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INVESTIGATIVE REPORT

Bacteria Aerosol Spread and Wound Bacteria Reduction with Different Methods for Wound Debridement in an Animal Model

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Debridement is essential in wound treatment to remove necrotic tissue and wound bacteria but may lead to bacteria spread by aerosolization. This study investigated the wound bacterial reduction and bacterial transmission induced by debridement using curette, plasma-mediated bipolar radiofrequency ablation (Coblation®) or hydrodebridement (Versajet®). Full thickness dermal wounds in porcine joint specimens inoculated with *S. aureus* were debrided with curette, Coblation, Versajet, or were left untreated. During and after debridement, aerosolized bacteria were measured and to assess wound bacterial load, quantitative swab samples were taken from each wound. Only Coblation was able to reduce the bacterial load of the wound significantly. Versajet debridement resulted in a significant bacterial aerosolization, but this was not the case with Coblation and curette debridement. This study shows that Coblation is a promising wound debridement method, which effectively reduces the wound bed bacterial load without the risk of bacterial aerosolization. *Key words: ablation techniques; bacterial spread; bactericidal; coblation; electrosurgery; hydrosurgery*.

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Chronic wounds are defined as wounds that have failed to proceed through an orderly and timely reparative process to produce anatomic and functional integrity over a period of 3 months (1). With an increasing life expectancy and increasing prevalence of diabetic disease and venous insufficiency the frequency of chronic wounds can be expected to increase (2, 3). It is estimated that approximately 1% of the general population has active or healed venous leg ulcers, contributing to substantial costs for society (4, 5).

Wound healing is an intricate process, demanding good nutritional status as well as recruitment of immune cells. Presence of bacteria can, depending on strain and amount of bacteria, delay wound healing (6). One of the mechanisms suggested is that bacteria form biofilms. The biofilm can lead to an increase in the bacterial resistance to local and systemic antibiotic treatment as well as to the innate antibacterial immune response (7). Chronic bacterial colonization of the wound can also lead to recurrent infections, which delay wound healing and often necessitate repeated antibiotic treatments. Furthermore, this increases the risk of antibiotic resistance, which poses a problem in wound infections as well as in other bacterial infections (8).

Surgical debridement is important in the care of acute and chronic wounds and assists in removing barriers that impair wound healing (6, 9). The aim of debridement is to promote wound healing by removing devitalized tissue and reducing the bacterial load, which impair the wound healing process (10).

A cold steel curette is the most common method used for wound debridement, as it only requires a curette and water for wound cleansing and removal of visible necrotic wound material.

Plasma-mediated bipolar radiofrequency ablation (Coblation®) is a method for volumetric soft tissue removal established in several surgical fields, such as arthroscopy, spinal surgery, tumour resection, and ear, nose and throat surgery (11–14). The technique is based on inducing a bipolar radiofrequency current between two electrodes in a conducting medium, such as saline, to initiate dissociation of water molecules and the formation of a gaseous plasma at the probe tip. The plasma is in an excited energy state, and therefore it has the ability to dissolve adjacent tissue in a controlled manner with limited thermal effect. It is therefore very different from the effect produced by conventional electrosurgical devices that use radiofrequency to generate heat and disintegrate tissue at high temperatures without plasma formation (15, 16). A previous study has shown that this technique has a microbicidal effect on microbes involved in wound infection, which seems to be a direct effect of the plasma field (17). The Coblation probe used for wound debridement flushes saline over the electrodes and has a suction line for evacuation of saline and debrided tissue material.

The Versajet® equipment is based on jet lavage, – a hydrosurgery type of technology: a water jet is focused with high intensity and speed to transfer mechanical energy to the tissue resulting in debridement (10).

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In recent decades there has been an increased focus on nosocomial infections as well as hospital hygiene (18). There is also a rising concern about perioperative spread of bacteria aerosols (19, 20). A recent study has shown that hydrosurgical debridement of wounds induces a significant risk of bacterial aerosol spread (19) and a report by Maragakis et al. (20) describes the potential clinical consequences of using wound debridement equipment with inadequate protection against the potential for bacteria transmission and environmental contamination. The report by Maragakis et al. describes a hospital outbreak with a multidrug-resistant strain with *Acetinobacter baumannii* caused by cross infection between patients treated with pulsed lavage wound debridement. Daeschlein et al. (21) as well as Angobaldo et al. (22) have, in two separate clinical setting trials, showed that unprotected hydrodynamic pulsatile debridement produces emissions of bacteria, including multiresistant bacteria strains, throughout the whole operating time, and have concluded that this poses a high risk of infection and contamination for patients and medical staff.

Reducing bacterial aerosol spread is therefore of interest both with regards to decreasing the dispersion of resistant strains to the immediate surroundings, as well as reducing the time to prepare the operation theatre for the next surgery session, and minimizing the risk of cross-infection between patients.

A previous pilot study by our research group has indicated that there may be differences between the methods available for wound debridement with regard to both the bacterial removal effect and the bacterial aerosol spread (23).

The aims of the present study were: 1) to determine the reduction of wound bacterial load, 2) to compare the amount of bacterial aerosolization induced by debridement, using either cold steel curette, Coblation or Versajet hydrodebridement in an *ex vivo* porcine wound model inoculated with *S. aureus*, and 3) to confirm presence of a bacterial biofilm in the porcine wound model used in the study.

METHODS

Thirty-two fresh porcine joint specimens were used for the study and divided into different treatment groups with 6 specimens in each group, with the exception of the control wound group, which contained two specimens. Six different treatment regimens were used; I) Untreated control wound (positive control), II) Cold steel curette, III) Coblation® (WoundWand®, ArthroCare corp., Austin, USA) at default setting (setting 7), IV) Coblation at maximum setting (setting 10), V) Versajet at default setting (setting 1), and VI) Versajet Versajet® (Versajet® Exact 14mm 45° hand piece, Smith & Nephew plc, London, UK) at maximum setting (setting 10). Active and passive aerosol sampling was also performed with no biological sample present (negative control). The default setting of the Coblation and Versajet devices is the recommended start setting set by the manufacturers. The maximum settings of the devices have a higher effect with higher bipolar voltage output of the Coblation device and higher saline jet flow for the Versajet. The rationale for the maximum setting is to achieve a more aggressive tissue removal effect.

Sample preparation, debridement and aerosol bacteria sampling were performed as described by Sönnergren et al. (23). During and after each debridement the bacterial aerosol was measured by active and passive sampling. Active sampling was performed with the bacterial air sampler Sartorius MD8 Airscan (Sartorius Stedim Biotech GmbH, Goettingen, Germany) with the air inlet manifold positioned 0.2 m from the specimen and a set air throughput of 6.0 m^3/h. One minute samples of 100 l of air were obtained at 0, 5, 15, 30, and 60 min post debridement initiation. Passive sampling was performed by placing four 90-mm diameter non-selective blood agar plates (Clinical Microbiology, Sahlgrenska University Hospital, Sweden) in the corners of the lab box. The plates were placed out directly prior to each debridement and collected 60 min post the start of debridement. To assess wound bacterial load, two quantitative bacterial swabs were taken from each wound at baseline (pre inoculation), post incubation, and post debridement. Swabs were obtained using the Levine's technique for quantitative culture (24) and processed as described by Sönnergren et al. (23).

Histology

One 8-mm punch biopsy for histology was taken from two wounds in each group at each time point (baseline, post incubation and post debridement) and fixed in neutral buffered 4% formaldehyde. The biopsies were processed using a Gram's stain protocol and examined as described by Sönnergren et al. (23).

Biofilm evaluation

A separate study was performed with scanning electron microscopy (SEM) for evaluation of biofilm formation, where 4 porcine leg wounds were prepared, inoculated and incubated as described above. Eight-mm punch biopsies for histology were taken from the wounds at baseline and post incubation, and fixed in a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.05 M sodium cacodylate buffer, pH 7.2, for 24 h. Samples were then subjected to a triple treatment with osmium tetroxide according to the OTOTO protocol (25), followed by dehydration in ethanol, ending in hexamethyldisilazane (HMDS). The HMDS was allowed to evaporate in a fume hood. The dried tissue blocks were mounted on aluminium stubs and sputter coated with palladium before examination in a Zeiss 982 Gemini scanning electron microscope. Digital images were recorded at a pixel resolution of 1,024 × 1,024. The samples were evaluated for presence of bacteria and bacterial biofilm formation.

Statistical analysis

For bacterial swabs, comparisons were made in the changes in wound bacterial load post incubation and post debridement for the untreated control wound and each debridement group. For active and passive aerosol samples, comparisons were made between the control wound and negative controls and each debridement group. For active aerosol samples, two sample *t*-tests were used for comparisons of mean amount of bacteria over all 5 measurements between treatment groups. For swabs and passive aerosol samples, linear mixed effects models were used for comparisons of treatment groups.

The microbiological results were statistically processed with suitable logarithmic transformations for the respective measurement types. The R version 2.14.2 statistical package was used for statistical analysis. The significance level was $p \leq 0.05$ and all tests were two-tailed. The histological and biofilm results were not statistically analysed but only qualitatively evaluated.

RESULTS

Wound bed bacteria results

At baseline, before *S. aureus* inoculation, the wounds had very low bacterial counts with 0.9 ± 1.1 log cfu/ml, compared to 9.8 ± 1.2 log cfu/ml post incubation. For the wound bacterial load after debridement (Fig. 1), Coblation default and max settings both significantly ($p < 0.0001$) reduced the bacterial counts compared to control wounds as measured by swabs. Versajet also gave a minor but significant ($p = 0.04$) reduction in wound bacterial counts at the max setting. Curette and the Versajet default setting did not significantly reduce the wound bacterial counts.

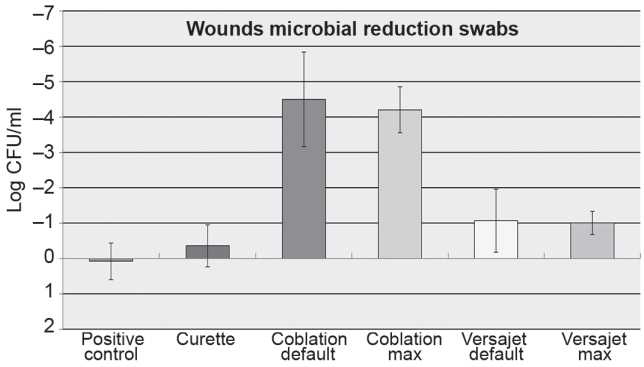

Fig. 1. Difference in wound bed bacterial counts post incubation and post debridement as measured by swabs.

Bacterial aerosol results

The active air sampling (Fig. 2) showed significantly higher bacterial counts for Versajet default ($p < 0.0001$) and max ($p = 0.0003$) settings compared to both negative and untreated controls. The bacterial counts for Versajet were initially markedly higher than for other groups, and subsequently decreased at 15, 30 and 60 min. However, Versajet default had the highest counts at each time point throughout the measurement period. For measurements at 0 min, the majority of both Versajet default and Versajet max bacteria plates were too numerous to count in terms of cfu, and the cfu number was thus assessed to be at least 1,000/plate. Compared to the controls, Versajet increased the air bacterial amount with up to at least 20,000%. Also, the passive sampling of bacterial air fallout (Fig. 3) was significantly higher for Versajet default ($p = 0.0002$) and max ($p = 0.002$) settings compared to negative and untreated positive controls.

Curette debridement, Coblation default and Coblation max groups did not show any significant difference in active or passive sampling compared with the controls.

Histology results

At baseline, before *S. aureus* inoculation, bacteria could not be detected in any of the samples. Post incubation, bacteria were present in all samples in focal clusters and in 58% of samples also in diffuse layers, with deep tissue involvement in 25% of the samples. Post debridement, all Coblation default samples had focal bacteria clusters but 50% showed no diffuse bacteria layers. Fifty percent of Coblation max samples had no detectable bacteria (Fig. 4b), and 50% showed only focal bacteria clusters. No Coblation-treated samples showed any deep tissue bacteria. In the Curette, Versajet default and Versajet max groups bacteria were still present in all samples in the form of both diffuse layers and focal clusters (Fig. 4a and c). In the Control group, all samples had focal bacteria clusters but 50% of samples had no diffuse bacteria layers. Measurements of bacterial penetration depth showed either increased bacterial penetration depth or no clear difference between post incubation and post debridement for the Control, Curette, Versajet default and Versajet max groups.

Biofilm results

The SEM analysis of the wound surface at baseline before bacteria inoculation showed a dense meshwork of collagen fibre bundles partly covered by flattened cell profiles in the low power micrograph (Fig. 5a), the collagen arrangement is enlarged in Fig. 5b. No biofilm forming bacteria were identified in the baseline samples. The SEM analysis of *S. aureus* inoculated specimens showed a dense growth of both staphy-

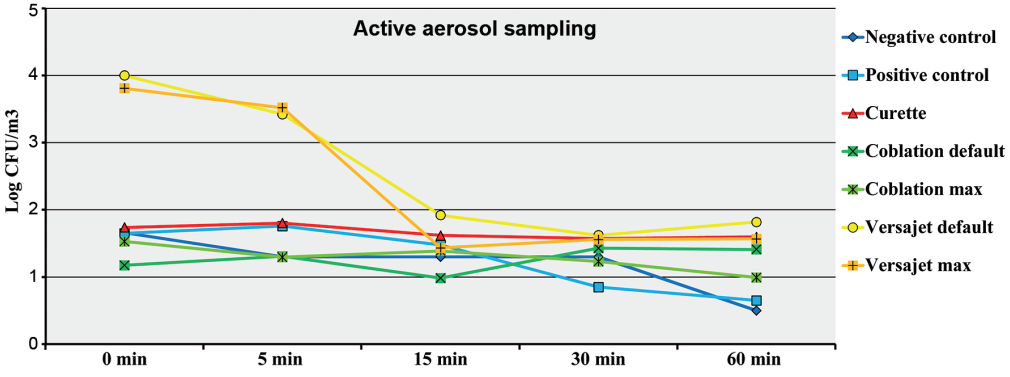

Fig. 2. Bacterial amounts in the air during and after debridement as measured with the Sartorius MD8 Airscan.

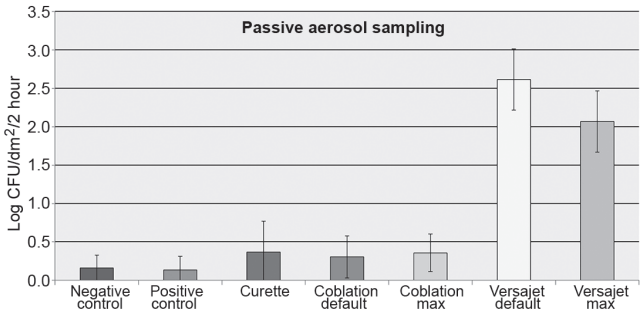

Fig. 3. Bacterial fallout on settle plates for the duration of 60 min during and after debridement.

lococci-like bacteria and also of coliformbacteria, morphologically most likely to be *Escherichia coli*, on the specimen surface. Thus, a mixed population of bacteria was found, forming large aggregates with regions containing extracellular networks of filaments, compatible with biofilm development (26) (Fig. 5c).

DISCUSSION

This study found that wound debridement with Coblation reduced the wound bacterial load by more than 4 logs in a porcine *ex vivo* wound model, while curette and Versajet debridement resulted in minor or no wound bacteria reduction. Versajet gave rise to a significant bacterial spread from the wound within the operating environment, while Coblation and curette debridement did not. These results are in agreement with the results of Bowling et al. (19) which evaluated the effect of Versajet on bacterial spread in an operating room setup. A previous study showed that Coblation *in vitro* has a bactericidal effect of 4–5 log reduction on planktonic solutions of *S. aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *E. coli* at 0.5–2.0 s exposure (17). The current study confirms the bactericidal effect of Coblation and a similar 4–5 log reduction of *S. aureus* in an *ex vivo* porcine wound biofilm model, and a likely reason for this effect is that Coblation has a direct bactericidal effect, rather than merely removing the bacteria from the wound bed as would be the aim of Versajet and curette debridement.

The SEM results confirm the formation of a biofilm after inoculation with *S. aureus* and incubation for 24 h. The SEM also showed the presence of coli-form bacteria. This is not unexpected since the wound surfaces are not entirely sterile at baseline, and additional bacterial growth of these contaminating strains may have taken place during incubation. This is also compatible with clinical wound colonization since clinical studies have shown that chronic wounds are in most cases colonized with multiple bacterial strains, including *S. aureus* and coli-form bacteria (27–30). Non-selective blood agar plates were used throughout the study, therefore the quantitative evaluation of wound bacterial colonization and aerosol spread included both *S. aureus* and aerobic coli-form bacteria.

This study has some limitations. Due to local laboratory regulations, a methicillin resistant *S. aureus* (MRSA) strain, or other multi-resistant strain, could not be used in the experiment. However, there is no reason why the results should differ between methicillin-sensitive and methicillin-resistant strains for the surgical debridement methods tested.

It is important to take into consideration that the present study is an *ex vivo* study and that wound healing as well as wound closure time could not be investigated *in vivo*. Future studies are needed to address this further. The clinical experience in the use of Coblation for wound debridement is limited. A recent study by Trial et al. (31) presented a number of clinical cases where the Coblation technique was used for wound debridement of venous leg ulcers, pressure ulcers and burn wounds. Out of the 25 cases presented, one Coblation debrided and skin grafted venous leg ulcer had heavy postoperative inflammation with a 30% postoperative skin loss, but otherwise no complications were reported. However no data on wound healing and wound closure was published in this report.

The current results emphasize that extra protective means should be used when utilizing Versajet debridement for infected and bacteria-colonized wounds,

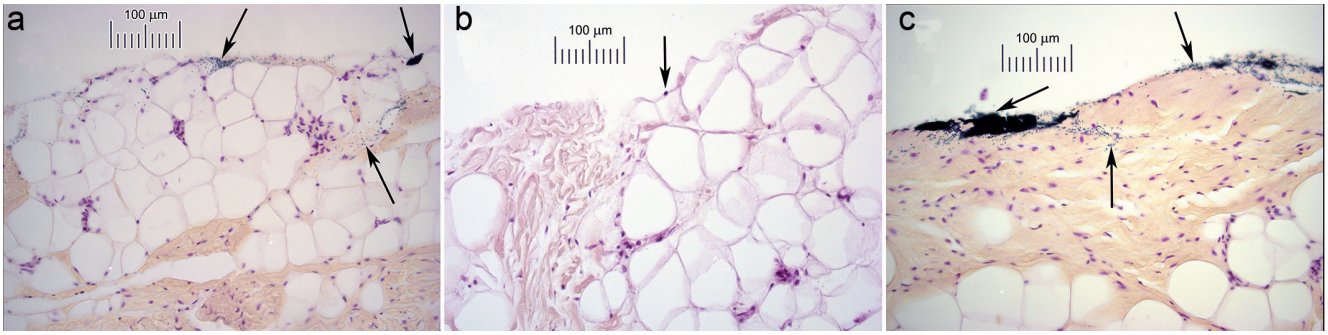

Fig. 4. Representative photos of histological slides, all taken using a 20× objective lens. a) Curette debrided wound with bacteria present in diffuse layers (left arrow), focal clusters (*right arrow*) and in deep tissue (*bottom arrow*). b) Coblation max debrided wound with no visible bacteria (only fibroblast cells are visible (*arrow*)). c) Versajet (max setting) debrided wound with bacteria present in focal clusters (*left arrow*), diffuse layers (*right arrow*) and in deep tissue (*bottom arrow*).

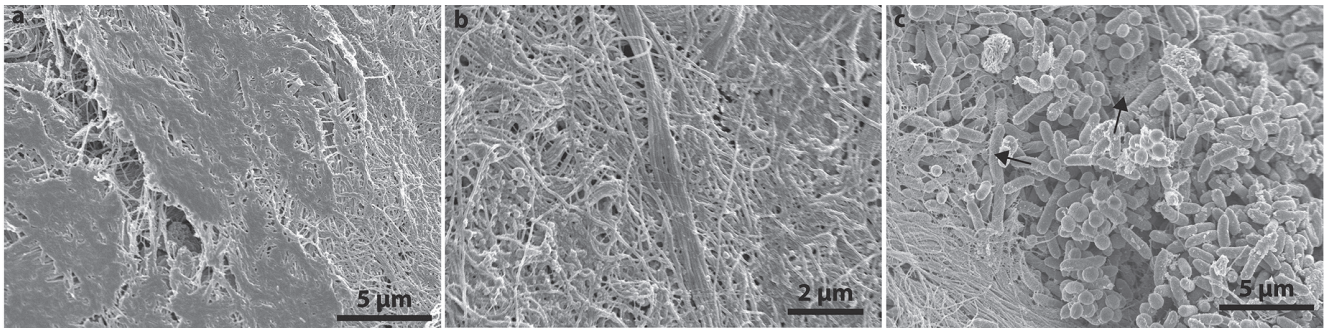

Fig. 5. Mount of scanning electron micrographs before (a, b) and after (c) bacteria inoculation. In (a) flattened, slightly elongated cells with an irregular contour cover partly a dense collagenous meshwork that is depicted at a higher magnification of a nude area in (b). A large colony of bacteria cover the wound surface in (c). Both spherical and elongated bacterial cells are identified indicating a mixed growth of added *Staphylococci* and a contaminating growth of coli-like species. Arrows indicate regions of biofilm character with fine meshworks of filaments connecting bacteria (visible only after enlargement of digital image file).

especially for wounds potentially contaminated with MRSA or other multi-resistant strains. The same level of precaution does not seem to be needed when using curette or Coblation for debridement.

In conclusion, this study shows that Coblation is a promising wound debridement method, which effectively reduces the wound bed bacterial load without the risk of bacterial aerosol spread. The Coblation method should be further evaluated in well-performed prospective clinical trials with evaluations of the clinical antibacterial effect and follow-up on wound healing progression after treatment.

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Conflict of interest: The first author previously had a consultancy agreement with ArthroCare corp.

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