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

Master Thesis in Medicine

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Abstract – English

Master Thesis/Degree Project, Programme of Medicine

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

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Background

Infection constitutes an important part of wound pathology and impede wound healing. Plasma-mediated bipolar radiofrequency ablation (Coblation®) is a tissue removal-technique suggested for use in wound treatment.

Aims

The study purposes 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.

Methods

Suspensions of 10^6 CFU/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 after that incubated aerobically. *E. coli* was also incubated anaerobically.

Results

Ablation was significantly ($p < 0.0001$) more microbicidal on all strains compared to untreated and thermal control. The reductions compared to untreated control were 99.87-99.99% for all strains.

Conclusions

In conclusion plasma-mediated bipolar radiofrequency ablation has a general microbicidal effect on microbes relevant to dermal wound infection independent of aerobic/anaerobic growth and thermal effect.

Introduction

Background

Localised infection is a common consequence in chronic wounds as well as in acute surgical wounds, leading to complications such as systemic infection and amputation, and result in significant socioeconomic burden and reduced quality of life [1-3]. Bacteria and fungi colonisation and infection is well recognised to constitute an important part of the wound pathology and 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 in 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 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 which 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.* for wound debridement in a case series of 25 chronic wound patients with good results and complete closure of all wounds within six to eight weeks. The series included both diabetic, post-traumatic and Charcot foot wounds [18]. However, no previous study has investigated the bactericidal or fungicidal potential of PbRf ablation.

Aims

Thus, the aims of the present study were; 1) To determine the direct antimicrobial effect of PbRf ablation exposure on bacteria and fungi strains relevant to wound infection, and 2) to

determine how the parameters of exposure time, temperature increase, and aerobic/anaerobic growth influence the antimicrobial effect.

Materials and methods

Data collection procedures

All microorganisms were obtained from CCUG (Culture Collection, University of Gothenburg, Sweden). 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^{o} C and *C. albicans* was maintained on Sabouraud's agar (Clinical bacteriology at Sahlgrenska University Hospital, Sweden) at 32^{o} C.

Bacteria and fungi from 24 hrs old cultures were dissolved in 0.9 % saline (pH 7.4) and adjusted to approximately 10^{6} cells/mL, as determined by OD 2.0 at 550 nm with a DEN-1 McFarland densitometer (Biosan, Riga, Latvia). The suspension was transferred to 96 well microtiter plates (Nunc A/S, Roskilde, Denmark) with 100 µL/well, with every second well and row left empty to avoid thermal effects between samples. The wells were divided into PbRf ablation, thermal control (TC) and untreated (normal) control groups. The two exposure groups were further subdivided into 500, 1000 and 2000 ms exposure time with six samples in each group. The experimental setup was repeated twice for each strain.

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, 1000 and 2000 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.

The generator was adjusted to setting 9 (of 1-9), equivalent to 300V 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 hrs and *C. albicans* at 32°C for 48 hrs. 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, England). The number of colony forming units (CFU) were 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.

To confirm that the ablation and coagulation modes generated comparable temperature increase a calorimetric trial was performed. 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 setup 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.

Statistical methods

All microbial data were analyzed 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. Already at 500 ms ablation there were significant decreases in bacteria/fungi counts compared to 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 to untreated control ($p < 0.0001$ for all comparisons). Ablation also significantly reduced CFU/mL compared to 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 500-1000 ms exposure but not at 2000 ms (Fig. 1a-f, Tables I and II).

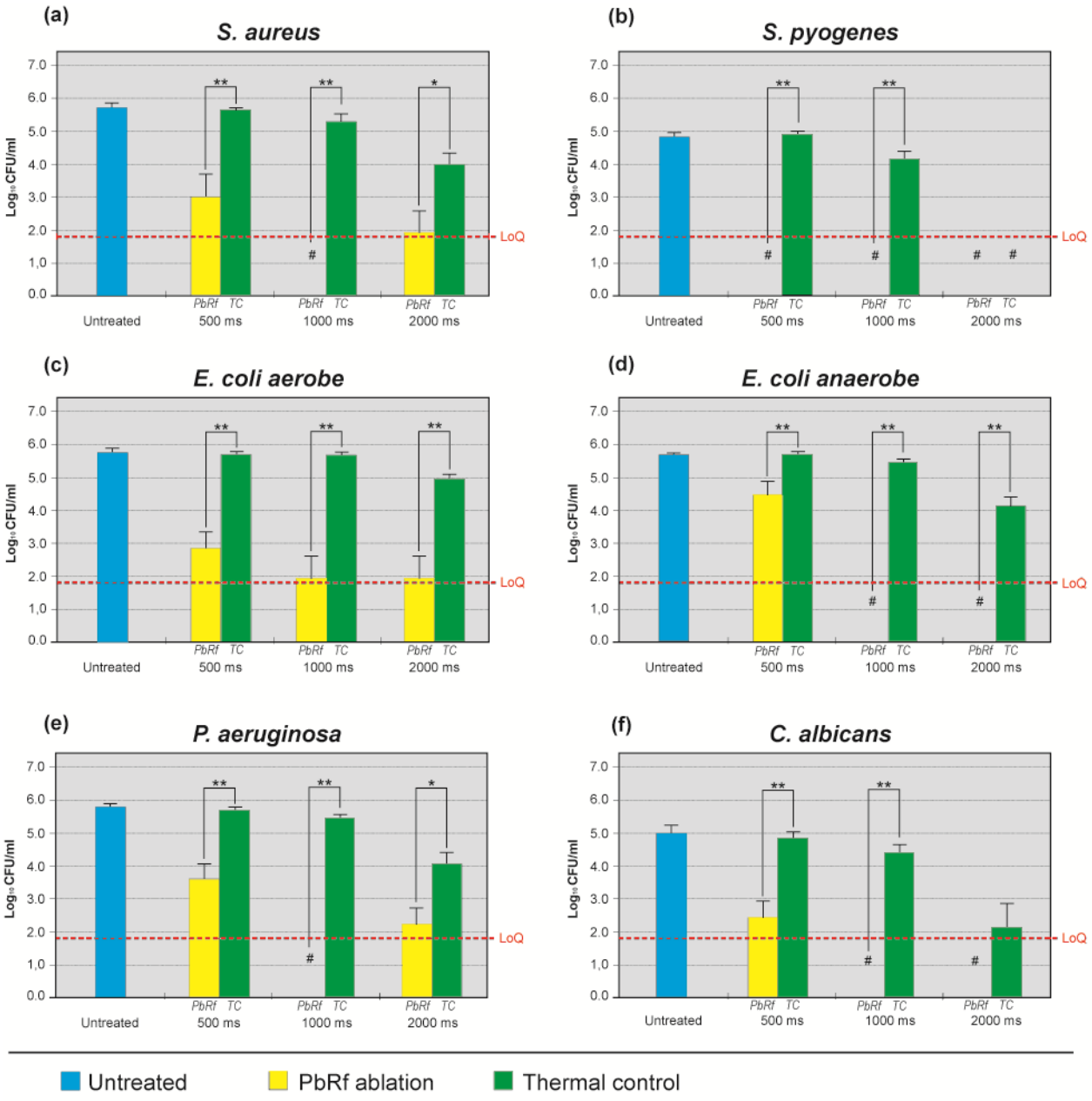

Figure 1. *The charts show the mean amount of CFU/mL + STD on a log scale for the different exposure times and strains. The red dotted lines indicate the limit of quantification (LoQ) of 1.92 log CFU/mL for the study setup. In groups indicated with # no CFU were detected. *p < 0.001 and **p < 0.0001.*

The absolute reductions compared to untreated control, considering the LoQ, were for *S. aureus* 99.77% at 500 ms and 99.98% at 1000-2000 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 1000-2000 ms ablation. *E. coli* grown in anaerobic conditions showed reductions of 94.33% at 500 ms, and 99.98% at 1000-2000 ms ablation. For *P. aeruginosa* the reductions were 99.52% at 500 ms, 99.99% at 1000 ms and 99.97% at 2000 ms ablation. With *C. albicans* the reductions were 99.73% at 500 ms, and 99.92% at 1000-2000 ms ablation. Compared to untreated control mean log reductions reached 4-5 log for all strains and results were consistent between strains (Fig. 2).

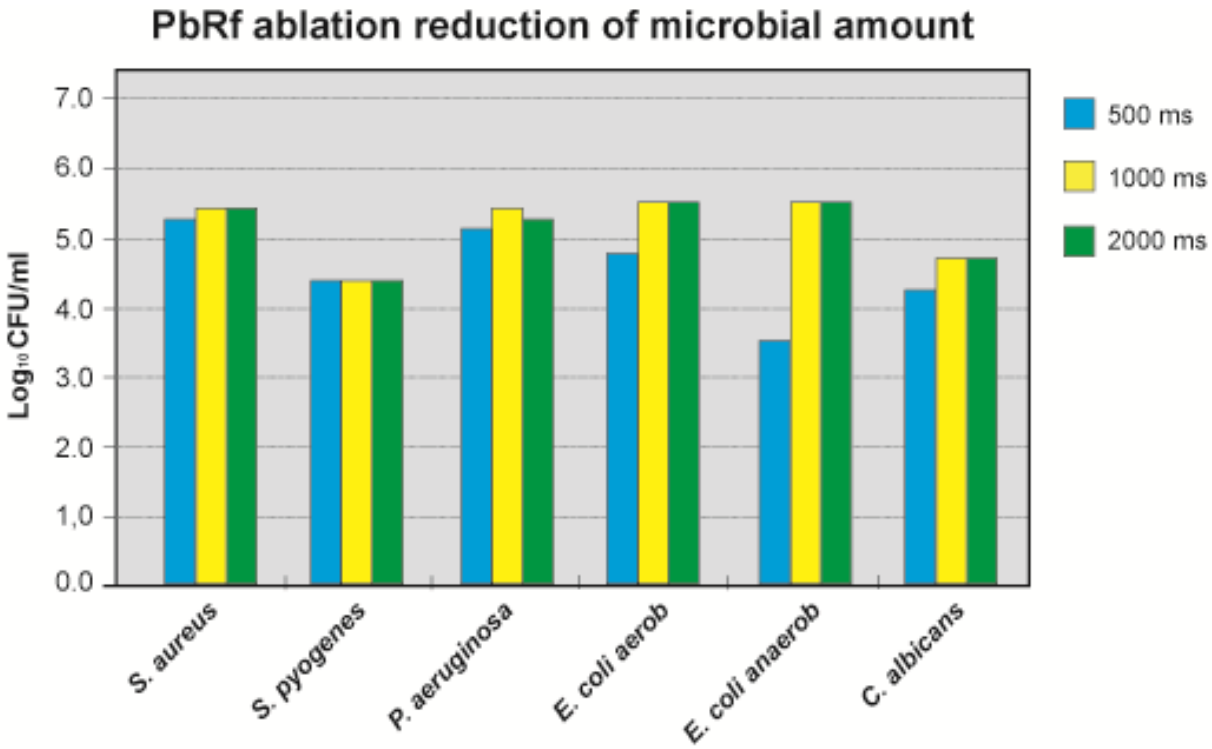

Figure 2. *The chart depicts the mean log reduction in microbial amount calculated as the difference between untreated control and each respective ablation exposure time, considering the limit of quantification.*

Results from the exposure time measurements of the Quantum generator showed differences of no more than three ms between set and measured activation times (Table III). 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.

Discussion

The study results clearly 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 to 2 seconds.

The four 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 and *C. albicans* has by Hansson *et al.* been identified as the most frequently found fungus in several clinical leg ulcer studies [20-23]. 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 colonisation of the wound bed and considered 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 1000 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 see* 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 of temperatures in the range of around 52-70°C [28-32].

The PbRf ablation has in technical studies been shown to produce hydroxyl radicals [17, 33] which are well recognised to have direct bactericidal and fungicidal effects [34, 35]. Other techniques based on gas plasmas, which are suggested for use in e.g. medical equipment sterilisation, have been shown to directly destruct the bacterial cell wall which also can be hypothesised as the potential mechanism of PbRf ablation [36].

In our setup 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 very similar decrease in bacterial load was seen for both aerobically and anaerobically grown *E. coli* show that the effect is, at least for *E. coli,* 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 most likely 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 1000 compared to 2000 ms ablation. While these results were close to the LoQ for the setup, the most probable conclusion is that these differences merely depend on statistical variation.

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

Further research is required to confirm whether the PbRf antimicrobial effect also is applicable *in vivo* and to evaluate 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 and determine how bacteria and fungi cells are affected on a molecular basis.

Conclusions

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 effectively remove tissue and the microbicidal capacity it is a promising technique for use in surgical areas such as chronic wound treatment or infection control.

UNIVERSITY OF GOTHENBURG
THE SAHLGRENSKA ACADEMY

Populärvetenskaplig sammanfattning – Svenska

Examensarbete 30 hp, Läkarprogrammet

Den Antimikrobiella Effekten av Plasma-medierad Bipolär Radiofrekvensablation på Bakterier och Svamp Relevanta för Sårinfektion

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Vad den här studien har kommit fram till

Denna studien visar att den elektrokirurgiska tekniken plasma ablation är effektiv för att döda bakterier och jästsvamp som är vanliga i hudsår. Denna teknik skulle därför eventuellt kunna användas för att behandla och rengöra svårläkta hudsår.

Bakgrund – Varför studien genomfördes

Svårläkta kroniska hudsår är ett stort hälsproblem för många äldre människor idag. Det kan finnas många anledningar till att vissa sår har svårt att läka. En anledning kan vara att bakterier och svamp växer i såret och hämmar sårläkningen. Bakterier och svamp i såret kan även leda till infektion med blodförgiftning och i värsta fall kan ett ben med svårläkta sår behöva amputeras.

Plasma-medierad bipolär radiofrekvens ablation (även kallad Coblation®) är en teknik som idag används inom flera olika typer av kirurgiska operationer. Tekniken har också föreslagits som en ny behandlingsmetod för sårbehandling.

Om plasma ablation skulle visa sig vara en bra behandlingsmetod för svårläkta och infekterade sår så skulle tekniken eventuellt kunna hjälpa dessa patienternas sår att läka fortare. På så sätt skulle problemen med sårinfektioner och amputationer kanske kunna undvikas hos dessa patienter.

Den här studien undersökte den bakteriedödande och svampdödande effekten av plasma ablation på olika bakteriearter och svamparter som är vanliga orsaker till sårinfektion. Studien undersökte också hur lång tids behandling som behövdes för att döda bakterierna och svampen samt om effekten var temperaturberoende.

Metoder – Hur studien genomfördes

I experimenten användes olika lösningar med bakterier av stammarna *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, Escherichia coli* och av jästsvampen *Candida albicans.* Studien gick till så att lösningarna behandlades med plasma ablation eller med en temperaturkontroll under 0,5-2 sekunder. En obehandlad grupp användes också som kontrollgrupp. Efter behandlingen så ströks lösningarna ut på plattor med tillväxtmedium och fick stå i värmeskåp ett dygn så att bakterierna och svamparna kunde räknas.

Resultat - Vad den här studien visar

Resultaten från studien visade att plasma ablation var mycket effektiv på att döda både bakterier och svamp av alla de testade stammarna. För alla de olika stammarna så dödade plasma ablation över 99% av bakterierna och svamparna. Det stora flertalet bakterier dödades redan efter 0,5 sekunders behandling. Plasma ablation var också betydligt bättre än temperaturkontrollen på att döda de olika bakterie- och svampstammarna.

Slutsatser – Vilka konsekvenser har studiens resultat

De slutsatser som kan dras från studien är att plasma ablation har en generell bakteridödande och svampdödande effekt. Denna effekt verkar också vara oberoende av den temperaturökning som tekniken kan ge. Studien tyder därför på att plasma ablation har en stor potential för att användas för att behandla infekterade och svårläkta sår. Ytterligare forskning behöver därför göras för att undersöka hur olika typer av sår kan behandlas med plasma ablations-teknik.

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References

1. Jeffcoate, W.J. and K.G. Harding, *Diabetic foot ulcers.* Lancet, 2003. **361**(9368): p. 1545-51.
2. Leaper, D.J., *Risk factors for and epidemiology of surgical site infections.* Surg Infect (Larchmt), 2010. **11**(3): p. 283-7.
3. Reichenberg, J. and M. Davis, *Venous ulcers.* Semin Cutan Med Surg, 2005. **24**(4): p. 216-26.
4. Breidenbach, W.C. and S. Trager, *Quantitative culture technique and infection in complex wounds of the extremities closed with free flaps.* Plast Reconstr Surg, 1995. **95**(5): p. 860-5.
5. Robson, M.C., *Wound infection. A failure of wound healing caused by an imbalance of bacteria.* Surg Clin North Am, 1997. **77**(3): p. 637-50.
6. Robson, M.C. and J.P. Heggers, *Delayed wound closure based on bacterial counts.* J Surg Oncol, 1970. **2**(4): p. 379-83.
7. Hopf, H.W., et al., *Guidelines for the treatment of arterial insufficiency ulcers.* Wound Repair Regen, 2006. **14**(6): p. 693-710.
8. Steed, D.L., et al., *Guidelines for the treatment of diabetic ulcers.* Wound Repair Regen, 2006. **14**(6): p. 680-92.
9. Whitney, J., et al., *Guidelines for the treatment of pressure ulcers.* Wound Repair Regen, 2006. **14**(6): p. 663-79.
10. Robson, M.C., et al., *Guidelines for the treatment of venous ulcers.* Wound Repair Regen, 2006. **14**(6): p. 649-62.
11. Dryburgh, N., et al., *Debridement for surgical wounds.* Cochrane Database Syst Rev, 2008(3): p. CD006214.
12. Gerszten, P.C. and E.A. Monaco, 3rd, *Complete percutaneous treatment of vertebral body tumors causing spinal canal compromise using a transpedicular cavitation, cement augmentation, and radiosurgical technique.* Neurosurg Focus, 2009. **27**(6): p. E9.
13. Gerszten, P.C., et al., *Plasma disc decompression compared with fluoroscopy-guided transforaminal epidural steroid injections for symptomatic contained lumbar disc herniation: a prospective, randomized, controlled trial.* J Neurosurg Spine, 2010. **12**(4): p. 357-71.

14. Shah, U.K. and B. Dunham, *Coblation for tonsillectomy: an evidence-based review.* ORL J Otorhinolaryngol Relat Spec, 2007. **69**(6): p. 349-57.
15. Spahn, G., et al., *Arthroscopic knee chondroplasty using a bipolar radiofrequency-based device compared to mechanical shaver: results of a prospective, randomized, controlled study.* Knee Surg Sports Traumatol Arthrosc, 2008. **16**(6): p. 565-73.
16. Stalder KR, M.D., Woloszko J, *Electrosurgical Plasmas.* J Phys D: Appl Phys, 2005. **38**: p. 1728-1738.
17. Stalder KR, W.J., Brown IG, Smith CD, *Repetitive plasma discharges in saline solutions.* Applied Physics Letters, 2001. **79**(27): p. 4503-4505.
18. Lee, D.K., et al., *Plasma-mediated Radiofrequency Debridement Stimulates Healing Mediators in Chronic Wounds: A Multi-center Retrospective Case Series,* in *SAWC*. 2009: Dallas, Texas.
19. Bowler, P.G., *Wound pathophysiology, infection and therapeutic options.* Ann Med, 2002. **34**(6): p. 419-27.
20. Hansson, C., et al., *The microbial flora in venous leg ulcers without clinical signs of infection. Repeated culture using a validated standardised microbiological technique.* Acta Derm Venereol, 1995. **75**(1): p. 24-30.
21. Mlinaric-Missoni, E., S. Kalenic, and V. Vazic-Babic, *Species distribution and frequency of isolation of yeasts and dermatophytes from toe webs of diabetic patients.* Acta dermatovenerologica Croatica : ADC / Hrvatsko dermatolosko drustvo, 2005. **13**(2): p. 85-92.
22. Hansson, C., J. Jekler, and G. Swanbeck, *Candida albicans infections in leg ulcers and surrounding skin after the use of ointment impregnated stockings.* Acta Derm Venereol, 1985. **65**(5): p. 424-7.
23. Hansson, C., J. Faergemann, and G. Swanbeck, *Fungal infections occurring under bandages in leg ulcer patients.* Acta Derm Venereol, 1987. **67**(4): p. 341-5.
24. Levine, N.S., et al., *The quantitative swab culture and smear: A quick, simple method for determining the number of viable aerobic bacteria on open wounds.* J Trauma, 1976. **16**(2): p. 89-94.
25. Raahave, D., et al., *The infective dose of aerobic and anaerobic bacteria in postoperative wound sepsis.* Archives of surgery (Chicago, Ill. : 1960), 1986. **121**(8): p. 924-9.

26. Robson, M.C., et al., *Quantitative bacteriology and delayed wound closure.* Surgical forum, 1968. **19**: p. 501-2.
27. Laidler, K.J., *The world of physical chemistry*. 1995, Oxford ; New York: Oxford University Press. xii, 476 p.
28. Arguelles, J.C., *Thermotolerance and trehalose accumulation induced by heat shock in yeast cells of Candida albicans.* FEMS Microbiol Lett, 1997. **146**(1): p. 65-71.
29. Rodriguez-Calleja, J.M., et al., *Variation in resistance of natural isolates of Staphylococcus aureus to heat, pulsed electric field and ultrasound under pressure.* J Appl Microbiol, 2006. **100**(5): p. 1054-62.
30. Sorqvist, S., *Heat resistance in liquids of Enterococcus spp., Listeria spp., Escherichia coli, Yersinia enterocolitica, Salmonella spp. and Campylobacter spp.* Acta Vet Scand, 2003. **44**(1-2): p. 1-19.
31. Spinks, A.T., et al., *Thermal inactivation of water-borne pathogenic and indicator bacteria at sub-boiling temperatures.* Water Res, 2006. **40**(6): p. 1326-32.
32. Tsuji, A., et al., *The effects of temperature and pH on the growth of eight enteric and nine glucose non-fermenting species of gram-negative rods.* Microbiol Immunol, 1982. **26**(1): p. 15-24.
33. Woloszko J, S.K., Brown IG, *Plasma characteristics of repetitively-pulsed electrical discharges in saline solutions used for surgical procedures.* IEEE transactions on plasma science 2002. **30**(3): p. 1376-1383.
34. Iwasawa, A., et al., *Fungicidal action of hydroxyl radicals generated by ultrasound in water.* J Clin Biochem Nutr, 2009. **45**(2): p. 214-8.
35. Moisan, M., et al., *Low-temperature sterilization using gas plasmas: a review of the experiments and an analysis of the inactivation mechanisms.* Int J Pharm, 2001. **226**(1-2): p. 1-21.
36. Joaquin, J.C., et al., *Is gas-discharge plasma a new solution to the old problem of biofilm inactivation?* Microbiology, 2009. **155**(Pt 3): p. 724-32.

Tables

Table I. Microbial amount in each group in absolute values[a]

	Untreated control	PbRf ablation (ms)			Thermal control (ms)		
		500	1000	2000	500	1000	2000
S. aureus	4.8e5 ± 1.0e5	1.1e3 ± 3.6e3	0 ± 0	8.3e1 ± 2.9e2	3.6e5 ± 5.4e4	2.1e5 ± 1.2e5	9.9e3 ± 1.3e4
S. pyogenes	6.5e4 ± 2.2e4	0 ± 0	0 ± 0	0 ± 0	8.7e4 ± 2.0e4	1.5e4 ± 9.9e3	0 ± 0
E. coli aerobe	7.7e5 ± 1.2e5	6.7e2 ± 1.2e3	8.3e1 ± 2.9e2	8.3e1 ± 2.9e2	6.4e5 ± 7.8e4	5.8e5 ± 8.7e4	9.2e4 ± 5.1e4
E. coli anaerobe	5.3e5 ± 5.7e4	3.0e4 ± 4.9e4	0 ± 0	0 ± 0	4.6e5 ± 5.6e4	3.1e5 ± 7.9e4	1.5e4 ± 1.3e4
P. aeruginosa	6.1e5 ± 1.3e5	2.9e3 ± 8.9e3	0 ± 0	1.7e2 ± 3.9e2	4.8e5 ± 9.0e4	3.6e5 ± 1.0e5	1.2e4 ± 1.3e4
C. albicans	1.0e5 ± 7.7e4	2.7e2 ± 6.5e2	0 ± 0	0 ± 0	7.1e4 ± 4.7e4	2.1e4 ± 2.4e4	1.7e2 ± 5.8e2

[a] Data presented as mean CFU/mL ± STD

PbRf, Plasma-mediated bipolar radiofrequency

Table II. Statistical results as *p*-values for each comparison

	Thermal control (ms)			Untreated control
	500	1000	2000	
S. aureus				
500 ms PbRf	<0.0001	<0.0001	0.0032	<0.0001
1000 ms PbRf	<0.0001	<0.0001	0.00046	<0.0001
2000 ms PbRf	<0.0001	<0.0001	0.00015	<0.0001
Untreated control	0.00090	0.0044	<0.0001	
S. pyogenes				
500 ms PbRf	<0.0001	<0.0001	1	<0.0001
1000 ms PbRf	<0.0001	<0.0001	1	<0.0001
2000 ms PbRf	<0.0001	<0.0001	1	<0.0001
Untreated control	0.037	<0.0001	<0.0001	
***E. coli* aerobe**				
500 ms PbRf	<0.0001	<0.0001	<0.0001	<0.0001
1000 ms PbRf	<0.0001	<0.0001	<0.0001	<0.0001
2000 ms PbRf	<0.0001	<0.0001	<0.0001	<0.0001
Untreated control	0.0060	<0.0001	<0.0001	
***E. coli* anaerobe**				
500 ms PbRf	<0.0001	<0.0001	0.34	<0.0001
1000 ms PbRf	<0.0001	<0.0001	<0.0001	<0.0001
2000 ms PbRf	<0.0001	<0.0001	<0.0001	<0.0001
Untreated control	0.032	<0.0001	<0.0001	
P. aeruginosa				
500 ms PbRf	<0.0001	<0.0001	0.010	<0.0001
1000 ms PbRf	<0.0001	<0.0001	<0.0001	<0.0001
2000 ms PbRf	<0.0001	<0.0001	0.00085	<0.0001
Untreated control	0.0026	<0.0001	<0.0001	
C. albicans				
500 ms PbRf	<0.0001	<0.0001	0.48	<0.0001
1000 ms PbRf	<0.0001	<0.0001	1	<0.0001
2000 ms PbRf	<0.0001	<0.0001	1	<0.0001
Untreated control	0.0027	<0.0001	<0.0001	

PbRf, Plasma-mediated bipolar radiofrequency ablation

Table III. Measured exposure times of the Quantum generator

Set time (ms)	**Measured time (ms)**	
	PbRf ablation mode	**Coagulation mode**
500	499 ± 1	499 ± 0
1000	1000 ± 1	999 ± 1
2000	2000 ± 0	1997 ± 2