Flaminal®: a novel approach to wound bioburden control

It is now widely accepted that wound bioburden, in chronic wounds in particular, will often require control. This is best achieved by the use of appropriate topical agents, available in safe, sustained release, broad-spectrum forms. The ancillary functions, if any, of a topical antimicrobial are becoming important health economic factors. Resistance and its development is also of great interest. The new Flaminal® products are novel formulations with broad-spectrum antimicrobial activity which have the capacity to control exudate and promote rapid wound healing. These will be available on the Drug Tariff from October 2006.

Richard White

KEY WORDS
Topical antimicrobial
Glucose oxidase
Lactoperoxidase
Hydrogen peroxide
Wound bioburden

Modern wound management requires that systematic assessment and treatment approaches be adopted in order to address the needs of the wound in a logical way. The frameworks of wound bed preparation using the TIME acronym (Dowsett and Ayello, 2004) and Applied Wound Management (Gray et al, 2006) are two such systems. Both require the assessment and management of the tissues in the wound (e.g. slough, necrosis), the control of exudate and an assessment and control of bioburden, as appropriate.

In recent years, research has increased our understanding of the role bacteria play in the chronic wound. For example, the wound bioburden has been ranked according to the Wound Infection Continuum (Kingsley, 2001) and to the principles of Wound Bed Preparation (Schultz et al, 2003). It is recognised that under certain circumstances, the bioburden will be a contributing factor to delayed healing (White, 2006) in the state now known as critical colonisation (White et al, 2006), or to overt infection. Wound bioburden in these states can be controlled by topical or systemic therapy with topical antimicrobials being the approach of choice for critical colonisation and local infection (Kingsley, 2003; White et al, 2006).

Bacteria in chronic wounds have been associated with the development of an 'immunopathological' state (Heinzelmann et al, 2002). The various virulence determinants of typical wound bacteria e.g. *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Bowler et al, 2001) have been listed and linked, to some degree, with the signs and symptoms of the wound (Cooper, 2003). Notable among these is the development of the biofilm.

Complex communities of micro-organisms encased in slime and attached to surfaces are known as biofilms (Costerton et al, 1995; 1999). They probably represent the most common form of existence for microbes in natural environments (Cooper, 2006). Biofilms have been described in wounds (Serralta et al, 2001; Boutli-Kasapidou et al, 2006). It is likely that many — possibly all — chronic wounds will harbour biofilms (Costerton, 2006), and in the typical chronic wound, slough is associated with biofilm formation. However, not all biofilms in wounds will necessarily be formed in the presence of slough. It has been shown that some wound antimicrobials, notably silver, iodine compounds, honey (Akyama et al, 2004; Chaw et al 2005; White, 2005) maggots and electrical current (Van der Borden et al, 2004a,b) have the capacity to disrupt the biofilm.

Antimicrobial wound dressings

Hydrogel dressings are either sheet presentations or amorphous gels supplied in tubes. They are intended to create a moist wound environment in dry wounds, and absorb exudate in exuding wounds to promote autolytic debridement, i.e. they either donate or absorb moisture (Thomas and Hay,

Figure 1. Flaminal® and Flaminal® Hydro are available in 15g tubes.

Richard White is Scientific Editor, Wounds UK and Senior Research Fellow, Aberdeen Royal Infirmary

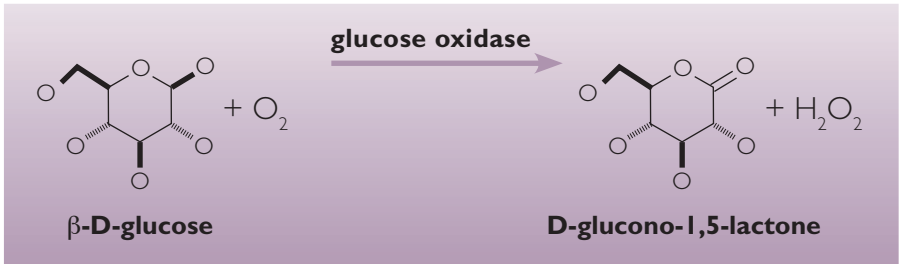

Figure 2. Glucose oxidase and its mode of action.

1996). Both presentations have been available in the UK for more than 20 years and both have become widely used in clinical practice (Flanagan, 1995). Flaminal® hydrogels are different because they are based upon gelled alginate and not on other polymers (*Figure 1*).

The recent developments in antimicrobial wound dressings have tended to follow the prevailing fashion, i.e. the use of silver (Silver et al, 2006). The arrival of medical-grade honey, with a CE mark and Drug Tariff listing, has been a welcome alternative (White, 2005). Flaminal® hydrogels use the enzymes glucose oxidase and lactoperoxidase to control the bioburden in a similar way to honey. Honey works as an antimicrobial by using its 'built-in' glucose oxidase enzyme system for generating hydrogen peroxide (*Figure 2*) (Molan, 2005). Hydrogen peroxide is a non-specific antimicrobial that kills all micro-organisms (Russell, 2002). This agent, used for many years as a solution to clean sloughy wounds (O'Brien 2002), is an antimicrobial by virtue of its oxidising activity. The use of hydrogen peroxide in solution form is now largely history. It is, however, the basic mechanism employed by phagocytic cells in fighting micro-organisms (Baboir, 1984; O'Brien, 2000).

Phagocyte antimicrobial action

Phagocytes (neutrophils, macrophages, eosinophils) destroy pathogens in part by 'respiratory burst' which is the rapid production of oxygen metabolites such as superoxide, peroxide, hydroxyl radicals and possibly singlet molecular oxygen (Baboir, 1984; Clark, 1990). The antimicrobial effectiveness of hydrogen peroxide is increased greatly by peroxidase. One enzyme that can be used for this purpose is myeloperoxidase; this is present in neutrophils and monocytes but is lost when the monocyte matures into a macrophage. Further, peroxidase-coated organisms are more readily killed when ingested by macrophages than are uncoated organisms (Klebanoff et al, 1983).

Flaminal® and Flaminal® Hydro both contain an enzyme complex containing glucose oxidase and lactoperoxidase that protects against microbial colonisation and combats infection. Thus both gels can be used clinically to address the tissues in the wound, exudate or moisture, and bioburden.

Flaminal® and Flaminal® Hydro alginate gel dressings

Flaminal® and Flaminal® Hydro (Flen Pharma, Belgium; Ark Therapeutics, UK) are new antimicrobial, amorphous alginate gel dressings which are based upon alginate, an agent well-known in modern wound management for its capacity to form moist gels in the presence of fluids such as wound exudate. Both Flaminal® and Flaminal® Hydro are available in 15g tubes (*Figure 1*). Flaminal® is designed for use on moderate to heavily exuding wounds and has a high alginate content, whereas Flaminal® Hydro is intended for more lightly exuding wounds and contains less alginate. Both contain an enzyme complex containing glucose oxidase and lactoperoxidase that protects against microbial colonisation and combats infection. Thus both gels can be used clinically to address the tissues in the wound, exudate or moisture, and bioburden.

Flaminal® hydrogels possess some of the attributes of the ideal antiseptic, such as slow-release and sustained release of the antimicrobial, non-toxic, and unlikely to select for resistance.

Both are intended to promote moist wound healing and, by association, autolytic debridement. In addition, and by virtue of the content of glucose oxidase and lactoperoxidase both will also have the capacity to control the bioburden of the wound in a safe, sustained fashion. This is useful for the management of all wounds left to heal by secondary intent, particularly chronic wounds, as they are invariably colonised.

Lactoperoxidase

Flaminal® contains lactoperoxidase which is an enzyme extracted from milk and acts as an important natural antimicrobial (Banks et al, 1986). It has been shown to be bacteriostatic against Gram-positive organisms and exhibits pH-dependent bactericidal action against Gram-negative organisms in the presence of hydrogen peroxide and thiocyanate. Lactoperoxidase offers the following benefits in chronic wound management:

- Antimicrobial properties
- Resistance to proteolysis.

Peroxidases are enzymes that belong to the natural non-immune defence systems (Tafazoli and O'Brien, 2005) found in milk and in the secretions of exocrine glands such as saliva, tears, intestinal secretions, cervical mucus and the thyroid.

The mammary fluids colostrum and milk, deliver nature's first host defences upon the first feed after birth. Lactoperoxidase also helps maintain the sterility of milk (Clare et al, 2003; Florisa et al, 2003). In tears it provides, together with lysozyme, mechanisms for keeping the eye free from infection (Bron and Seal, 1986). In the airways the secretion of mucus provides a defence against infection. Mucus contains enzymes, including lactoperoxidase which is

identical to that found in breast milk; this, in conjunction with secretions containing thiocyanate (SCN^-), form a peroxidase system that protects against infection from bacteria, fungi and viruses (Conner et al, 2002).

The salivary gland is a rich source of peroxidases (Banerjee and Datta, 1986; Ihalin et al, 2006). Lactoperoxidase in saliva (Bannerjee and Datta, 1986) serves to combat bacteria, particularly *Streptococcus mutans* (Thomas et al, 1983) and to inhibit acid formation in dental plaque (Tenovuo et al, 1981).

Activity

Peroxidases have no antimicrobial activity themselves, but in the presence of the specific co-factors they constitute an important defence system when they are in liquid solution. These co-factors are hydrogen peroxide and a halide (e.g. iodine, chlorine) or pseudo-halides (e.g. thiocyanate) depending on the specific enzyme. The oxidation product OX^- is a short-lived oxidizing agent which will react with thiol groups (-SH) of the enzymes essential for the metabolism of bacteria. This defence mechanism plays a key role in protecting mucus membranes against bacterial invasion.

Lactoperoxidase system

The lactoperoxidase system can produce, in appropriate conditions, molecules such as hypothiocyanite ($OSCN^-$), hypoiodide (OI^-) or a mixture of both (Ghibaudi and Laurenti, 2003). These molecules are powerful antimicrobial agents against bacteria, viruses and yeasts. A non-exhaustive list of susceptible organisms includes the following:

Bacteria:

- *Escherichia coli*
- *Yersinia enterocolitica*
- *Klebsiella pneumoniae*
- *Streptococcus agalactiae*
- *Streptococcus mutans*
- *Staphylococcus aureus* (including methicillin-resistant *S. aureus*)
- Salmonella species
- *Shigella sonnei*
- *Listeria monocytogenes*
- Acinetobacter species
- Neisseria species
- *Haemophilus influenzae*
- *Campylobacter jejuni*
- *Aeromonas hydrophila*
- *Pseudomonas aeruginosa*
- *Enterobacter cloacae*

Viruses:

- Herpes simplex virus
- Immunodeficiency virus
- Respiratory syncytial virus

Yeast:

- *Candida albicans.*

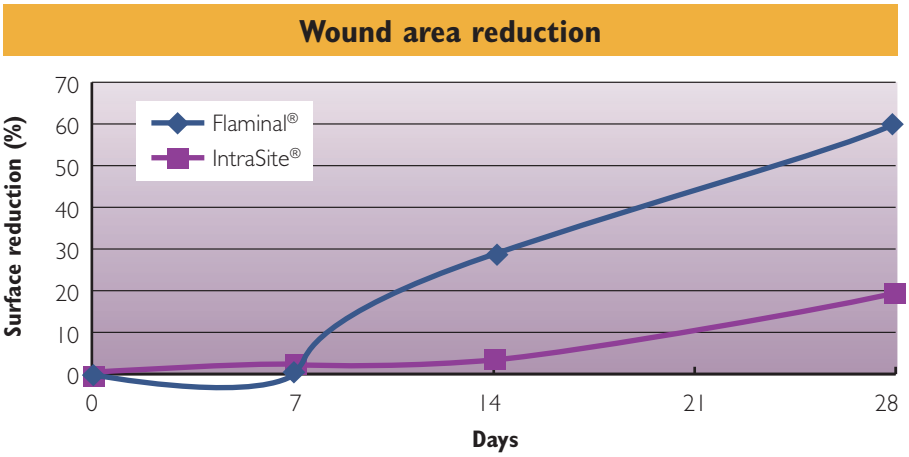

Figure 3. Flaminal® and IntraSite's impact on wound area on two comparable groups of 10 patients.

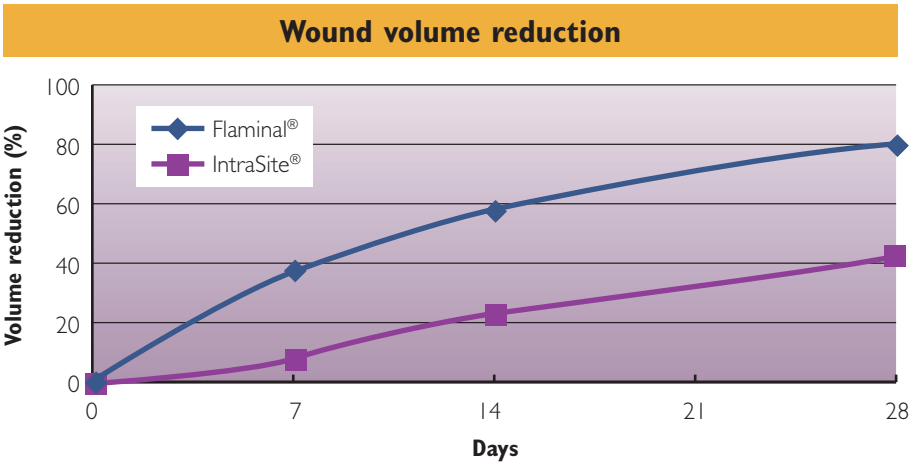

Figure 4. Flaminal® and IntraSite's impact on wound volume on two comparable groups of 10 patients.

Clinical evidence for the effectiveness of Flaminal® gels

Both Flaminal® gels have been tested in vitro and in vivo for antimicrobial activity, and for cytotoxicity in vitro (Vandenbulcke et al, 2006) and in a randomised comparative clinical trial. In the clinical trial, de la Brassinne et al (2006) compared Flaminal® with IntraSite gel® (Smith & Nephew, Hull) in patients with leg ulcers. Two groups of 10 patients that had a balanced total wound size were treated for 28 days. Each wound was assessed weekly for the surrogate endpoints of area and volume. Results show both groups to have area reduction over time with Flaminal® having a statistically significant reduction at day 14 ($p<0.01$) and at day 28 ($p<0.01$), representing a 63% reduction in size for Flaminal® *vs* 19% for IntraSite (*Figure 3*). Similarly, the volumes of both groups also decreased over time. However, for this parameter the difference was greater for Flaminal® at day 7 ($p<0.001$), and at days 14 ($p<0.001$) up to day 28 ($p=0.02$), representing an 80% *vs* 41% total volume reduction. The correlation between volume and area was highly significant, expressed as a Pearson coefficient for the Flaminal® group 0.843; $p<0.001$ (*Figures 3 and 4*).

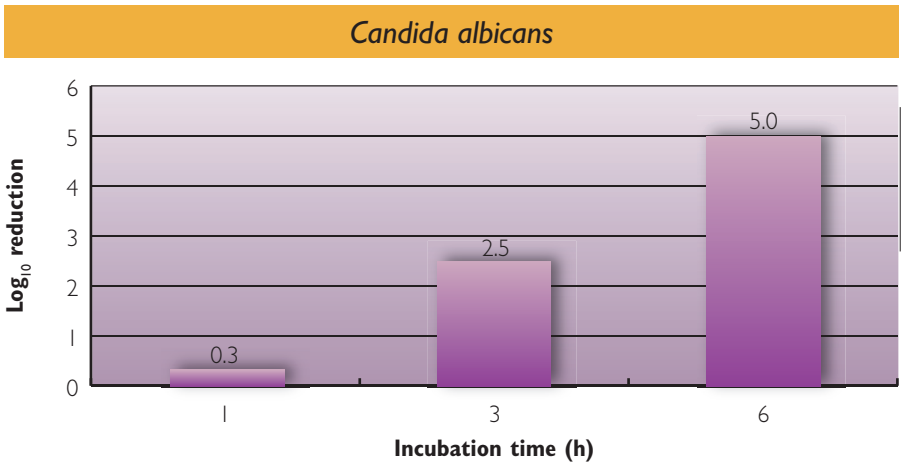

Figure 5. Flaminal®'s effect on *Candida albicans* in vitro.

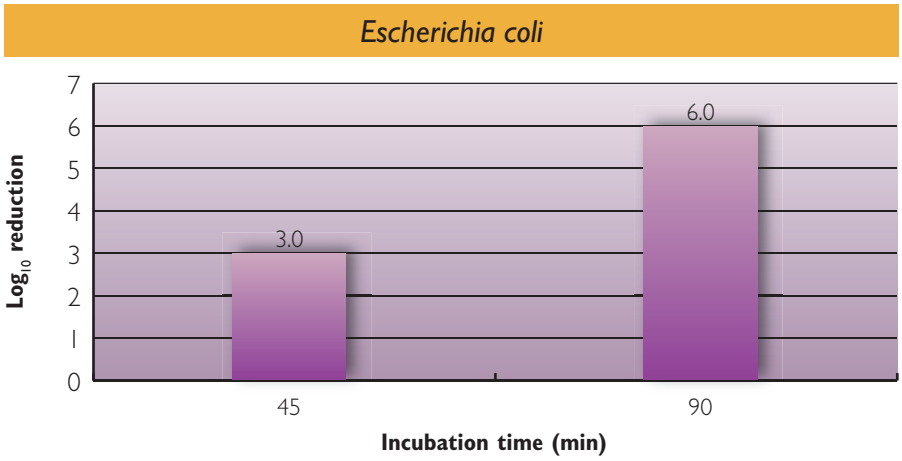

Figure 6. Flaminal®'s effect on *Escherichia coli* in vitro.

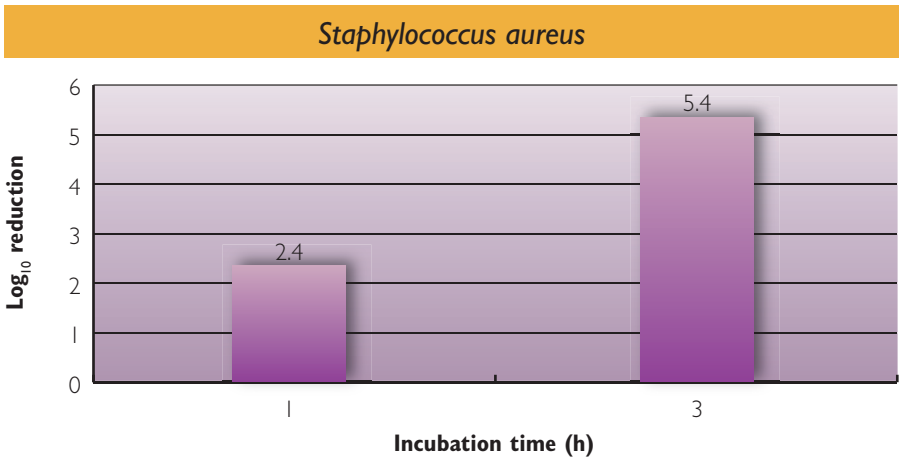

Figure 7. Flaminal®'s effect on *Staphylococcus aureus* in vitro.

In vitro cytotoxicity assays, conducted on human keratinocytes in culture, showed Flaminal® gels to be essentially non-toxic. The antimicrobial activity in vitro showed that both Flaminal® gels reduced a range of Gram-negative and Gram-positive organisms by more than seven $\log_{10}$ units in 48 hours. *E. coli* and *S. aureus* were both reduced by over two $\log_{10}$ values in 45–60 minutes while *C. albicans* was reduced by two $\log_{10}$ in three hours (*Figures 5,6,7*). In vivo, the sampling of wounds before and after treatment with Flaminal® showed a significant decrease in the eradication of species isolated ($p=0.018$). This included complete eradication of *C. albicans*, *P. aeruginosa* and S. *pyogenes* among others (Vandenbulcke et al, 2006).

Conclusions

From the available laboratory and clinical evidence it is clear that the Flaminal® products are safe and effective both clinically and microbiologically. They offer an alternative to the current antimicrobials insofar as they have exudate absorptive capacity, they promote autolytic debridement, they have a broad spectrum of antimicrobial activity with a very low propensity for resistance, and are safe to use on the newly-growing tissues of the wound bed.

The combination of glucose oxidase with lactoperoxidase serves to provide a sustained source of safe and effective broad-spectrum antimicrobial action in a manner similar to our own natural white cell defences. These new and unique additions to the UK Drug Tariff offer the clinician practical alternatives to existing wound treatments. WUK

Akiyama H, Oono T, Saito M, Iwatsuki K (2004) Assessment of cadexomer iodine against Staphylococcus aureus biofilm in vivo and in vitro using confocal laser scanning microscopy. *J Dermatol* **31:** 529–34

Baboir BM (1984) The respiratory burst of phagocytes. *J Clin Invest* **73:** 599–601

Banerjee RK, Datta AG (1986) Salivary peroxidases. *Mol Cell Biochem* **70(1):** 21–9

Banks JG, Board RG, Sparks NH (1986) Natural antimicrobial systems and their

potential in food preservation. *Biotechnol Appl Biochem* **8(2–3):** 103–47

Boutli-Kasapidou F, Delli F, Avgoustinaki N, Lambrou N, Tsatsos M, Karakatsanis G. (2006) What are biofilms? Evaluation and management in open skin wounds. *J Eur Acad Dermatol Venereol* **20(6):** 743–5

Bowler PG, Duerden BI, Armstrong DG (2001) Wound microbiology and associated approaches to wound management. *Clin Microbiol Rev* **14(2):** 244–69

Bron AJ, Seal DV (1986) The defences of the ocular surface. *Trans Ophthalmol Soc UK* **105(part 1):** 18–25

Chaw KC, Manimaran M, Tay FEH (2005) Role of silver ions in destabilization of intermolecular adhesion forces measured by atomic force microscopy in Staphylococcus epidermidis biofilms. *Antimicrob Agents Chemother* **49(12):** 4853–9

Clare DA, Catignani GL, Swaisgood HE (2003) Biodefense properties of milk: the role of antimicrobial proteins and peptides. *Curr Pharm Res* **9(16):** 1239–55

Clark RA (1990) The human neutrophil respiratory burst. *J Infect Dis* **161(6):** 1140–47

Conner GE, Salathe M, Forteza R (2002) Lactoperoxidase and hydrogen peroxide metabolism in the airway. *Am J Respir Crit Care Med* **166(12 part 2):** S57–S61

Cooper RA (2003) The contribution of microbial virulence to wound infection. In: RJ White (ed) *The Silver Book*. Quay Books, Dinton: 38–45

Cooper RA, Okhiria O (2006) Biofilms, wound infection and the issue of control. *Wounds UK* **2(3):** 52–61

Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin-Scott HM (1995) Microbial biofilms. *Annu Rev Microbiol* **49:** 711–45

Costerton JW, Stewart PS, Greenberg EP (1999) Bacterial biofilms: a common cause of persistent infections. *Science* **284:** 1318–22

Costerton JW (2006) *The Complexity of Bacterial Biofilms.* Oral presentation SAWC San Antonio USA. 1st May 2006

de la Brassinne M, Thirion L, Horvat L-I L (2006) A novel method of comparing the healing properties of two hydrogels in chronic leg ulcers. *J Eur Acad Dermatol Venereol* **20(2):** 131–5

Dowsett C, Ayello E (2004) TIME principles of chronic wound bed preparation and treatment. *Br J Nurs* **13(15 Suppl):** S16–S23

Flanagan M (1995) The efficacy of a hydrogel in the treatment of wounds with non-viable tissue. *J Wound Care* **4(6):** 264–7

Florisa R, Recio I, Berkhout B, Visser S (2003) Antibacterial and antiviral effects of milk proteins and derivatives thereof. *Curr Pharm Des* **9(16):** 1257–75

Ghibaudi E, Laurenti E (2003) Unravelling the catalytic mechanism of lactoperoxidase and myeloperoxidase. *Eur J Biochem* **270(22):** 4403–12

Gray D, White R, Cooper P, Kingsley A (2006) Applied wound management. In: *Wound Healing: a systematic approach to advanced wound healing and management.* Wounds UK Books, Aberdeen: 59–100

Heinzelmann M, Scott M, Tan T (2002) Factors predisposing to bacterial invasion and infection. *Am J Surg* **183:** 179–90

Ihalin R, Loimaranta V, Tenuovo J (2006) Origin, structure, and biological activities of peroxidases in human saliva. *Arch Biochem Biophys* **445(2):** 261–8

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

Kingsley AR (2003) The wound infection continuum and its application to clinical practice. *Ostomy Wound Manage* **49(7 suppl A):** 1–7

Klebanoff SJ, Locksley RM, Jong EC, Rosen H (1983) Oxidative response of phagocytes to parasitic invasion. *Ciba Found Symp* **99:** 92–112

Molan P (2005) Honey mode of action. In: White RJ, Cooper RA, Molan P (Eds) *Honey, A Modern Wound Management Product.* Wounds UK Books, Aberdeen: 1–23

O'Brien PJ (2000) Peroxidases. *Chem Biol Interact* **129:** 113–39

O'Brien M (2002) Exploring methods of wound debridement. *Br J Community Nurs* **Dec:** 10–18

Russell AD (2002) Introduction of biocides into clinical practice and the impact on antibiotic-resistant bacteria. *Symp Ser Soc Appl Microbiol* **31:** 121S–135S

Schultz G, Sibbald RG, Falanga V, et al (2003) Wound bed preparation: a systematic approach to wound management. *Wound Repair Regen* **11(Suppl 1):** S1–S28

Silver S, Phung le T, Silver G (2006) Silver as biocides in burn and wound dressings and bacterial resistance to silver dressings. *J Ind Microbiol Biotechnol* **33(7):** 627–34

Tafazoli S, O'Brien PJ (2005) Peroxidases. *Drug Disc Today* **10(9):** 617–25

Tenovuo J, Mansson-Rahemtulla B, Pruitt KM, Arnold R (1981) Inhibition of dental plaque acid production by the salivary lactoperoxidase antimicrobial system. *Infect Immun* **34(1):** 208–14

Thomas EL, Pera KA, Smith KW, et al (1983) Inhibition of Streptococcus mutans by the lactoperoxidase antimicrobial system. *Infect Immun* **39(2):** 767–78

Thomas S, Hay NP (1996) In vitro investigations of a new hydrogel dressing. *J Wound Care* **5(3):** 130–1

Vandenbulcke K, L-I Laenen Horvat, de Mil M et al (2006) Evaluation of the antibacterial action and toxicity of two new hydrogels: a pilot study. *Lower Extrem Wounds* **5(2):** 109–14

Van der Borden AJ, van der Werf H, van der Mei C, Busscher HJ (2004a) Electric current-induced detachment of *Staphylococcus epidermidis* biofilms from surgical stainless steel. *Appl Environ Microbiol* **70(11):** 6871–74

Van der Borden AJ, van der Werf H, van der Mei C, Busscher HJ (2004b) Electric current-induced detachment of Staphylococcus epidermidis biofilms from surgical stainless steel. *J BiomedMater Res* **68B:** 160–4

White RJ (2005) The benefits of honey in wound management. *Nurs Stand* **20(10):** 57–64

White RJ (2006) Delayed wound healing: what, when, why and in whom? *Nurs Stand* **(Suppl)1(1):** 47–54

White RJ, Cutting KF, Kingsley AR (2006) Topical antimicrobials in the control of wound bioburden. *Ostomy Wound Manage* **52(8):** 26–58

Key Points

- Flaminal® hydrogels offer a novel antimicrobial action with exudate control.
- The control of wound bioburden by topical applications is important in critical colonisation, and local infection in wounds healing by secondary intent.
- Flaminal® has a broad spectrum of antimicrobial action including rapid kill of MRSA.
- Clinical data indicates that Flaminal® is effective in reducing wound depth and area in chronic leg ulcers.
- Flaminal® has a very low propensity for resistance selection.