Aim: The aim of this poster is to present the preliminary results of a literature review about the use of antiseptics in pressure ulcer.

Method: We made a literature review, searching in databases Cochrane, MedLine, Cinhal and PubMed. We looked for full-text articles, published after 2000, in Portuguese and English. We used the descriptors antiseptic*, wound OR pressure ulcer OR bed sore; wound bed preparation. The research was made in 4 different days.

Results / Discussion: We found 29 articles, but only 13 answered the investigation question. Four articles talk about the use of antiseptics to prevent biofilm; nine articles described that we should use antiseptics when local infection, critical colonization or sepsis are present; and six articles refer that the non careful use of antiseptics may cause bacterial resistance or tissue tolerance. Eleven articles refer that this is a dichotomous subject and more research is necessary.

Conclusion: The use of antiseptic in infection prophylaxy is a controversial subject. If some authors advocate their use eliminate the plantonic cells, others refer that the non careful use could make antiseptics ineffective through many strains and will gave them genetically resistance. Other authors refer two that antiseptics can destroy granulation tissue and delay cicatrization. There’s no RCT that compare the procedures of use antiseptics. Their achievement would facilitate the achievement of standards.

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A CLOSER LOOK AT SILVER: NEW FORMULATIONS WITH AG2+/3+ HAVE ENHANCED ANTIBIOFILM ACTIVITY IN VITRO AND IN VIVO

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E-poster session: Antimicrobials 1

Aim: Ionic silver (Ag+) is widely used for its antimicrobial effects in treatment of stalled wounds. New technology incorporates silver oxysalts into dressings at higher oxidative states (Ag2+ and Ag3+). This study aimed to quantify and differentiate the anti-biofilm efficacy of different Ag species found in wound dressings.

Method: AgCl(s), Ag2SO4(s), Ag(s), Ag2O(s), and Ag7NO11(s) were compared using a range of equivalent Ag concentrations. Anti-biofilm activity was measured by determining the minimum biocidal concentrations (MBEC), minimum biofilm inhibition concentration (MBIC) and minimum biofilm eradication concentrations (MBEC) of planktonic and biofilm populations. In vivo a porcine burn wound biofilm model was used to evaluate anti-biofilm activity. For all in vitro testing a panel of pathogenic organisms was tested including multi-drug resistant bacteria such as Carbapenem-resistant Enterobacteriaceae.

Results / Discussion: Relative to other Ag compounds, Ag7NO11(s) required a 3-50 fold lower silver concentration to inhibit or kill each organism tested. In vivo, dressing with Ag7NO11(s) had a log reduction 2 and 3 log greater than silver sulfadiazine and a dressing containing AgCl, EDTA and benzethonium chloride respectively after 48 hour exposure to established Pseudomonas aeruginosa biofilm.

Conclusion: The physiochemical properties including oxidation state of silver ions impact antimicrobial efficacy observed. Ag2+ and Ag3+ ions effectively eradicate organisms growing planktonically or in a mature biofilm state, and prevent biofilm re-formation at low concentrations, which reduces the risk of toxicity as well as the overall exposure to silver.
Aim: When selecting antimicrobial dressings, primary and secondary requirements must be considered. Primarily infection control and secondarily ability to manage exudation, remove necrotic tissue and malodour, conform to the wound bed, perform wound bed preparation functions and satisfy patients’ expectations. A clinical evaluation of a foam and a hydropolymer gel dressing, covered with a bacterial binding mesh, was performed.

Method: 10 Patients suffering from infected arterial and venous leg ulcers (classical and additionial signs and symptoms) were included and treated with the foam or hydropolymer gel dressing.

Results / Discussion: The bacteria binding mesh effectively controls wound infection. The hydropolymer gel dressing performed best with low/moderate exudate levels, a necrotic wound bed and focus on reliable fixation. The foam dressing performed best with moderate/high exudate levels, compression therapy and prevention of maceration. Both supported a moist wound environment, stayed in place up to four days, conformed to the wound bed, enabled atraumatic, painless dressing changes and were easy to apply.

Conclusion: It is possible to combine primary and secondary goals in one product (simultaneously reducing costs).
**Aim:** To assess clinical efficacy and safety of topical combination of bacitracin/ neomycin in comparison to topical chloramphenicol in the treatment of surgical SSTIs in adult out-patients.

**Method:** A total of 309 adult out-patients from 6 cities with mild to moderate surgical SSTIs (abscesses, furuncles, hydadenitisis, infected posttraumatic and surgical wounds, infected burns) were randomized to receive either topical powder and/or ointment of combination bacitracin/neomycin (n=156) or topical 0.75% chloramphenicol ointment (n=153) BID. Deep swabs, biopsy or puncture were performed in all patients before the start of therapy. Identification of pathogens and susceptibility testing were performed according to EUCAST guidelines, 2012.

**Results / Discussion:** The total cure rate was comparable in both group (97.4%, p=0.98), but cure period was significantly shorter for bacitracin/neomycin regimen (cure rate by day 8 – 82.7% vs. 68.6%, p=0.004; by day 15 – 97.4% vs. 94.8%, respectively, p=0.23). The total of 228 strains were isolated. The main causative agent was *S. aureus* (82.5%), less frequently *S. pyogenes* (5.3%) and other (<2% each). 22% of *S. aureus* strains were resistant to chloramphenicol, 7.7% to tetracycline, 7.2% to macrolides, 3.3% to ciprofloxacin, 2.2% to clindamycin and gentamicin, 1.7% to oxacillin, mupirocin and levofloxacin, 1.1% to fusidic acid and moxifloxacin, 0.6% to co-trimoxazole, while all isolated strains were susceptible to vancomycin and linezolid.

**Conclusion:** Topical combination bacitracin/neomycin has high clinical efficacy in the treatment of mild to moderate surgical SSTIs with significantly more rapid recovery in comparison to topical chloramphenicol and therefore can be recommended for the treatment of this group of infections in adult out-patients.
A REVIEW OF CURRENT IN VITRO BIOFILM MODELS

Aim: Various in vitro biofilm models are used to study biofilm behaviour in response to antimicrobial exposure; however, there is no single currently accepted model. The type of model used and assay conditions can substantially affect the results achieved\(^1\). A review has been conducted on currently published in vitro biofilm models.

Method: A review of published literature was performed and a summary of in vitro wound biofilm models was prepared. The review outlines the similarities and differences between models, and equates them to specific wound conditions seen clinically, with an overview of current in vitro biofilm data.

Results / Discussion: There are a wide and varied range of in vitro biofilm models currently in use, ranging from extremely simple (single species, <24h old) to very challenging (multiple species, high exudate conditions, mature biofilms, >72h old). In addition, experiments are conducted in various media and under a range of incubation conditions which can also lead to differing experimental outcomes. It may be useful to assess what type of wound the experimental conditions of a model are most similar to when evaluating in vitro biofilm data.

Conclusion: The variability in design of in vitro biofilm models may contribute to variability in results. Understanding the relationship between biofilm model design, environmental conditions and experimental outcome is crucial to interpreting the results of in vitro testing. Many biofilm models mimic specific clinical conditions, which may explain conflicting data. Using a variety of different models may therefore give a better understanding of biofilm behaviour in wounds.

1. Bourdillon, K., Westmoreland, M., Simmons, R., Regan, S. (2014) Media Composition Affects the Ability of Antimicrobial Dressings to Disrupt Pre-Formed Bacterial Biofilms in a Novel in-Vitro Biofilm Model. SAWC Fall, Las Vegas (Poster presentation)
Aim: Infection and high bacterial bioburden in wounds are major contributors to delayed healing and can have a detrimental impact on patient quality of life. Poly(hexamethylene) biguanide hydrochloride (PHMB) is a well-tolerated antiseptic with low toxicity and a broad spectrum of activity against both Gram-negative and Gram-positive bacteria. An overview of PHMB in wound care has been prepared, encompassing mechanism of action, formulation, spectrum of activity, safety and toxicity with comparison to other commonly used antimicrobials.

Method: A review of the current use of PHMB in wound care has been conducted and its properties compared to other commonly used antimicrobials. In addition, in vitro studies investigating the ability of PHMB containing dressings and gels to reduce bacterial populations in both planktonic culture (log₁₀ reduction assay) and when the bacteria is present as a biofilm (cellulose disc assay) have been conducted.

Results / Discussion: PHMB has previously been demonstrated as having broad spectrum activity against microorganisms while being well tolerated making it an attractive option to manage wound bioburden. In vitro testing confirmed that PHMB containing dressings/solutions have potent bactericidal activity against planktonic bacteria and bacterial biofilms grown on cellulose discs.

Conclusion: The evidence presented, which is consistent with previously reported data, demonstrates the potential usefulness of PHMB in the management of wounds where bioburden is an issue. The in vitro data demonstrating efficacy against biofilms is particularly interesting, as biofilms are often less susceptible to antimicrobial activity.

Aim: It is widely recognised that sloughy, necrotic tissue may impair wound healing. Debridement of such tissue is considered essential in preparing the wound bed, allowing granulation tissue to form. The use of hydrogels is an excellent way to gently debride devitalized tissue while simultaneously keeping the wound moist. The inclusion of Propylene glycol (PEG) as a preservative in some hydrogel formulations may give the products bacteriostatic properties. This in vitro study determines the bacteriostatic properties of six commercially available hydrogels (H1 –H6).

Method: Hydrogels were evaluated in triplicate against S. aureus (SA) and P. aeruginosa (PA) in separate experiments. Hydrogels were inoculated with $10^3$ CFU bacteria in the presence of foetal bovine serum. Gels were incubated at 32°C for 72 hours, and sampled at 0, 4, 24 and 72 hours with samples subjected to viable counting to enumerate bacteria.

Results / Discussion: H2 and H5 of the six hydrogels showed bacteriostatic properties against PA with H2 also active against SA. The remaining four hydrogels demonstrated no bacteriostatic activity, allowing bacteria to proliferate, despite some containing PEG. It is likely that the relative concentration of PEG in each product is responsible for the varying degree of bacteriostatic activity observed.

Conclusion: The data in this study showed that only one out of the six hydrogels tested exhibited bacteriostatic properties against both Gram-positive and Gram-negative test organisms. These results suggest that the bacteriostatic properties of hydrogel H2 may make it useful in the management of bioburden in addition to its known debridement properties.

H1: Granugel; H2: NU-Gel; H3: Duoderm; H4: Purilon; H5: Intrasite; H6: Suprasorb
Aim: Silver has a well-established ability to reduce bioburden. However some reports have raised concerns over the efficacy of silver-containing products. Recently, a range of alternative antimicrobial dressings have emerged which do not contain a recognized antimicrobial. This study compared the antimicrobial efficacy of two silver-containing dressings; a silver impregnated activated charcoal dressing (SIAC)* and a silver non-adherent alginate dressing (SNA)‡, an antimicrobial bacterial binding dressing (ABB)¥ and a honey calcium alginate dressing (HCA)† against clinically significant organisms S. aureus (SA) and P. aeruginosa (PA) in vitro.

Method: The antimicrobial efficacies of the dressings were evaluated in triplicate by log₁₀ reduction assay, which exposes a small sample of dressing to a bacterial culture. Samples of culture were removed at various time-points over 24 hours and total viable counts (TVC) determined.

Results / Discussion: Both SIAC and SNA were highly active against both bacterial strains tested, with a ≥4.5 log₁₀ reduction in TVC observed within 3 hours. In contrast, ABB had only a minor effect on TVC, with log₁₀ reductions of ≤2 log₁₀ units observed for both bacterial strains tested. HCA achieved only a 1-1.5 log₁₀ reduction of bacteria within 3 hours.

Conclusion: The two silver dressings showed high antimicrobial efficacy, reducing TVC by ≥4.5 log₁₀ units within 3 hours. Equivalent antimicrobial efficacy was not achieved by either of the alternative antimicrobial dressings. These results should be considered when determining the appropriate dressing to use on wounds at risk of high bioburden.

*ACTISORB 220 (Systagenix), ‡SILVERCEL Non-adherent (Systagenix), ¥ Cutimed Sorbact (BSN Medical), †Medihoney Calcium alginate (Derma Science)
Aim: This study aimed to develop a representative wound biofilm model to assess the effect of antimicrobial dressings in direct contact with preformed established biofilms. There is currently no standardised accepted model.

Method: A novel in-vitro wound biofilm model was developed, which established mature Pseudomonas aeruginosa biofilms on cellulose discs. Biofilms were challenged with test dressings (silver non-adherent alginate dressing (SNA)*, honey dressing (HCA)† or control dressing‡). After exposure, biofilm total viable counts (TVC) were determined. Scanning electron microscopy (SEM) was also performed. The assay was conducted in low challenge, medium challenge and high challenge media (containing increasing levels of serum) to investigate the effect of media composition on results.

Results / Discussion: A 24 hour application of SNA or HCA to 72 hour old P. aeruginosa biofilms in low challenge media led to >4 log_{10} unit reduction in TVC. While SNA achieved a similar reduction in medium challenge media, HCA demonstrated no reduction in biofilm TVC compared to the control. In high challenge media the efficacy of SNA was reduced, while HCA again had no effect on TVC.

Conclusion: SEM visualized stable, mature biofilms on discs. Both antimicrobial dressings showed disruptive activity in this in vitro biofilm model, however efficacy of dressings was affected by the media composition. Performing the assay in a complex nutritional environment rendered HCA inactive against 72h old Pseudomonas biofilms. SNA remained active against Pseudomonas biofilms when levels of serum were increased to mimic more clinically relevant conditions, although activity was reduced.

*SILVERCEL® Non-adherent (Systagenix)
† Medihoney calcium alginate dressing (Derma Science)
‡N-A Ultra (Systagenix)
Aim: The ability to test the antibiofilm activity of a dressing in-vitro, requires a suitable model that will enable the dressing sample to be applied to a biofilm and subsequently assessed for its antibiofilm properties.

Method: An in-vitro model was developed utilising gauze as a substrate for surface attached biofilm bacteria. Evidence of a mature, viable biofilm, is observed using a stain*. A separate gauze biofilm is transferred onto a suitable growth medium, which allows for a dressing to be applied as it would be in a clinical situation, then left in situ at 37°C. Enumeration of the biofilm present on the gauze is assessed, at certain time points, using a combination of homogenisation and total viable counts (TVCs) in order to determine the level of biofilm on the gauze prior to and following treatment.

Results / Discussion: The in-vitro biofilm model enables a mature biofilm to grow on the gauze substrate, which can be visualised and quantified. This enables for the reduction and/or eradication of the biofilm on the gauze to be determined following application of an antibiofilm dressing.

Conclusion: The biofilm model demonstrates that viable, reproducible biofilms can attach to the gauze substrate, which, once mature, can allow for dressing application to be administered in a manor closely replicating a clinical situation. The method to quantify and visualise the biofilm present on the gauze following treatment, enables for the antibiofilm properties of the dressing to be determined, therefore providing valuable data when evaluating potential antibiofilm products.

* LIVE/DEAD BacLight stain
Aim: To investigate the efficacy of new potential antifungal/antibiofilm agents on *Trichophyton rubrum* biofilms.

Method: Three 3 cm² samples of different acrylic adhesives containing different antifungal/antibiofilm agents, and various positive and negative controls, were placed into Petri dishes. Each acrylic sample was inoculated with 100µl of approximately $1 \times 10^4$ cfu/ml of *T. rubrum* in poloxamer (biofilm model). All biofilm models were incubated at 35°C (±3°C) for the following time periods: 24h, 48h, 72h, 96h and 7 to 14 days. Following incubation all acrylic samples were evaluated for kill rates using confocal laser scanning microscopy (CLSM) and standard microbiological plate count assays.

Results / Discussion: All antifungal/antibiofilm agents tested were found to elute out of the acrylic adhesives and demonstrate activity at the adhesive surface. For a number of agents antimicrobial activity was maintained over a 7 day test period. At day 14 one specific agent remained active and prevented the growth of *T. rubrum*.

Conclusion: Interestingly a number of potential agents demonstrated very good antibiofilm ability on *T. rubrum* when grown in a novel *in-vitro* biofilm model.
Aim: To investigate the antimicrobial and anti-biofilm ability of a novel silicone gel adhesive construct.

Method: Antimicrobial Efficacy Testing (Method 1) - 55mm diameter antimicrobial silicone constructs (in triplicate) were placed into sterile containers containing 9ml tryptic soy broth (TSB) and 1ml of challenge organism (Staphylococcus aureus, Pseudomonas aeruginosa or Candida albicans - 1 x 106 cfu/ml). Each model was incubated on a shaking platform set at 100rpm. After 4, 24, 48, 72 and 96 hours samples of the suspension from each container were plated onto agar.

Antimicrobial Activity Testing (Method 2) - 3cm² samples of silicone constructs (in triplicate) were added to Petri dishes with 144µl of challenge organism (S. aureus or P. aeruginosa or C. albicans - 6 x 105 cfu/ml). The silicone samples were covered with a film and incubated for 24hours before plating out.

Poloxamer (Biofilm Model) - 3 cm² samples of silicone constructs (in triplicate) were added to Petri dishes. Samples were inoculated with 100µl of the challenge organism in Poloxamer. At 24, 48, 72, 96 hours microbial viability was determined in all biofilm samples.

Results / Discussion: Overall, the antimicrobial silicone adhesives demonstrated superior efficacy when compared to the positive and negative control in all American Society for Testing and Materials (ASTM), International Organization for Standardization (ISO) and biofilm models employed.

Conclusion: These studies have demonstrated that a novel silicone gel adhesive construct has the ability to deliver an antimicrobial that has efficacy on planktonic and biofilm grown Gram positive, Gram negative and yeasts over a 96hr test period.
Aim: This study examined the influence of silicone, acrylic and polyurethane adhesives on the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilms.

Method: Three x 3cm² of silicone, acrylic and polyurethane adhesive samples were inoculated with 100μl (10⁸ cfu/ml of *S. aureus* and/or *P. aeruginosa*) of bacterial or poloxamer (biofilm inducer) based bacterial suspensions, and incubated at 35°C (±3°C). At various time periods, up to 72 hours, images (using confocal laser microscopy) of the microbes, attached to the adhesives and growing in the poloxamer hydrogels were taken. Total viable counts were also performed.

Results / Discussion: Similar numbers of viable cells were isolated from *S. aureus* and *P. aeruginosa* poloxamer hydrogel biofilms grown in the presence of silicone and acrylic adhesives. Visualisation of *S. aureus* and *P. aeruginosa* biofilms were demonstrated on all adhesive samples.

Conclusion: To date little work has been done to investigate the effects of adhesives on the formation of biofilms. In this study biofilms were clearly evident growing on acrylic adhesives. Further studies are on-going to fully understand the effects of adhesives on biofilm formation and their maintenance.
Aim: The aim of the study is to assess the application efficiency of new silver foam hydrofiber dressing which combine healing benefits of a hydrofiber with the comfort of foam and the bacteria-killing power of ionic silver in management of donor-site wounds.

Method: In the prospective clinical study carried out in the Burn Department, 17 patients were enrolled between April and July 2014, with a mean age of 27.12 years. Donor sites ranged from 200 to 350 cm². The wounds were inspected daily until complete reepithelization and were analyzed with respect to epithelization time, antibacterial effect, ease of dressing change, pain, patient comfort, scar quality, pharmacologic and cost-effective characteristics. Photographs were taken before, during, and after epithelization. After the reepithelization all donor sites were evaluated using the Vancouver Scar Scale. The overall cost of treatment was assessed using material cost, labor cost, frequency of dressing changes and epithelization time.

Results / Discussion: All donor wounds were epithelized after 8 to 11 days. The average pain score is 2.94. A dressing change was needed in only two patients due to exudate saturation. The patient comfort was measured through patient questionnaire classified into three categories. The obtained results were statistically validated. All the wounds were without bacterial growth in the beginning and remained sterile until healing. No side effects were observed. No hypertrophic scars were observed.

Conclusion: The ease of use, non-adhesion, high absorbency, powerful antibacterial effect, lower frequency and painless dressing change are the key advantages of the new silver hydrofiber foam dressing.
**Aim:** Bacteria resistant to common antibiotics are increasingly isolated from chronic wounds and care has to be taken to prevent their spread. Concerns have been raised whether antimicrobial dressings are indeed effective against e.g. MRSA and NMD-1-carrying strains. Here, we have rated the antibacterial activity of various dressings against *S. aureus*, MRSA, *E. coli*, NMD-1-carrying *E. coli*, *K. pneumoniae*, and NMD-1 carrying *K. pneumoniae* using the JIS L 1902 standard test.

**Method:** Determination of antimicrobial activity was performed according to JIS L 1902. For experiments, 400mg samples of the dressings* were incubated with each test for 24 hours at 37°C under aerobic conditions.

**Results / Discussion:** All dressings containing an antimicrobial substance like PHMB or silver exerted a distinct antimicrobial effect against all test strains (log reduction > 3). Furthermore, the alginate dressing was able to efficiently bind and inhibit bacteria progeny. Similarly, strong antibacterial activity was observed for the SAP-containing dressing against the gram-negative bacteria while it demonstrated a significant activity against *S. aureus* and MRSA. However, no effect on bacterial growth was found for the dressing containing just activated carbon in a viscose matrix.

**Conclusion:** It was shown that dressings with an inherent antibacterial activity such as alginate or SAP-dressings or those with antimicrobial substances like PHMB or silver are equally effective against sensitive strains of *S. aureus, E. coli and K. pneumonia* as well as their resistant kinsmen MRSA and NMD-1 strains. Hence, it seems to be safe to use these dressings in the treatment of infected chronic wounds.

*Vliwasorb®, Vliwaktiv®, Vliwaktiv® Ag, Suprasorb® A, Suprasorb® A + Ag, Suprasorb® X + PHMB; Lohmann & Rauscher
Aim: Inflammatory skin diseases are the most common issues in dermatology, ranging from mild dermatological disorders (redness or itching) up to chronic conditions. Preliminary studies have conferred to Chlorella, a green microalga rich in nutrients, potential benefits in the context of inflammation-related diseases. The aim of the study was to investigate the effect of oral supplementation of Chlorella* on prevention of skin inflammation in hairless mice.

Method: At D1, Skh-1 mice were randomly assigned to 4 groups (n=6/group) and received daily by gastric intubation either 125, 250, 500 mg/kg body weight of Chlorella or water (control), respectively, for 14 days. From D8 to D14, skin inflammation was induced by topical application of an inflammatory agent on the back. Macroscopic scoring of skin inflammation was daily performed from D8 to D15. At D15, mice were euthanized. Histological and microscopic inflammation scoring was performed. Statistical difference was defined at p<0.05 level for all comparisons.

Results / Discussion: From D11 to D15, mean macroscopic score of skin inflammation was significantly reduced in the 250 and 500mg/kg Chlorella groups compared to the control group (p<0.05). Although histological scores tended to be lower at the end of the observation in the Chlorella groups as compared to the control group, the results were not statistically significant.

Conclusion: Oral supplementation with Chlorella may have a preventive impact on skin inflammation induced in mice. Chlorella may be of potential medical use in the treatment of various skin disorders.

*Algility™ chlorella, ROQUETTE (Lestrem - France)
Aim: In a chronic wound the surrounding tissue environment changes with the presence of bacterial biofilms. The wound pH is increasingly alkaline compared to an acidic profile in healthy skin. The aim of this study was to determine the effects of pH on the growth and attachment of bacterial biofilms.

Method: $10^6$ CFU/mL of *S. aureus* and *P. aeruginosa* in pH-adjusted Tryptone Soya Broth (TSB; pH 6, 7.5 and 9) was inoculated into a 96 well pegged lid plate and incubated at 37°C on a shaking platform for 24, 48 and 72 hours. Pegs were rinsed to remove any planktonic bacteria and then placed in phosphate buffered saline (PBS) and sonicated for 10 minutes to remove the attached biofilm. The PBS was then serially diluted and plated onto Tryptone Soya Agar (TSA) plates and incubated at 37°C. After 24 hours the colonies were counted. After rinsing, the peg lid was also stained with Crystal Violet for 15 minutes. The lid was washed in PBS before being placed in 30% acetic acid for 1 minute. The resulting acetic acid solution was measured on a plate reader at an absorbance of 550nm.

Results / Discussion: It was shown that pH does have an effect on the growth and attachment of both *S. aureus* and *P. aeruginosa* biofilms.

Conclusion: A change in pH does affect biofilm growth and formation. In a chronic wound environment changing the pH could result in disruption of already formed biofilms and the prevention of further attachment and growth.
Aim: To investigate the effects of antimicrobial wound dressings on biofilm growth and destruction.

Method: Three 1cm² samples of different antimicrobial wound dressings were inoculated with 200µl of 1 x 10⁶ cfu/ml of *Staphylococcus aureus* and/or *Pseudomonas aeruginosa* and/or *Candida albicans*. Each dressing was then incubated at 35°C (±3°C) for 1hr to allow for biofilm growth. After 1hr, each dressing was placed within a 2ml syringe so that the dressing samples filled up to 0.5ml of the syringe. A needle and tubing was connected to the syringe and tryptic soy broth (TSB) only was slowly dripped (a continuous flow model) through each biofilm ‘infected’ wound dressing (16ml/hr). Over the seven day test period the numbers of microbes disseminating from each wound dressing was taken. In addition confocal laser scanning microscopy (CLSM) images were taken to visualise the biofilm within each wound dressing.

Results / Discussion: It was found that the antimicrobial wound dressings supported the growth of biofilm over the test period. However, the antimicrobial wound dressings did initially reduce biofilm development when compared to the non-antimicrobial dressing.

Conclusion: Biofilm growth and dissemination of microbes from wound dressings increased over time in all wound dressings impregnated with antimicrobials.
CASE SERIES TO EVALUATE THE USE OF AN IODINE BASED DRESSING* IN THE MANAGEMENT OF PATIENTS WITH CHRONIC VENOUS LEG ULCERS

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E-poster session: Antimicrobials 1

**Aim:** Iodine dressings* are indicated for use on many different wound types as a means of managing local or spreading infection or prophylactically in high risk patients. This case series concentrated on chronic leg ulcers.

**Method:** Nineteen patients with difficult-to-heal lower leg ulcers of between 4 months and 5 years duration were followed for 4 weeks. All patients included had either indolent wounds with a history of recurrent wound infections or localised infection as evidenced by an unhealthy wound bed. Iodine dressings were applied in conjunction with standard therapy, such as compression bandages. Patients were assessed weekly and dressings were changed at least twice weekly depending on exudate levels. Iodine use was monitored and discontinued when appropriate.

**Results / Discussion:** Three patients achieved complete re-epithelialisation of their venous leg ulcers after 4-6 weeks use of Iodine dressings. Ten patients’ wounds decreased in size after between 4 and 6 weeks of using Iodine, with increased percentage of granulation tissue to the wound bed. Four patients developed either a local or systemic wound infection after commencing iodine dressings with either no improvement or deterioration in size or condition of wound bed which warranted discontinuation of the dressing.

**Conclusion:** 68% of the patients included had a successful outcome when using Iodine dressings alongside standard therapy over a sustained period of time- While acknowledging that four patients continued to experience symptoms of wound infection during use, this is an encouraging result in a complex group of patients with particularly chronic episodes of ulceration prone to infection.

*INADINE® (Systagenix)
Aim: Silicone soft skin adhesives are widely used in advanced dressings for the treatment of chronic wounds. In order to prevent bacterial infection, the feasibility to incorporate antimicrobial agents into a silicone soft skin adhesive was evaluated.

Method: Chlorhexidine was incorporated at levels ranging from 1.5 to 6% into a silicone soft skin adhesive. The gels were cured and tested for peel release, adhesion, gel penetration and antimicrobial activity using ISO 22196 against controls.

Results / Discussion: Depending on the level, the addition of chlorhexidine to the silicone soft skin adhesive resulted in similar cure profile and peel release, slightly higher adhesion and slightly reduced gel softness, when compared to the control formulation. Antimicrobial activity was evidenced by a minimum of a four log reduction of S.aureus, C. albicans and S. aureus for all tested chlorhexidine loaded formulations.

Conclusion: Through addition of chlorhexidine to silicone gels, the advantages of gentle silicone adhesives can be combined with those of silver-free antimicrobial agents. This holds promise to prevent infection of chronic wounds.

Dow Corning® MG7-9850 Soft Skin Adhesive
**Aim:** Here we show how to enhance the antimicrobial efficacy of currently used silicone contact layers in wound dressings. Physical properties of silicone such as hydrophilicity and permeability can be improved via integration of nanstructured modified silicone (poly (N-vinyl pyrrolidinone--graft-dimethyl siloxane) (P (NVP-g-PDMS) 'micelles', which assists the delivery of antimicrobials by supporting a separate aqueous phase.

**Method:** PDMS functionlised macromonomers were prepared by reaction of allyl chloroformate with PDMS siloxanes. N-vinylpyrrolidone (NVP) and PDMS macromonomer were copolymerised. The product was precipitated and repeatedly washed with diethyl ether. Two silicone adhesives were modified by adding copolymers dissolved in a minimum volume of hexamethyldisiloxane before being combined with an antimicrobial agent. The adhesives were drawn down using 200µm K-bars onto release paper and set in an oven at 70°C.

**Results / Discussion:** The copolymer content increased the surface morphology which changed from being smooth to more rough with the particles protruding from the surface of the silicone. Confocal micrographs were taken to highlight evidence of encapsulation of antimicrobials within the silicone adhesive suggesting that this hydrophilic molecule, was held within an aqueous phase stabilised by the interfacial properties of the graft copolymers.

**Conclusion:** Poly NVP-g-PDMS can be used to stabilise an aqueous phase in PDMS adhesives to enable the release of antimicrobials into a wound.
Aim: Prevention and treatment of infectious causes related to the presence of gastrostomy for nutritional purposes.

Method: Gastrostomy has as main indication the placement of a probe for nutritional purpose. The presence of biofilm is documented, regardless of the material used with infectious consequences caused by the migration of bacteria and/or fungi from device to organs, even distant ones.

The patients followed carried gastrostomy devices. The replacement and monitoring of the probes was carried out by professionals of the Nutritional Team and a nurse with expertise in stoma therapy and wound care. Gastrostomy probes were analyzed with cultural tests; attention was paid to distillation water in the balloon/bumper, and to the permeability of the bumper itself, with possible passage of microorganisms from the inside to the outside or vice versa. A group of patients was treated with PHMB Wound Gel 0.1% applied on the peristomal skin, with the aim of reducing the skin microbial load through biofilm disruption, with reduction of its migration into the stoma.

Results / Discussion: The analysis of the preliminary data showed a reduction of the microbial load in the peristomal area, resulting in the absence or reduction in the peristomal and stomal areas, on the device surface.

Conclusion: As anti-biofilm gastrostomy devices are not yet available, the use of skin cleansers, having no contraindications and compatible with materials and medications used, can help reduce the risk of migration of bacterial colonies resistant to antibiotics and antiseptics, with possible increase of the device lifetime.
Aim: Evaluate the clinical performance of a new foam with Silver sulfadiazine.

Method: 10 pts was enrolled in a period of 6 months, inclusion criteria was superficial wounds without necrosis or cellulitis, not infected. Observational period for 4 weeks. Data collected was: Area, VAS, WBP score, infection according the Cutting and Harding criteria, Bates Jensen score and any side effects.

Results / Discussion: 10 patients on 10 concluded the observational period. 7 was females and 3 males, the medium age was 71,7 years (32-88); the wound’s age was 8.6 month (4-18). 3 patients achieve closure, in the remaining 7 the mean of area’s reduction was 31,5%, only 1 case had an enlargement. The VAS was performed on 9 patients (1 neuropathic) and the mean reduction was of 3 point. WBP score was improved in 6 cases, but no worsening was noted. B-J score shown a mean reduction of 18,5%. On perilesional skin 2 episode of minimal maceration was noted. No any side effect was revealed during the observational period.

Conclusion: A good clinical performed was noted. The observational period was short but all the parameters collected show an improvement.
[EP322] TEN TIPS TO REDUCE ANTIBIOTIC RESISTANCE

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E-poster session: Antimicrobials 1

**Aim:** To increase the awareness of and help reduce the misuse and over use of antibiotics leading to antibiotic resistance.

**Method:** Seven experts in wound management and infection reviewed the literature and have developed the ten tips to help reduce antibiotic resistance.

**Results / Discussion:** The paper will present and discuss the ten tips and how, in particular health professionals in wound care can both in their practices and by influencing others help improve the use of antibiotics and thus rescue the growing problem of antibiotic resistance.

**Conclusion:** Urgent action is necessary to reverse the trend to antibiotic resistance for without such actions and interventions we will within 10 to 15 years according to the World Health Organization have no antibiotics to treat infection.
**Aim:** To determine *in vitro* and *in vivo* percutaneous penetration of silver.

**Method:** In the *in vivo* study 15 healthy subjects and 15 patients with atopic dermatitis (AD) wore a sleeve containing 13% silver (Silver nylon technology) on their lower arms for 8 hours during 5 consecutive days. The percutaneous penetration has been determined from the silver concentrations in the stratum corneum (SC) layers collected by adhesive tape. Furthermore, silver was measured in urine samples collected before and after exposure.

*In vitro* percutaneous penetration through human cryopreserved full thickness skin has been investigated by using a Franz diffusion cell. Silver material (1.77 cm²) was placed in the donor chamber filled with 1.5 mL of synthetic sweat. The receptor solution was collected at, 2, 4, 8, 16 and 24 hours of exposure. The concentration of silver in the SC, urine and receiving phase was determined by ICP-MS.

**Results / Discussion:** Steady state flux in healthy subjects and AD patients was 0.2 ng Ag/cm²/h. There was no significant difference in dermal flux between AD patients and healthy subjects. Dermal exposure did not result in an increased levels of silver in urine.

In the *in vitro* study, the amount of silver that penetrated across the skin into receptor fluid was low and amounted 0.07 ng/cm²/h.

**Conclusion:** *In vitro* and *in vivo* study revealed low penetration of silver through the atopic and healthy skin. Dermal absorption of silver extrapolated to the “in use” scenario was lower than the current reference dose for silver uptake.
Aim: To evaluate the clinical efficacy of Propylbetaine–Polihexanide* solution associated to best clinical practice in subjects with pressure ulcers or vascular leg ulcers, with particular attention to the reduction of inflammatory signs.

Method: Adult patients recruited had either pressure ulcers stage II or III or vascular leg ulcers with inflammatory signs and/or fibrin on the wound bed. Ethics authorisations were obtained for all the participating centres. Follow-up period for each patient was 4 weeks.

Wound management included using local best practice protocols. Our intervention at each dressing change was the 10-minutes application over the wounds of a packing containing either Propylbetaine-Polihexanide solution (EG) or Normal saline solution (CG). Outcomes were measured using the Bates-Jensen-Wound-Assessment-Tool (BWAT), focusing on inflammatory signs (peripheral oedema and induration, peri-wound skin colour, exudate type and amount). Investigators assessing the wounds did not know what product was being used.

Results / Discussion: 289 patients were included, 143 in the EG and 146 in the CG. The comparative analysis of data collected showed a statistically significant difference in the BWAT score for inflammatory signs (p=0.0248), reduction of the wound surface (p=0.049), and formation of granulation tissue (p=0.049), all in favour of the treatment with Propylbetaine-Polihexanide (EG).

Conclusion: The study results are in favour of a significant higher efficacy of Propylbetaine-Polihexanide when associated to best clinical practice to reduce the inflammatory signs and the time to healing of vascular leg wounds (venous and mixed) and pressure ulcers.

*Prontosan® Wound Solution (B.Braun)
Aim: To investigate the correlation between silver content, silver availability, dressing technology and antibiofilm efficacy of commercially available antimicrobial silver dressings.

Method: A previously developed, reproduceable, in-vitro biofilm model was used. Prior to, and after the application of silver-containing dressings: the thickness, viability and specific elemental composition of the biofilm were measured by confocal laser scanning microscopy (CLSM), biofilm matrix specific staining, BacLight Live/Dead bacterial staining, image analysis and elemental analysis by inductively-coupled plasma mass spectrometry*. Silver availability and content were determined by elution into isotonic media and chemical digestion respectively, followed by assay by mass spectrometry*.

Results / Discussion: The calcium content of biofilm correlates with its thickness as measured using sophisticated microscope methods. The removal of biofilm (as measured by thickness) is not a function of silver content or concentration, but it is related to dressing technology. The efficacy of bacterial kill within the biofilm is a function of the silver concentration within the biofilm and is not a function of the dressings silver content. The addition of anti-biofilm agents to one specific dressing* results in significantly greater silver concentration within the biofilm which is associated with a faster and more extensive bacterial kill.

Conclusion: Silver alone is an inefficient anti-biofilm agent. Dressing technology plays a major role in biofilm removal. There is no correlation between silver content, silver availability and anti-biofilm activity. The addition of biofilm-disrupting agents significantly enhances the antimicrobial efficacy of silver against biofilm bacteria.

*ICP-MS

*ICP-MS

* AQUACEL® Ag+ EXTRA dressing
**Aim:** Three silver-containing wound dressings were evaluated *in vitro* for their ability to disrupt and kill a mixed biofilm comprised of clinical wound isolates, *Klebsiella pneumoniae* and *Staphylococcus aureus*.

**Method:** *S. aureus* and *K. pneumoniae* were inoculated and cultured on anodisc membrane filters for 24 hours to create mature, polymicrobial biofilm. These biofilms were separately treated with the NGAD and two traditional silver-containing antimicrobial dressings for 48 hours. Biofilm visualisation was undertaken using CLSM before and after treatment. Peptide nucleic acid fluorescence *in situ* hybridisation (PNA FISH) was used to differentiate the two organisms using fluorescent labels and enable visualisation of the mixed biofilm population. Additional fluorescent markers were also used, namely calcofluor white to establish the effect of the dressings on the outer biofilm layer (e.g. extracellular polymeric substance layer) and BacLight Live/Dead stain to ascertain the viability of the bacteria after treatment.

**Results / Discussion:** CLSM images observed showed an established polymicrobial biofilm containing both clinical isolates, and overall viability was confirmed with total viable counts (TVC). Using computer software* analysis the maximum thickness of the biofilm was determined before and after 48 hour treatment with the test dressings. This *in vitro* analysis showed that the NGAD** was superior to the control and other test antimicrobial dressings in reducing biofilm and killing associated bacteria.

**Conclusion:** NGAD was shown to be superior to traditional silver-containing antimicrobial dressings in disrupting polymicrobial biofilm and killing the associated bacteria *in vitro*.

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* Image-Pro® Premier 3D
** AQUACEL® Ag+ EXTRA dressing
**Aim:** Negative pressure wound therapy (NPWT) has established successfully as a treatment strategy to support wound healing in a number of clinical indications. However, systematic research investigating the bacterial kinetics on wounds is lacking and just a few studies are available comparing the microbiological difference of antiseptic dressings or rinsing solutions in combination with NPWT. The aim of this study was to investigate the antimicrobial effect of different antiseptic options (dressing or rinsing solution) in combination with negative pressure wound therapy on standardized experimental porcine wounds colonized with Gr+ or Gr- bacteria (*Staphylococcus aureus* or *Escherichia coli*).

**Method:** Wounds were treated with a NPWT system capable to instill liquid solutions*, and negative pressure was applied in combination with antiseptic dressings (silver, polyhexanide) or intermitted instillation of a wound irrigation solution (octenidine). The device was operated at 125 mm Hg sub-atmospheric pressure. In the silver and polyhexanide group continuous negative pressure was applied, whereas in the octenidine group automated intermittent instillation for about 3 minutes every 240 minutes were performed. Bacterial load per gram tissue were measured before intervention and after 24 or 48 hours, respectively.

**Results / Discussion:** In this artificial wound model, no treatment option was able to reduce bacterial burden, but a significant reduced bacterial growth compared to traditional NPWT was observed after 48 hours, when silver dressings or instillation of a wound irrigation solution** were used.

**Conclusion:** This study demonstrated that management of wounds with NPWT plus instillation of octenidine or silver dressings significantly delay the growth of *S. aureus* in artificial wounds compared to other antiseptic dressings after 48 hours.
* V.A.C. Ultra™
** Octenilin®
Aim: Examination of biofilm formation and the evaluation of a next-generation antimicrobial dressing* against standard topical antimicrobials.

Method: *Pseudomonas aeruginosa* biofilm was grown on inanimate surfaces that allowed examination by a combination of Light Microscopy, (LM), Transmission Electron Microscopy (TEM), and Scanning Electron Microscopy (SEM). A second in vitro model used sterile porcine belly tissue to produce a mixed biofilm of *P. aeruginosa* and *Staphylococcus aureus*. A pre-mixed culture was added to the belly tissue, which was placed in a 6-well plate insert, with 1.5 ml of Tryptone Soy Broth added to each well on the outside of the insert. Dressings were then applied and left in place for a series of predetermined intervals up to 72 hours. A gauze dressing was used as the non-antimicrobial control. Dressing samples were removed at each time point and processed for microscopic observations.

Results / Discussion: Biofilm formation was shown to be independent of the initial inoculum concentration, and was well established within 5-6 hours of incubation. In the meat model biofilm was observed under all dressings except NGAD within a 24 hour period. Histological evaluations were done at 24 and 72 hours.

Conclusion: SEM analysis demonstrated differences in the external appearance of bacterial cells forming either biofilm or remaining planktonic. In this in vitro model, NGAD was the only dressing tested observed to prevent biofilm formation over a 24 hour period. Standard antimicrobial dressings containing silver, iodine or PHMB were observed not to have controlled biofilm formation compared to NGAD.

*AQUACEL® Ag+ EXTRA dressing*
Aim: Growing evidence of increasing antibiotic resistance and continued over-prescribing represents a major threat to public health, and is especially pertinent to wound care as infection is the most frequent complication of non-healing wounds and often requires antibiotic treatment. Alternative products and methods are required to prevent and manage wound infection.

Wound cleansing can be easily incorporated into wound bed preparation (WBP) to manage bioburden and potentially decrease antibiotic use. Hypochlorous acid (HOCl) is produced naturally as part of our immune response and is widely used in industry for cleansing and disinfection due to its broad-spectrum antimicrobial properties.

A commercially available wound irritation solution* containing purified water and 250ppm (parts per million) HOCl is a simple but effective option for wound cleansing to remove debris, microorganisms and devitalised tissue. 

Method: In-vitro USP-51 testing of the solution at 250ppm of HOCl and 80ppm was performed at an external accredited microbiology laboratory to determine antimicrobial efficacy against common wound pathogens.

Results / Discussion: Fifteen of 17 organisms tested showed a ≥99.99% reduction in microbial count after 15 seconds exposure time to the 250ppm solution, and fourteen pathogens with the dilute 80ppm solution.

HOCl irrigation solution is an effective and rapid-acting microbiocidal agent in-vitro, even at dilute concentrations, providing clinicians with an efficient method of combating wound bioburden without facilitating resistance.

Conclusion: The HOCl Solution is non-irritating, non-sensitising and non-cytotoxic and does not negatively impact on wound healing, providing a viable method of integrating cleansing into WBP for wound management without causing microbial resistance.

*Woundox Irrigation Solution, Martindale Pharma, UK