INVESTIGATIVE REPORT

Antimicrobial Effects of Plasma-mediated Bipolar Radiofrequency Ablation on Bacteria and Fungi Relevant for Wound Infection

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Infection constitutes an important part of wound pathology and impedes wound healing. Plasma-mediated bipolar radiofrequency ablation (Coblation®) is a tissue-removal technique suggested for use in wound treatment. The aims of this study were to determine the antimicrobial effect of ablation exposure on bacteria and fungi relevant to wound infection, and how exposure time, temperature and aerobic/anaerobic growth influence the effect. Suspensions of 10^6 colony-forming units/ml of *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans* were exposed to ablation or thermal control for 500, 1000 or 2000 ms, or left untreated, and incubated aerobically. *E. coli* was also incubated anaerobically. Ablation was significantly ($p < 0.0001$) microbicidal on all strains compared with untreated and thermal control. The reductions compared with untreated control were 99.87–99.99% for all strains. In conclusion, plasma-mediated bipolar radiofrequency ablation has a general microbicidal effect *in vitro* on microbes relevant to wound infection independent of aerobic/anaerobic growth and thermal effect. *Key words: ablation techniques; bactericidal; coblation; debridement; fungicidal; ulcer.*

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Localized infection is a common consequence in chronic as well as acute surgical wounds, leading to complications such as systemic infection and amputation, and resulting in significant socioeconomic burden and reduced quality of life (1–3). Bacteria and fungi colonization and infection is well-recognized to constitute an important part of wound pathology and to impede the wound healing process (4–6). To decrease the microbial wound load is therefore of vital importance, and removal of non-viable tissue and microorganisms is central to the treatment regime for both surgical, venous, pressure, diabetic and arterial insufficiency wounds (7–11).

Plasma-mediated bipolar radiofrequency ablation (PbRf ablation), commonly referred to as Coblation®, is a method for volumetric soft tissue removal, which is established in several surgical fields, such as arthroscopy, spinal surgery, tumour resection, and ear, nose and throat surgery (12–15). The technique is based on inducing a bipolar radiofrequency current between two electrodes in a conductive medium, such as saline, and thus creating a physical plasma field that is able to break molecular bonds and dissolve tissue at relatively low temperatures (16, 17). Physical plasma is regarded as a distinct state of matter and is not to be confused with the physiologically well-known blood plasma. PbRf ablation has been clinically associated with safe and effective tissue removal and has been suggested for use in wound treatment (12–15). The technique has also been used by Lee et al. (18) for wound debridement in a case series of 25 chronic wound patients with good results and complete closure of all wounds within 6–8 weeks. The series included both diabetic, post-traumatic and Charcot foot wounds. However, no previous study has investigated the bactericidal or fungicidal potential of PbRf ablation.

Thus, the aims of the present study were: (*i*) to determine the direct antimicrobial effect of PbRf ablation exposure on bacteria and fungi strains relevant to wound infection; and (*ii*) to determine how the parameters of exposure time, temperature increase, and aerobic/anaerobic growth influence the antimicrobial effect.

MATERIALS AND METHODS

Microbial experimental set-up

All microorganisms were obtained from Culture Collection, University of Gothenburg, Sweden (CCUG). The microorganisms used were *Staphylococcus aureus* (CCUG 17621), *Streptococcus pyogenes* (CCUG 4207T), *Pseudomonas aeruginosa* (CCUG 17619), Escherichia coli (CCUG 24T) and *Candida albicans* (CCUG 5594). *S. aureus*, *S. pyogenes*, *P. aeruginosa* and *E. coli* were maintained on horse blood agar plates at 37°C and *C. albicans* was maintained on Sabouraud's agar (clinical bacteriology at Sahlgrenska University Hospital, Sweden) at 32°C.

Bacteria and fungi from 24 h old cultures were dissolved in 0.9% saline (pH 7.4) and adjusted to approximately 10^6 cells/ml, as determined by optical density (OD) 2.0 at 550 nm with a DEN-1 McFarland densitometer (Biosan, Riga, Latvia). The suspension was transferred to 96-well microtitre plates (Nunc A/S, Roskilde, Denmark) with 100 μl/well, with every second well and row left empty to avoid thermal effects between samp-

les. The wells were divided into PbRf ablation, thermal control (TC) and untreated (normal) control groups. The two exposure groups were further subdivided into 500, 1,000 and 2,000 ms exposure time with six samples in each group. The experimental set-up was repeated twice for each strain.

Ablation and thermal control equipment

For the exposure, Microblator 30 ICW probes (ArthroCare, Austin, USA) connected to a specifically programmed Quantum generator (ArthroCare) was used. The system can be used in ablation mode where a physical plasma field is generated around the tip of the probe through bi-polar radiofrequency conduction between the probe electrodes applied in a conductive medium, such as saline, or in coagulation mode where the medium is only thermally heated. Both modes use the same electrical waveform, but a certain voltage threshold is required to heat the saline to induce a vapour layer, which in turn enables plasma formation. Voltages for the coagulation mode are below this threshold, and the power delivered only generates thermal increase. In ablation mode, the power delivered generates both plasma and thermal increase.

The generator used was specifically programmed by the producer to allow set activation times of 500, 1,000 and 2,000 ms and the output voltage for the coagulation mode to provide essentially the same energy and thermal induction per time unit as the ablation mode. Exposure time and mode of activation were controlled via a foot pedal.

Sample exposure

The generator was adjusted to setting 9 (of 1–9), equivalent to 300 V output, and the probes were applied in the wells in ablation mode for the PbRf ablation group and coagulation mode for the TC group, for the preset exposure times. The probe tips were disinfected in 70% ethanol and washed in isotonic saline between samples. A new probe was used for each group, and for a maximum of six samples. Plasma formation, as confirmed by light emission and bubble formation for PbRf ablation samples and a typical fizz in TC samples, were monitored to confirm proper probe activation. The normal control samples were left untreated.

Post-exposure each sample was serially diluted in 0.9% saline to 1/100, and plated onto Sabouraud's agar for *C. albicans* and horse blood agar for the bacterial strains. Bacterial plates were incubated at 37°C for 24 h and *C. albicans* at 32°C for 48 h. All strains were incubated aerobically, except *E. coli*, which was incubated in both aerobic and anaerobic conditions using anaerobic jars with AnaeroGen sachets (Oxoid Ltd, Basingstoke, Hampshire, UK). The number of colony-forming units (CFU) was counted and minimal log reduction and relative reduction (expressed as percent of absolute amount of CFU reduced by treatment) for ablation exposed groups were calculated, considering a limit of quantification (LoQ) of 1 CFU/group.

Temperature and exposure time measurements

To confirm that the ablation and coagulation modes generated comparable temperature increases a calorimetric trial was performed. The maximum temperature rise of each exposure was measured with a fibre optic temperature sensor (Neoptix Inc., Québec, Canada) at 100 Hz in 100 µl of saline using the same set-up as for the microbial trials.

Measurements of the pre-programmed radiofrequency activation times were performed with a DPO4034 Digital Oscilloscope (Tektronix Inc., Oregon, USA), and a P5200 High Voltage Differential Probe (Tektronix), to confirm correct activation times equal between modes. Each measurement was repeated six times.

Statistic analysis

All microbial data were analysed using R version 2.10.1 (The R Foundation for Statistical Computing, Vienna, Austria) using the coin package. The exact permutation form of Wilcoxon-Mann-Whitney's test stratifying for measurement occasion was used for comparison of CFU/ml-values between groups. The values were ranked within each strata. All tests were two-sided and statistical significance was taken at $p < 0.05$.

RESULTS

The PbRf ablation exposure had a direct microbicidal effect on all tested strains. At 500 ms ablation there were already significant reductions in bacteria/fungi counts compared with both untreated control and 500 ms TC for all strains.

For all strains tested there were significant reductions in CFU/ml for all PbRf ablation groups compared with untreated control ($p < 0.0001$ for all comparisons). Ablation also significantly reduced CFU/ml compared with each respective TC exposure time for all strains ($p < 0.001$ or less), except for *S. pyogenes* and *C. albicans* where significant differences could be seen only at 500–1,000 ms exposure but not at 2,000 ms (Fig. 1a–f, Tables SI and SII (available from http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1171).

The absolute reductions compared with untreated control, considering the LoQ, for *S. aureus* were 99.77% at 500 ms and 99.98% at 1,000–2,000 ms ablation. For *S. pyogenes* the reductions were 99.87% for all ablation groups. The reductions for aerobically grown *E. coli* were 99.91% at 500 ms, and 99.99% at 1,000–2,000 ms ablation. *E. coli* grown in anaerobic conditions showed reductions of 94.33% at 500 ms, and 99.98% at 1,000–2,000 ms ablation. For *P. aeruginosa* the reductions were 99.52% at 500 ms, 99.99% at 1,000 ms and 99.97% at 2,000 ms ablation. With *C. albicans* the reductions were 99.73% at 500 ms, and 99.92% at 1,000–2,000 ms ablation. Compared with untreated control mean log reductions reached 4–5 log for all strains and results were consistent between strains (Fig. 2).

Results from the exposure time measurements of the Quantum generator showed differences of no more than 3 ms between set and measured activation times (Table SIII (available from http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1171). The temperature measurements showed that total temperature increase did not differ significantly ($p > 0.05$) between the two modes of activation used for PbRf ablation and TC for any of the exposure times (data not shown).

DISCUSSION

The study results show that PbRf ablation has a direct local microbicidal effect on the tested bacteria and fungi strains *in vitro* at exposure times of 0.5–2 s.
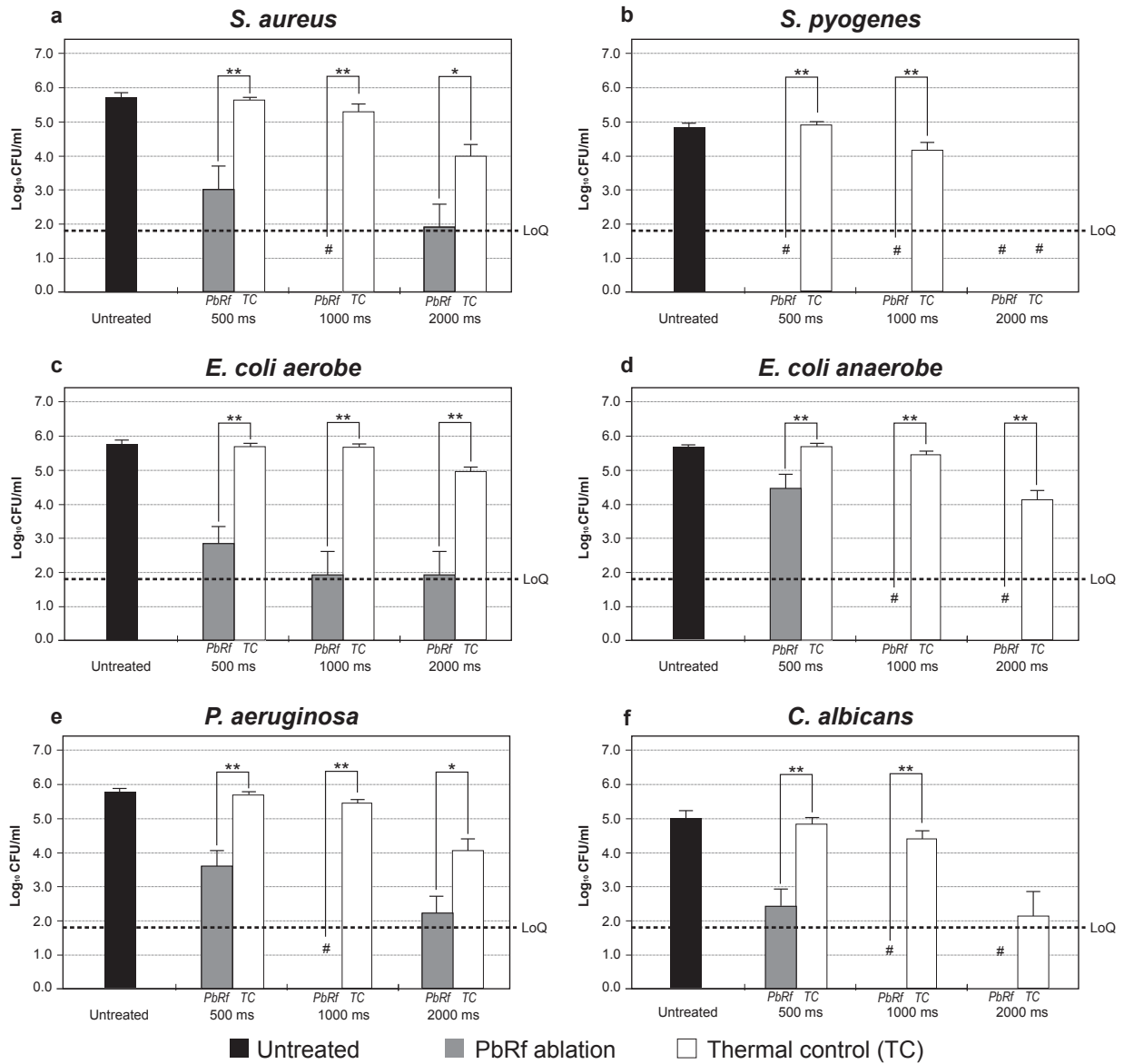
Fig. 1. The mean amount of colony-forming units (CFU)/ml ± standard deviation on a log scale for the different exposure times and strains. Dotted lines indicate limit of quantification (LoQ) of 1.92 log CFU/ml for the study set-up. #Groups in which no CFU were detected. *$p<0.001$ and **$p<0.0001$.
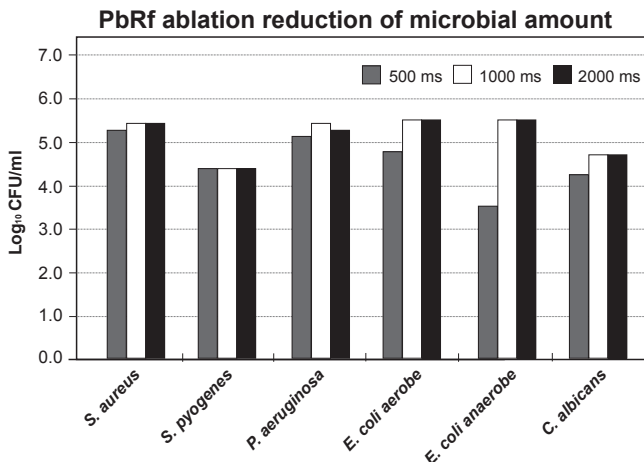

Fig. 2. Mean log reduction in microbial amount calculated as the difference between untreated control and each respective ablation exposure time, considering the limit of quantification.

The 4 tested bacteria strains are among the most frequently found in both infected and non-infected wounds, and *S. aureus* is considered to be the most important pathogen (19). Different *Candida* species have been concluded to be the most common fungus found in diabetic feet (20), and *C. albicans* has been identified by Hansson et al. as the most frequently found fungus in several clinical leg ulcer studies (21–23). The choice to use 10^6 CFU/ml in this study was based on the established concept that 10^5 or 10^6 microorganisms per ml or gram of tissue, depending on the type of wound, is characterized as a clinically relevant colonization of the wound bed and considered to be a key factor in wounds that fail to heal (6, 19, 24–26).

In our comparison of the ablation and TC mode of the device with regards to thermal increase, it can be concluded that the TC was a relevant thermal and energy

control, while thermal difference is directly correlated to energy according to the specific heat formula (27). For all strains the 500 ms ablation was significantly more microbicidal than both 500 and 1,000 ms TC. It can thus be concluded that the antimicrobial effect of the ablation is not only thermal or due to the energy input in the suspension *per se*, but instead associated with the plasma and its characteristics. At the longest TC exposure time a clear reduction in CFU/ml was seen for all strains, as would be expected with the thermal increase in a small amount of fluid. It is well established that the different strains utilized are sensitive to short exposures to temperatures in the range of approximately 52–70°C (28–32).

In technical studies PbRf ablation has been shown to produce hydroxyl radicals (17, 33), which are well-recognized to have direct bactericidal and fungicidal effects (34, 35). Other techniques based on gas plasmas, which are suggested for use in, for example, medical equipment sterilization, have been shown directly to destroy the bacterial cell wall, which also can be hypothesized as the potential mechanism of PbRf ablation (36).

In our set-up we tested Gram-negative and Gram-positive bacteria as well as fungi and, in general, the same effect could be seen. The fact that a similar decrease in bacterial load was seen for both aerobically and anaerobically grown *E. coli* shows that the effect is also independent of bacterial metabolism. The maximum reduction in microbial load detected for the different strains varied between 4.5 log for *S. pyogenes* (99.87%) and 5.6 log for *E. coli* grown aerobically (99.99%). As the microbicidal effect in this case is probably a purely physiochemical or mechanical process, these differences could depend on factors such as the microbe shape, size, cell wall constitution, and disposition to form aggregates. However, as the relative maximal reductions detected are very similar, it is reasonable to conclude that the microbicidal effect is general and independent of factors such as cellular wall and microbe size.

The general trend was that a longer ablation exposure time gave a higher reduction. For *S. aureus* and *P. aeruginosa* the reduction was slightly higher at 1,000 ms ablation compared with 2,000 ms. While these results were close to the LoQ for the set-up, the most probable conclusion is that these differences depend merely on statistical variation.

The probe used for this study creates a plasma field approximately 100 μm thick around the active electrode (33). From the results we can thus conclude only that the microbicidal effect is local within the direct vicinity of the probe tip within the microtitre well. The absolute distance of the effect is therefore yet to be determined.

Further research is required to confirm whether the PbRf antimicrobial effect is also applicable *in vivo* and to evaluate the safety and efficacy of the technique for wound treatment in the clinical situation. Additional basic research is also needed to verify the precise mechanism of action of PbRf ablation and to determine how bacteria and fungi cells are affected on a molecular basis. This study demonstrates that PbRf ablation has a general microbicidal effect on bacteria and fungi common in wound infection. Thus, with the combination of its previously shown ability to remove tissue effectively and its microbicidal capacity, it is a promising technique for use in surgical areas such as chronic wound treatment or infection control.

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