

4. CLINICAL EVALUATION

A patient was treated with Mepilex Ag; the healing was evaluated qualitatively; SEM analysis and bacterial counts were undertaken on the dressing samples.

METHODS

A patient with a venous leg ulcer (previously treated with a control foam dressing without silver) was treated with Mepilex Ag for 4 weeks, changed approximately twice a week in conjunction with compression therapy. At inclusion, and after 1 and 4 weeks, 4mm punch biopsies were taken from the dressings (n=3) and were fixed for SEM (0 and 1 week material) and vigorously shaken in 5ml 10mM phosphate buffer with 0.05% Tween (10 min) (0 and 4 week materials). Bacterial counts in the control foam dressing (without silver) at inclusion of the study were compared with the counts obtained after 4 weeks of treatment with Mepilex Ag.

RESULTS

The dressing was well tolerated. The pictures (Figure 8) show the status at inclusion (1); at 1 week (2); week 3 (3); and week 4 (4).

The bacteria detected were *S. aureus*, *Xanthomonas maltophilia*, and *S. agalacticae*. There was a reduction in the bacterial counts after using Mepilex Ag. Although bacteriological analysis detected these three microbes after 4 weeks, there was a marked predominance of *S. aureus* after the 4-week treatment period (Figure 7).

Analysis by SEM of the dressing surface demonstrated various Gram-negative rods were detected on the control dressing surface, which were not detectable on the Mepilex Ag dressing after 1 week.

Figure 7: Colony Forming Unit counts from Mepilex (at baseline) and Mepilex Ag after 4 weeks

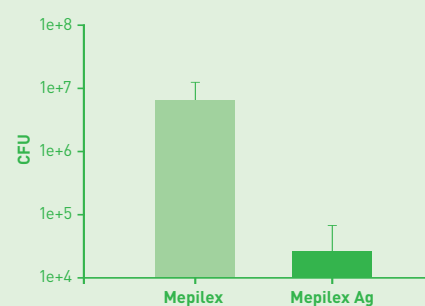


Figure 8: Sequential (9 weekly) photographs of VLU treated with Mepilex Ag



CASE STUDY IN VITRO STUDY

Multiple Actions of Mepilex Ag®, a New Antimicrobial Wound Dressing

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INTRODUCTION

Mepilex Ag is an antimicrobial soft silicone foam dressing that absorbs exudate and maintains a moist wound environment. It combines the proven benefits of soft silicone technology (i.e. ability to prevent pain and trauma to the patient) with a silver component that provides instant and sustained, broad-spectrum antimicrobial activity. Mepilex Ag begins inactivating wound-related pathogens within 30 minutes of application to the wound, with sustained effect up to seven days. By reducing the number of microorganisms, it may also reduce wound odour. Mepilex Ag is designed for a wide range of exuding wounds such as leg and foot ulcers, pressure ulcers and partial thickness burns. It may also be used under compression bandaging.

AIM

A series of in vitro, ex vivo and in vivo tests were undertaken to:

1. Evaluate the antimicrobial activity of Mepilex Ag
2. Compare the antimicrobial activity of Mepilex Ag and other silver dressings
3. Evaluate the ability of Mepilex Ag to reduce the effect of bacterial proteinases
4. Evaluate Mepilex Ag in a clinical setting

METHODS

1. Evaluation of antibacterial activity:
 - 1a Growth assays using *Pseudomonas aeruginosa* and *Staphylococcus aureus*.
 - 1b Radial diffusion assay.
 - 1c Growth assays using *Pseudomonas aeruginosa* and *Staphylococcus aureus* in the presence of human serum.
2. Comparison of antibacterial activity with other silver dressings: growth assays using *Pseudomonas aeruginosa*.
3. Evaluation of effects of dressing on protein degradation and proteinases.
4. Preliminary clinical evaluation (case study) – wound size and bacterial content of dressing analysed.

RESULTS

1. Mepilex Ag demonstrated a significant antibacterial effect in relevant physiological environments. The dressing was active against 19 isolates derived from chronic wounds, including *S. aureus* bacteria.
2. The antimicrobial activity of Mepilex Ag was shown to be comparable to that of other silver dressings.
3. Mepilex Ag blocked protein degradation and abolished release of bacterial enzymes.
4. Clinically, a venous leg ulcer (VLU) treated with Mepilex Ag showed a healing response, and analysis showed a reduction of bacterial contamination within the dressings after 4 weeks' treatment.

KEY POINTS

Mepilex Ag represents a silver dressing, having:

1. Significant and sustainable activity against relevant wound bacteria.
2. Established capacity for exudate control.
3. Ability to block proteinase release and protein degradation.

 **Mepilex Ag®**

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1. ANTIBACTERIAL ACTIVITY

1A. GROWTH ASSAYS

A growth assay was undertaken to evaluate the antimicrobial effects of Mepilex Ag eluates on *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

METHODS

Mepilex Ag dressings were incubated in bacterial medium overnight at 37°C to give different eluate concentrations. These eluates were incubated with *Pseudomonas aeruginosa* and *Staphylococcus aureus* bacteria (2 x 10⁵ cfu) in a total volume of 200µl and their subsequent growth measured at different time periods. The growth was recorded by measuring absorbance (y-axis).

RESULTS

The results demonstrate that both *Pseudomonas aeruginosa* (Figure 1) and *Staphylococcus aureus* (Figure 2) showed inhibitions of growth at the higher concentrations of eluates.

Figure 1: Growth curves for *Pseudomonas aeruginosa* treated with Mepilex Ag eluates

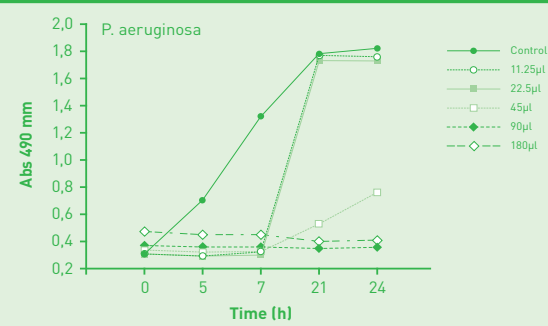


Figure 2: Growth curves for *Staphylococcus aureus* treated with Mepilex Ag eluates

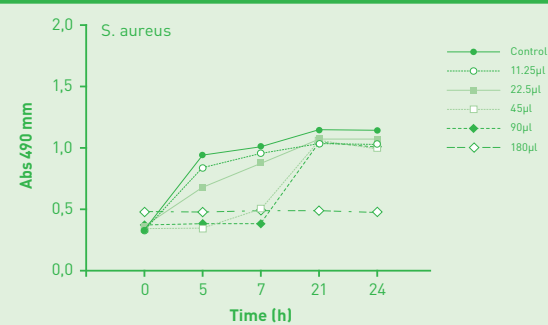


Figure 3: Measurement of zone of inhibition up to 48hrs of Mepilex Ag eluates against *P. aeruginosa*

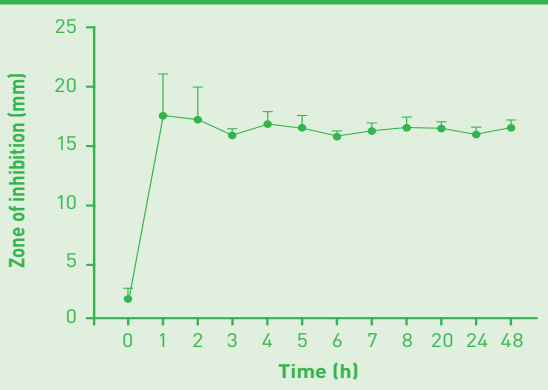
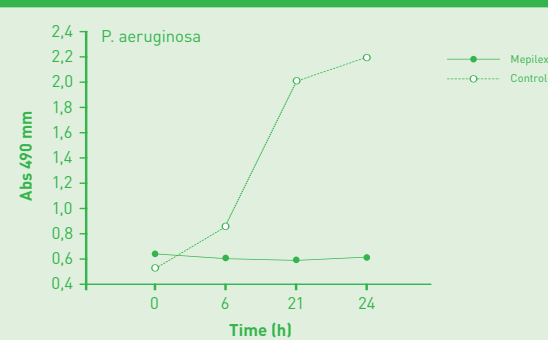


Figure 4: Growth of *P. aeruginosa* with Mepilex Ag eluate



blocked growth of *P. aeruginosa* (Figure 4) and *S. aureus* (Figure 5). Nineteen different *P. aeruginosa* isolates derived from

2. COMPARISON OF DIFFERENT DRESSINGS

METHODS

The dressings listed in Table 1 were incubated in bacterial medium overnight at 37°C to give different eluate concentrations (corresponding to a 20mm diameter dressing in 10ml TH or 10ml 50% human serum in TH). Aliquots of these eluates (90 or 180µl) were inoculated with wound-derived *P. aeruginosa*.

RESULTS

Table 2 shows the results of growth curves of the *P. aeruginosa* treated with aliquots obtained from the different dressing elutions, with or without human serum. The data indicates that TH medium negated the effects of some dressings (Column 2), whereas addition of human serum facilitated the antimicrobial effects (Column 3).

Table 1: Dressings evaluated

Mepilex Ag	Contreet
Silcercel Ag	Aquacel Ag
Tegaderm Ag	Acticoat 7
Cellosorb Ag	Acticoat Moisture Control

3. PROTEIN DEGRADATION

Evaluation of the blocking of plasma protein degradations and the blocking of elastase synthesis from *P. aeruginosa* was undertaken.

METHODS

Dressings fluids containing 50% human serum (180µl) obtained after an overnight incubation with the respective

Table 2: Results of growth assays of *P. aeruginosa* incubated with eluates of different dressings: with and without human serum

Dressing aliquot (µl)	Growth – TH only	Growth TH + Human Serum
Mepilex Ag 90	No growth up to 48 hrs	No growth up to 48 hrs
Mepilex Ag 180	No growth up to 48 hrs	No growth up to 48 hrs
Contreet 90	Growth after 18 hrs	No growth up to 48 hrs
Contreet 180	Growth after 24 hrs	No growth up to 48 hrs
Acticoat MC 90	Growth after 18 hrs	Growth after 24 hrs
Acticoat MC 180	Growth after 24 hrs	No growth up to 48 hrs
Cellosorb Ag 90	Growth after 10 hrs	Growth after 10 hrs
Cellosorb Ag 180	Growth after 10 hrs	Growth after 10 hrs
Silvercel Ag 90	Growth after 10 hrs	Growth after 10 hrs
Silvercel Ag 180	Growth after 10 hrs	Growth after 10 hrs
Acticoat 7 90	Growth after 10 hrs	No growth up to 48 hrs
Acticoat 7 180	No growth up to 48 hrs	No growth up to 48 hrs
Tegaderm Ag 90	Growth after 6 hrs	Growth after 10 hrs
Tegaderm Ag 180	Growth after 6 hrs	Growth after 10 hrs
	Mepilex Ag and Acticoat 7 were associated with the best results	Mepilex Ag, Acticoat 7, Contreet, Aquacel Ag, Acticoat Moisture Control associated with good results

Cellosorb Ag, Silvercel and Tegaderm Ag did not show good results.

dressing were inoculated with wound-derived *P. aeruginosa*. After an incubation period overnight, the supernatants were subsequently analysed on SDS-PAGE (10% gels) and zymography (10% SDS-PAGE with gelatin).

RESULTS

The results showed that Mepilex Ag, as well as several other dressings, were able to block both protein degradation as well as release of *P. aeruginosa* elastase.

1B. RELEASE KINETICS

A radial diffusion assay was undertaken in order to investigate the release kinetics from Mepilex Ag.

METHODS

Mepilex Ag dressing was incubated with 50% human serum (in TH medium, 20mm diameter in a total of 10ml solution) and 6µl aliquots (n=3) were analysed by radial diffusion assay using *P. aeruginosa* as the test organism.

RESULTS

A rapid, and then sustained, release was detected (Figure 3).

1C. GROWTH ASSAY

A growth assay was undertaken to evaluate the antimicrobial effects of Mepilex Ag eluates on *Pseudomonas aeruginosa* and *Staphylococcus aureus* in the presence of human serum (50%).

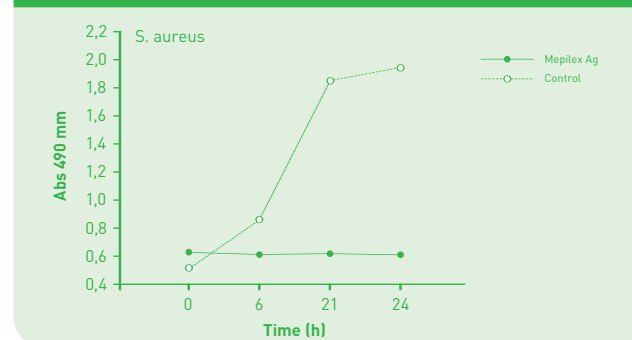
METHODS

Mepilex Ag dressings were incubated in bacterial medium overnight at 37°C to give different eluate concentrations (corresponding to a 20mm diameter dressing in 10ml 50% serum in TH).

RESULTS

In the growth assays, eluates from Mepilex Ag completely

Figure 5: Growth of *S. aureus* with Mepilex Ag eluate



chronic venous ulcers were completely inhibited by addition of eluates from Mepilex Ag (Figure 6).

Figure 6: Comparison of *P. aeruginosa* clinical isolates treated with Mepilex or Mepilex Ag

