespread abuse of antiseptic/antimicrobial agents is of concern. In recent years, many clinicians have relied heavily on these products to control perceived bacterial threat without considering the long-term consequences, and we are now faced with the spectre of resistance and escalating healthcare costs. Our dependence on these treatments is likely to increase as there appears to be no new genre of antibiotics in the development cycle to take over the mantle of systemic infection management (Sipahi, 2008). There are two actions which need to be undertaken:

- Control of the use of antiseptic/antimicrobial agents

- Development of new antiseptic/antimicrobial therapies.

Control of current usage is already being tackled by education, the development of treatment protocols and Best Practice statements. The development of new antiseptic/antimicrobial technologies and modalities is something where cooperation between health care, research and industry is now a priority.

Discussion

It would appear from the evidence available that PHMB has an important place within wound care. Research and testing has demonstrated that the compound has a good safety record, has a low toxicity to human tissue and is effective in reducing bacterial load. It has not demonstrated systemic absorption or the development of bacterial resistance. In addition, it can be relatively easily combined into a series of wound-related devices. The bioavailability of the compound and its kill speed are areas which need consideration. PHMB is indicated for the control of bacterial burden in wounds. Specifically, it should be used to reduce bacterial burden in the critically colonised wound and may be indicated as prophylaxis in immunocompromised individuals. Therapy with PHMB should also be considered as an adjunct to systemic treatment when treating serious wound sepsis. As with all topical antiseptic/antimicrobial therapies, if the wound is unchanged after ten days or deteriorates, alternative antiseptic/antimicrobial strategies should be considered (including systemic antibiotics). In most cases, treatment should not extend beyond 14 days unless previously agreed by a local specialist (Best Practice Statement, 2010).

The ability of PHMB to bind effectively to proteins is one of the key feature which has led to its success as an environmental disinfectant. However, this does have its drawbacks. While 'bonding' is beneficial in hard surface decontamination, in wound care where the product may need to be carried on a medium such as alginate, foam or gauze, this 'bonding' can mean that donation of the product to the wound bed is problematic. A number of products currently commercially available suffer this fate. By locking PHMB into the dressing matrix, availability of the active compound is reduced. This severely limits the beneficial effects one might expect to see. Dressing materials with this format are able to reduce bacterial load, but only as the bacteria (presumably as planktonic entities within wound exudate) come into contact with the dressing. This makes them ideal barriers to bacterial spread, either preventing bacterial ingress or cross-contamination from colonised wounds, but limits their ability to optimise total bacterial wound clearance. It may also limit their action on biofilm communities within the wound.

The use of wound irrigation fluids containing PHMB and liquid wound antiseptic/antimicrobial agents is one way to overcome the issue of PHMB availability to the wound surface. One other attribute of PHMB also poses a problem. The action of PHMB on bacteria is not immediate; the mode of action of the product means that contact between the chemical and the bacterial cell wall is required over a small but significant time. A study in Germany (Werner and Kramer, 1995) indicated that contact between the bacterium and PHMB needs to be maintained for 10–15 minutes to ensure maximum antibacterial action. In solutions this can pose a practical problem. Few clinicians are likely to be able to leave fluid in contact with the wound bed for the required timeframe. Continuous irrigation is a possible option, although this is undertaken relatively infrequently in the UK and would be limited to environments suited to such techniques (e.g. hospitals and specialist clinics). Such techniques could be considered when linked to other healthcare interventions such as topical negative pressure (TNP) and the technology is available to achieve this (V.A.C.® Instill, KCl). However, such products are currently not available in the UK. The use of PHMB irrigation-soaked gauze is another possibility, but, as previously indicated, PHMB molecules will bond to the gauze fibres, severely reducing their availability to the wound bed.

If dispersal of PHMB into the wound is a clinical priority, other mechanisms of delivery will need to be considered. Some 'donating' dressings are commercially available and it would seem appropriate that if this particular action is required in a clinical scenario, these products would be the mode of choice.

In the German-speaking sector of the European community where the use of PHMB has been accepted for a number of years, there have been two consensus meetings (2004, 2008), both of which named PHMB as the first

choice of antiseptic/antimicrobial therapy in the management of locally infected and critically colonised wounds. The findings from this group's review of the published studies indicate that PHMB offers a safe and effective method of antiseptic/antimicrobial activity. Commonly used concentrations of PHMB in this arena are 0.01%, 0.02% or 0.04%, available as antiseptic/antimicrobial solutions, wound rinsing agents, antiseptic/antimicrobial and cleansing gels and dressings. Within the dressing formulations, there are products containing PHMB which provide an antiseptic/antimicrobial barrier, and dressing materials capable of donating PHMB to the wound surface. The method of application and relative concentration of active constituent depends on the specific treatment indication.

In Europe, the following guidelines for use are given (Dissemond et al, 2010):

Antiseptic/antimicrobial solutions

- Acute, contaminated, severely purulent wounds: use a 0.04% solution
- Clinically infected chronic wounds: use a 0.04% solution
- For application in suction/rinse drainage: use a 0.02% solution
- Intraoperative wound contamination: use a 0.01% solution for decontamination
- Colonised chronic wounds (particularly in 'at risk' groups): use a 0.01–0.02% solution.

(Note: At present no antiseptic/antimicrobial solutions are commercially available in the UK.)

Wound rinsing solutions (medical devices)

Wound rinsing solutions are not considered to be an antiseptic/anticmicrobial agent, but a medical device with PHMB added as a preservative, i.e. the product claims are based on a purely physical cleansing effect.

Antiseptic/antimicrobial gel preparations (medicinal products)

Antiseptic/antimicrobial gels are used to deliver PHMB over a longer period of time for the prophylaxis and therapy of infected wounds and can be made up of similar concentrations to antiseptic/antimicrobial solutions. The common recommendation for infections with Gram-negative pathogens is to use the higher concentration (0.1%).
(Note: At present no antiseptic/antimicrobial gel preparations are commercially available in the UK.)

Wound dressings containing PHMB

Wound care products are available to reduce microbial counts within the wound. Some products are claimed to donate PHMB and are indicated for the treatment of critically colonised or infected wounds.

The use of PHMB has specific contraindications. According to current knowledge (Deutscher Arzneimittel Codex, 2008), PHMB must not be used:

- For peritoneal lavage
- For antiseptic/antimicrobial joint lavage (cartilage toxicity)
- In applications involving any part of the central nervous system (CNS), including the meninges, and intralumbal applications
- For applications involving the middle or inner ear, or for intraocular applications
- During the first four months of pregnancy (at any time thereafter, a strict benefit/risk assessment has to be performed)
- In patients allergic to PHMB

(Dissemond et al, 2010).

As can be seen, apart from a very small minority of patients who fall within the last two groups, PHMB does not have any contraindications for application within the wound care population.

The UK market is generally naive to the role of PHMB in wound bioburden management. To date, only a few dressing products containing PHMB have been launched and have made little market penetration. Therefore, the full range of products listed in the 'German document' is not appropriate for UK consideration at this time. In mainland Europe, many wound preparations are manufactured locally by pharmacists to approved recipes. While this model has historically been present in the UK, the lack of manufacturing facilities in many establishments, and issues over manufacturers' liability and licensing, particularly since the 'peppermint water case' (Taylor-Lloyd v Crown, 2000), means that this is generally no longer undertaken. Instead, the UK market relies on commercially available products.

Conclusion

The tone and advice offered within the German guidance points the way to how a similar document could be utilised within the UK sector. The consensus panel believes it is unlikely that

the UK healthcare market will adopt a single antiseptic/antimicrobial strategy — silver, iodine and honey-based products will continue to play a key role in clinical practice. However, there is a need to investigate alternative antiseptic/antimicrobial approaches. PHMB offers an opportunity to incorporate a new method of bacterial control which has been proven safe, efficient and cost-effective. This will provide benefits to patients and clinicians by offering alternative and additional tools to manage bacterial burden within the wound care environment.

The consensus group believes that by developing a framework for its introduction in the UK, clinicians will be able to ensure that future product development and PHMB's subsequent integration within existing policies and guidelines is achieved through the guise of evidence-based practice and cost-effectiveness, rather than by being driven by commercial considerations.

References

Akiyama H, Kanzaki H, Tada J, Arata J (1996) *Staphylococcus aureus* infection on cut wounds in the mouse skin: Experimental staphylococcal botryomycosis. *J Dermatol Sci* **11(3)**: 234–8

Allen MJ, Morby AP, White GF (2004) Cooperativity in the binding of the cationic biocide polyhexamethylene biguanide to nucleic acids. *Biochem Biophys Res Commun* **318(2)**: 397–404

Beneke MJ, Doner J (2005) Observation of nosocomial surgical-site infection rates with utilization of antimicrobial gauze dressing in an acute care setting. Abstract presented at the 18th Annual Symposium on Advanced Wound Care, San Diego. Available online at: www.kendallamd.com/pdf/H-5764ObservSSI_WP.pdf

Best Practice Statement: The use of topical antiseptic/antimicrobial agents in wound management (2010) Wounds UK, Aberdeen: in print

Bowler PG, Duerden BI, Armstrong DG (2001) Wound microbiology and associated approaches to wound management. *Clin Microbiol Rev* **14**: 244–69

Broxton P, Woodcock PM, Gilbert, P. (1984b) Injury and recovery of *Escherichia coli* ATCC 8739 from treatment with some polyhexamethylene biguanides. *Microbios* **40**: 161–2, 187–93

Broxton P, Woodcock P., Heatley F, Gilbert P (1984a) Interaction of some polyhexamethylene biguanides and membrane phospholipids in *Escherichia coli*. *J Appl Bacteriol* **57(1)**: 115–24

Cazzaniga A, Serralta V, Davis S, Orr R, Eaglstein W, Mertz PM (2002) The effect of an antimicrobial gauze dressing impregnated with 0.2-percent polyhexamethylene biguanide as a barrier to prevent *Pseudomonas aeruginosa* wound invasion. *WOUNDS* **14(5)**: 169–76

Ceri H, Olson ME, Stremick C, et al (1999) The Calgary biofilm device: New technology for rapid determination of antibiotic susceptibilities of bacterial biofilms. *J Clin Micro* **37(6)**: 1771–6

Cooper R, Okhiria O (2008) The issue of biofilms in wounds. In: White R, ed. *Advances in Wound Care*. Wounds UK, Aberdeen: 189–204

Cooper RA (2004) A review of the evidence for the use of topical antimicrobial agents in wound care. WorldWide Wounds. Available online at: www.worldwidewounds.com/2004/february/Cooper/Topical-Antimicrobial-Agents.html (last accessed April 2010)

Cutting KF, Harding KG (1994) Criteria for identifying wound infection. *J Wound Care* **3**: 198–201

Daeschlein G, Assadian O, Bruck JC, et al (2007) Feasibility and clinical applicability of polihexanide for treatment of second-degree burn wounds. *Skin Pharmacol Physiol* **20(6)**: 292–6

Davies A, Bentley M, Field BS (1968) Comparison of the action of vantocil, cetrimide and chlorhexidine on *Escherichia coli* and its spheroplasts and the protoplasts of Gram-positive bacteria. *J Appl Bacteriol* **31(4)**: 448–61

Davies A, Field BS (1969) Action of biguanides, phenols and detergents on *Escherichia coli* and its sphereoplasts. *J Appl Bacteriol* **32(2)**: 233–43

De Hann B, Ellis H, Wilkes M (1974) The role of infection in wound healing. *Surg Gyn Obstet* **138**: 693–700

Deutscher Arzneimittel-Codex DAC/Neues Rezeptur-Formularium NRF (2008) Govi-Verlag Eschborn

Dissemond J, Gerber V, Kramer A, et al (2010) A practice-orientated recommendation for treatment of critically colonised and locally infected wounds using polihexanide. *J Tissue Viability:* in print

Dow G, Browne A, Sibbald RG (1999) Infection in chronic wounds: controversies in diagnosis and treatment. *Ostomy Wound Manage* **45**: 23–40

Dowsett C, Edwards-Jones V, Davies S (2005) Infection control for wound bed preparation. *Br J Community Nurs* **9(9s)**: 12–17

Dworkin M, Shapiro J, eds (1997) *Bacteria as Multicellular Organisms*. Oxford University Press, New York

Eberlein Th, Wild Th (2008) Approaches to therapy and an outlook on improving the clinical data situation, with a special focus on the polihexanide-containing Suprasorb X + PHMB Hydrobalance dressing. EWMA conference extended abstract. Available online at: www.activahealthcare.co.uk/pdf/SXP009.pdf (last accessed April 2010)

Edwards R, Harding KG (2004) Bacteria and wound healing. *Curr Opin Infect Dis* **17(2)**: 91–6

European Wound Management Association (2006) Position Document: *Management of wound infection*. MEP Ltd, London

Galitz C, Hämmerle G, Signer M, et al (2009) Polyhexanide versus silver wound dressings — first interim results of a controlled, randomized, prospective, multicentric study. Poster presentation. European Wound Management Association Conference, Helsinki

Gethin G, (2009) Role of topical antimicrobials in wound management. *J Wound Care* **Activa Healthcare Supplement**: S4–S8

Gilliver S (2009) PHMB: a well-tolerated antiseptic with

no reported toxic effects. *J Wound Care* **Activa Healthcare Supplement**: S9–14

Hancock RE, Sahl HG (2006) Antimicrobial and host-defense peptides as new antiinfective therapeutic strategies. *Nat Biotechnol* **24(12)**: 1551–7

Harbs N, Siebert J (2007) *In vitro* efficacy of octenidine and polihexanide against biofilms composed of *Pseudomonas aeruginosa*. *GMS Krankenhaushyg Interdiszpl* **2(2)**: Doc.45 (20071228)

Harrison-Balestra C, Cazzaniga AL, Davis SC, Mertz PM (2003) A wound-isolated *Pseudomonas aeruginosa* grows a biofilm *in vitro* within 10 hours and is visualized by light microscopy. *Dermatol Surg* **29**: 631–5

Hunt TK, Hopf HW (1997) Wound healing and wound infection. What surgeons and anaesthesiologists can do. *Surg Clin North Am* **77**: 587–606

Ikeda T, Ledwith A, Bamford CH, Hann RA (1984) Interaction of a polymeric biguanide biocide with phospholipid membranes. *Biochim Biophys Acta* **769(1)**: 57–66

Ikeda T, Tazuke S, Watanabe M (1983) Interaction of biologically active molecules with phospholipid membranes. I. Fluorescence depolarization studies on the effect of polymeric biocide bearing biguanide groups in the main chain. *Biochim Biophys Acta* **735(3)**: 380–6

Kerstein MD (1997) The scientific basis of healing. *Adv Wound Care* **10**: 30–6

Kingsley A (2001) A proactive approach to wound infection. *Nurs Standard* **15(30)**: 50–8

Larkin DF, Kilvington S, Dart JK (1992) Treatment of *Acanthamoeba keratitis* with polyhexamethylene biguanide. *Ophthalmol* **99(2)**: 185–91

Lawrence JC (1998) The use of iodine as an antiseptic agent. *J Wound Care* **7**: 421–5

Lee WR, Tobias KM, Bemis DA, Rohrbach BW (2004) *In vitro* efficacy of a polyhexamethylene biguanide-impregnated gauze dressing against bacteria found in veterinary patients. *Vet Surg* **33(4)**: 404–11

Malaisrie SC, Malekzadeh S, Biedlingmaier JF (1998) *In vivo* analysis of bacterial biofilm formation on facial plastic bioimplants. *Laryngoscope* **108(11 Pt 1)**: 1733–8

McLean RJ, Whiteley M, Stickler DJ, Fuqua WC (1997) Evidence of autoinducer activity in naturally occurring biofilms. *FEMS Microbiol Letters* **154(2)**: 259–63

Mertz PM, (2003) Cutaneous Biofilms: friend or foe? *WOUNDS* **15(5)**. Available online at: www.medscape.com/viewarticle/456297

Michaels J A , Campbell B, King B Palfreyman SJ, Shackley P, Stevenson M (2009) Randomized controlled trial and cost-effectiveness analysis of silver-donating antimicrobial dressings for venous leg ulcers (VULCAN trial). *Br J Surg* **96(10)**: 1147–56

Moore K, Gray D (2007) Using PHMB antimicrobial to prevent wound infection. *Wounds UK* **3(2)**: 96–102

Motta GJ, Milne CT, Corbett LG (2004) Impact of antimicrobial gauze on bacterial colonies in wounds that require packing. *Ostomy Wound Manage* **50**: 48–62

Motta GJ, Trigilia D (2005) The effect of an antimicrobial drain sponge dressing on specific bacterial isolates at tracheostomy sites. *Ostomy Wound Manage* **51**: 60–6

Mueller M, de la Pe FA, Derendorf H (2004) Issues in pharmacokinetics and pharmacodynamics of anti-infective agents: Kill curves versus MIC. *Antimicrob Agents Chemother* **48(2)**: 369–77

Mueller SW, Krebsbach LE (2008) Impact of an antimicrobial-impregnated gauze dressing on surgical site infections including methicillin-resistant *Staphylococcus aureus* infections. *Am J Infect Control* **36(9)**: 651–5

Mulder GD, Cavorsi JP, Lee DK (2007) Polyhexamethylene biguanide (PHMB): an addendum to current topical antimicrobials. *WOUNDS* **19(7)**: 173–82

Müller G, Kramer A (2008) Biocompatibility index of antiseptic agents by parallel assessment of antimicrobial activity and cellular cytotoxicity. *J Antimicrob Chemother* **61(6)**: 1281–7

National Committee for Clinical Laboratory Standards (1997) *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*. 4th edn. Approved Standard. Villanova, PA: NCCLS Publication M7–A4

Pietsch T, Kraft R (2006) Antimikrobielle Wirksamkeit ausgewählter Substanzen für die Wasserdesinfektion in Dentaleinheiten mit Biofilmen. *Aseptica* **12(4)**: 3–4

Pinto F, Maillard JY Denyer SP (2009) Effect of surfactants, temperature, and sonication on the virucidal activity of polyhexamethylene biguanide against the bacteriophage MS2. *Am J Infect Control* [Epub ahead of print], available online at: www.ncbi.nlm.nih.gov/pubmed/20006410

Pollock S (1984) The wound healing process. *Clin Dermatol* **2**: 8

Reid G, Howard J Bing SG (2001) Can bacterial interference prevent infection? *Trends Microbiol* **9(9)**: 424–8

Schnuch A, Geier J, Brasch J, et al (2000) Polyhexamethylene biguanide: a relevant contact allergen? *Contact Dermatitis* **42(5)**: 302–3

Schnuch A, Geier J, Uter W, et al (2007) The biocide polyhexamethylene biguanide remains an uncommon contact allergen. *Contact Dermatitis* **56(4)**: 235–59

Schultz GS, Sibbald RG, Falanga V, et al (2003) Wound bed preparation: a systematic approach to wound management. *Wound Rep Regen* **11(Suppl 1)**: S1–28

Seipp HM, Hofmann S, Hack A, Skowronsky A, Hauri A (2005) Wirksamkeit verschiedener Wundspüllösungen gegenüber Biofilmen. *J Wound Healing* (ZfW) **4**: 160–4

Serralta VW, Harrison-Balestra C, Cazzaniga AL, Davis SC, Mertz PM (2001) Lifestyles of bacteria in wounds: Presence of biofilms? *WOUNDS* **13(1)**: 29–34

Sibbald RG, Browne AC, Coutts P, Queen D (2001) Screening evaluation of ionised nanocrystalline silver dressing in chronic wound care. *Ostomy Wound Manage* **47**: 38–43

Sipahi OR (2008) Economics of antibiotic resistance. *Expert review of Anti-infective Therapy* **6(4)**: 523–39. Available online at: www.medscape.com/viewarticle/580479 (accessed April 2010)

Sorensen OE, Cowland JB, Theilgaard-Monch K, Liu L, Ganz T, Borregaard N (2003) Wound healing and expression of antimicrobial peptides/polypeptides in human keratinocytes, a consequence of common growth factors. *J Immunol* **170(11)**: 5583–9

Taylor-Lloyd v Crown (2000) Details available online at:

www.pharmj.com/editorial/20020216/society/statcomm.html#3

Thomas S, McCubbin P (2003) An *in vitro* analysis of the antimicrobial properties of 10 silver-containing dressings. *J Wound Care* **12(8)**: 305–8

Vowden K, Cooper RA (2006) Managing wound infection. In: Position Document: *Management of wound infection*. MEP Ltd, London

Warriner R, Burrell R. (2005) Infection and the chronic wound: a focus on silver. *Adv Skin Wound Care* **18(Suppl 1)**: 2–12

Werner HP, Kramer A (1995) Mikrobiologische Anforderungen an lokale Antiinfektiva unter spezieller Berücksichtigung der antiinfektiven Wundbehandlung. In: Kramer A, Wendt M, Werner HP (Hrsg) *Möglichkeiten und Perspektiven der klinischen Antiseptik*. Wiesbaden mhp-Verlag: 26–30

Werthen M, Davoudi M, Sonesson A, et al (2004) Pseudomonas aeruginosa-induced infection and degradation of human wound fluid and skin proteins ex vivo are eradicated by a synthetic cationic polymer. *J Antimicrob Chemother* **54(4)**: 772–9

White RJ, Cooper RA, Kingsley A (2001) Wound colonization and infection: the role of topical antibacterials. *Br J Nurs* **10**: 563–78

White RJ, Cutting KF (2006) Critical colonization — the concept under scrutiny. *Ostomy Wound Manage* **52(11)**: 50–6

Wiegand C, Abel M, Kramer A, Müller G, Ruth P, Hipler U-C (2008) Viability and proliferation of fibroblasts, keratinocytes and HaCaT-cells influenced by polihexanide. Poster presentation Wounds UK Conference, Harrogate. Available online at: www.activahealthcare.co.uk/pdf/SXP004.pdf (last accessed April 2010)

Wiegand C, Abel M, Ruth P, Hipler U-C (2008) Protective effect of polihexanide on HaCaT keratinocytes in co-culture with *Staphylococcus aureus*. Poster presentation at Wounds UK Conference, Harrogate. Available online at: www.activahealthcare.co.uk/pdf/SXP008.pdf (last accessed April 2010)

Wild Th, Buckner M, Payrich M, Schwarz Ch, Eberlein Th (2009) Prospective, randomized study for eradication of MRSA with polihexanide containing biocellulose dressing compared with polihexanide wound solution. Poster presentation. European Wound Management Association Conference, Helsinki

Wright JB, Lam K, Olson ME, Burrell RE (2003) Is antimicrobial efficacy sufficient? A question concerning the benefits of new dressings. *WOUNDS* **15(3)**. Available online at: www.woundsresearch.com/article/1583 (accessed April 2010)

Yasuda K, Ohmizo C, Katsu T (2003) Potassium and tetraphenylphosphonium ion-selective electrodes for monitoring changes in the permeability of bacterial outer and cytoplasmic membranes. *J Microbiol Methods* **54(1)**: 111–15