

Antimicrobial Effect of Low-Frequency Ultrasound in an In Vitro Wound Model

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Introduction

Low-frequency ultrasonic wound treatment (UAW) is emerging as an alternative method for wound bed preparation and debridement. Low-frequency ultrasound when applied to the wound bed via a wound treatment solution, allows for deep tissue penetration of the solution, with associated micro-cavitations causing bacterial destruction. An In Vitro model was designed to evaluate the bactericidal effectiveness of UAW treatment in different wound types. An immersion model for deep, tunneling or undermining wounds and the surface model for superficial wounds was studied. Various bacterial strains (E. coli, S. pyogenes, S. aureus, P. aeruginosa) were cultured and treated at differing ultrasonic outputs and exposure times.

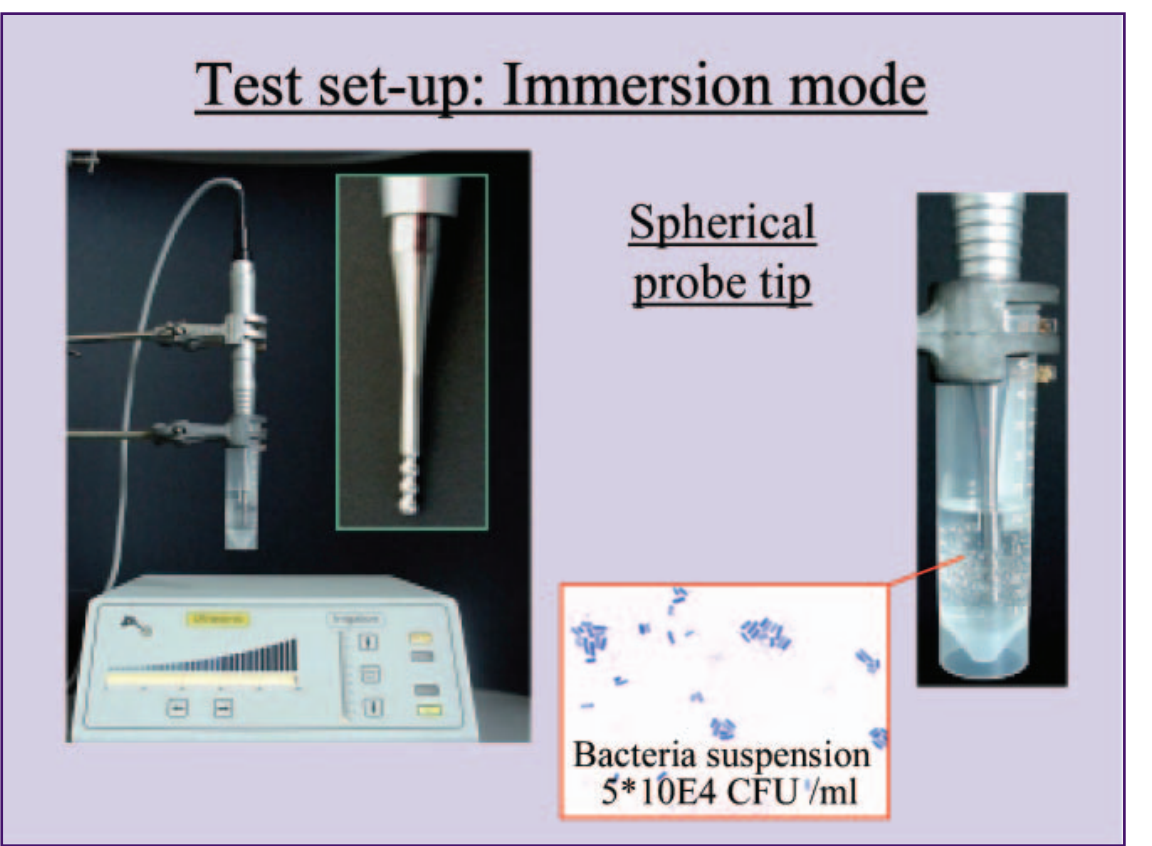


Figure 1. The immersion mode is applied to deep and pocket-type wounds. The type of application is comparable to an ultrasonic bath.

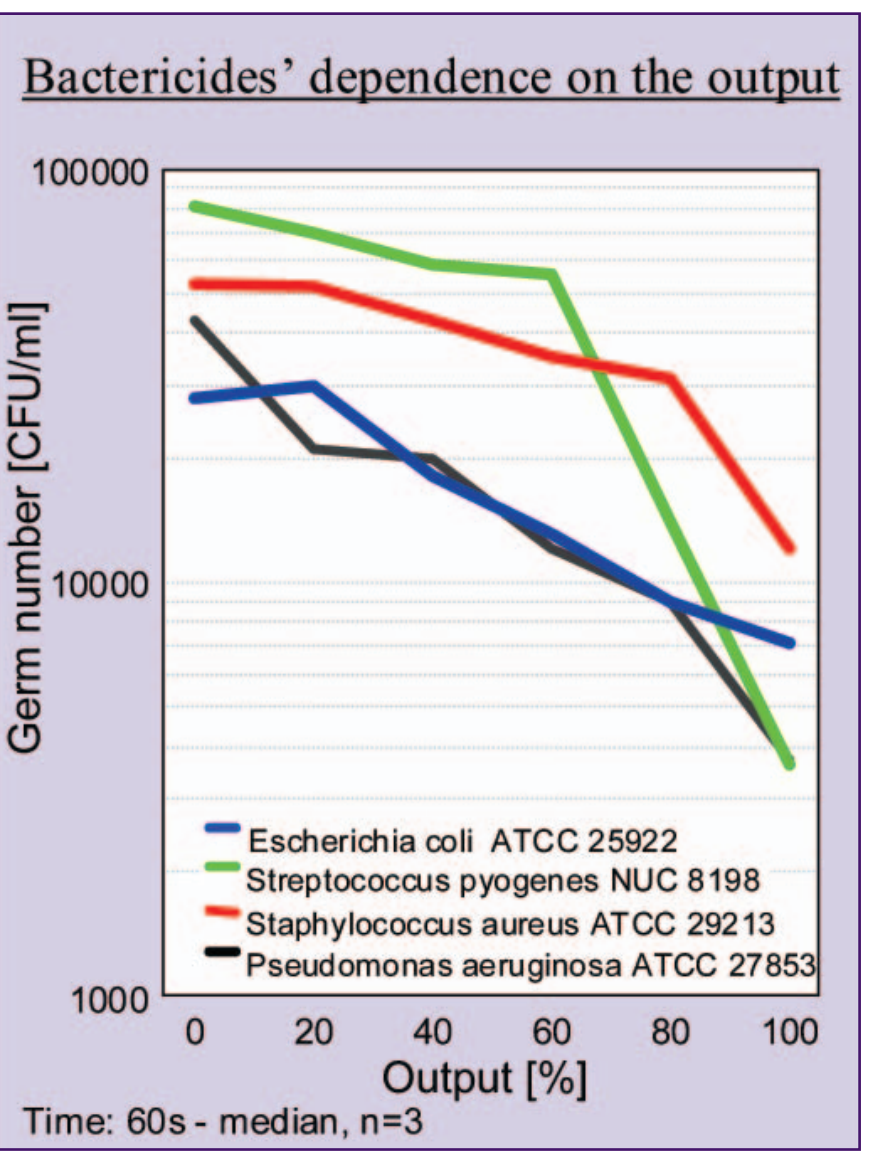


Figure 2. All bacterial strains demonstrated that the achieved bactericidal effect is dependent on the output level selected on the control panel.

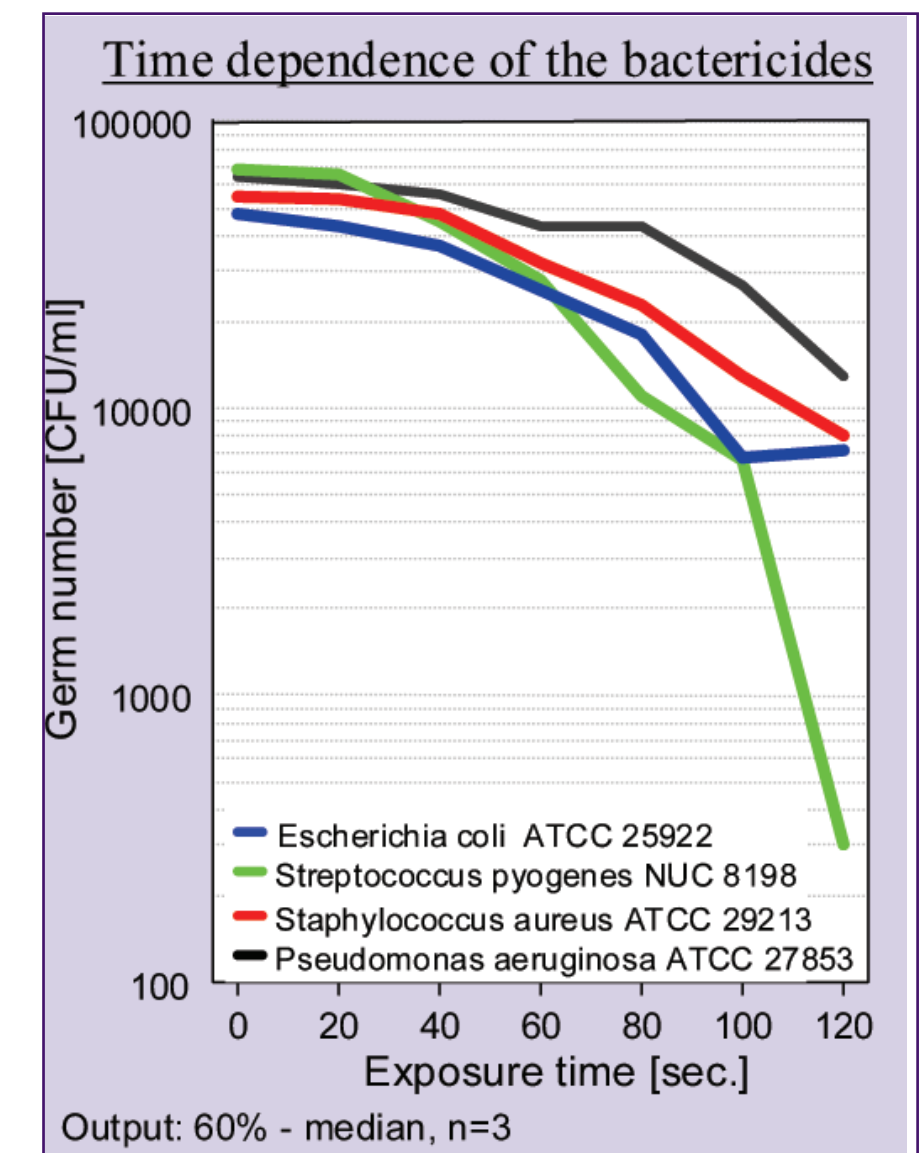


Figure 3. The examined bacterial strains also demonstrated that the achieved bactericidal effect depends on the exposure time as shown during clinic application.

Conclusion

Both wound models showed highly effective bactericidal effect when the UAW output was 100% for duration of 120 seconds. Fifteen out of sixteen bacterial matrix plates treated at 100% ultrasonic output for duration of 60 seconds were found to be sterile. These interesting and exciting results provide an excellent basis for In Vivo research and clinical trials.

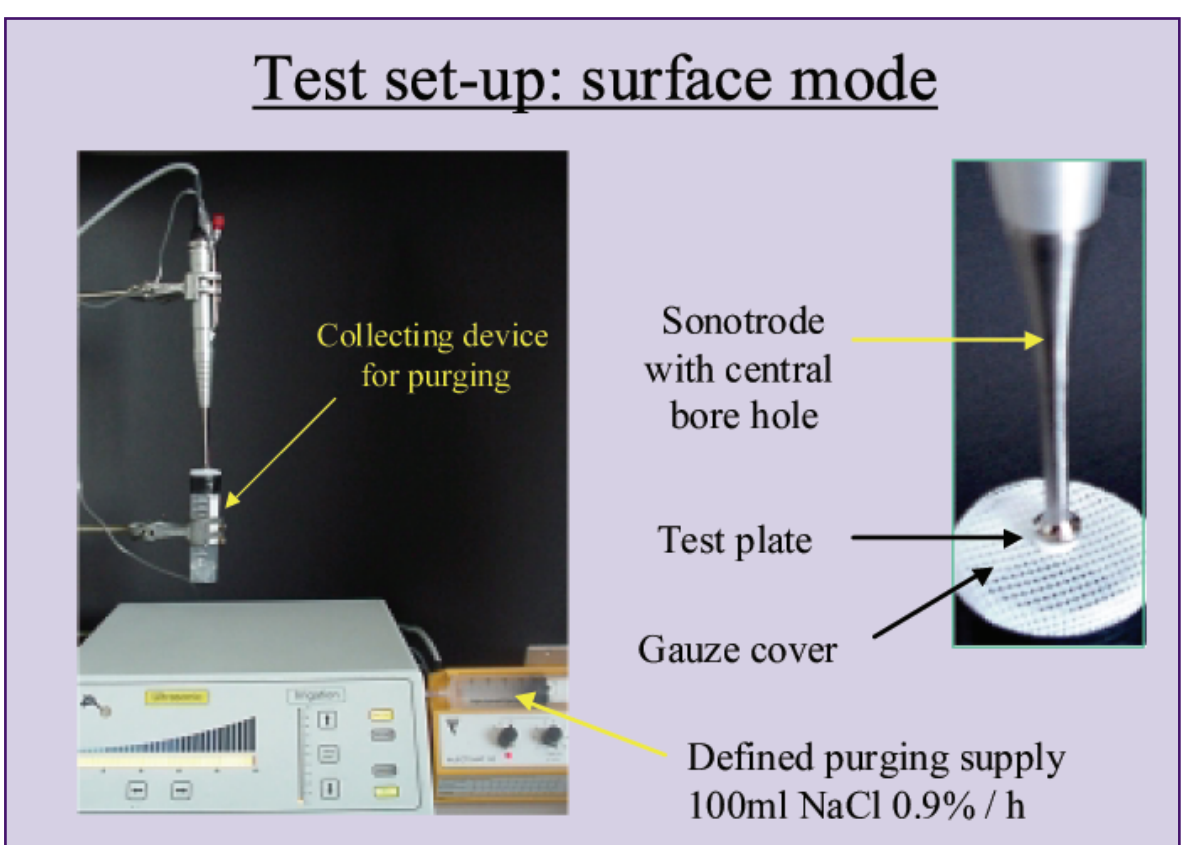


Figure 4. The Surface Mode is used for the majority of wounds as defects are generally of a planar type. In the surface mode, fluid is supplied via the handpiece, which establishes the sonic coupling between the probe and the wound surface.

Output [%] *	Germ No. [CFU]	Streptococcus pyogenes	Staphylococcus aureus	Escherichia coli	Pseudomonas aeruginosa
0	10	3/3	3/3	3/3	3/3
0	100	3/3	3/3	3/3	3/3
0	1000	3/3	3/3	3/3	3/3
0	10000	3/3	3/3	3/3	3/3
100	10	0/3	0/3	0/3	0/3
100	100	0/3	0/3	0/3	0/3
100	1000	0/3	0/3	1/3	0/3
100	10000	0/3	0/3	0/3	0/3

* Sonotrode excursion that can be set at the device

Figure 5. All matrix sections subjected to ultrasound were bred in a liquid culture. The samples shown in red background are samples that were not treated and produced positive results, as expected. With one exception (E. coli / 1000 CFU), all matrix plates subjected to 100% ultrasonic output for 60 seconds by the Sonoca 180 were found to be sterile. To rule out any temperature effects, the treatment was applied in fractions of 10 seconds each.

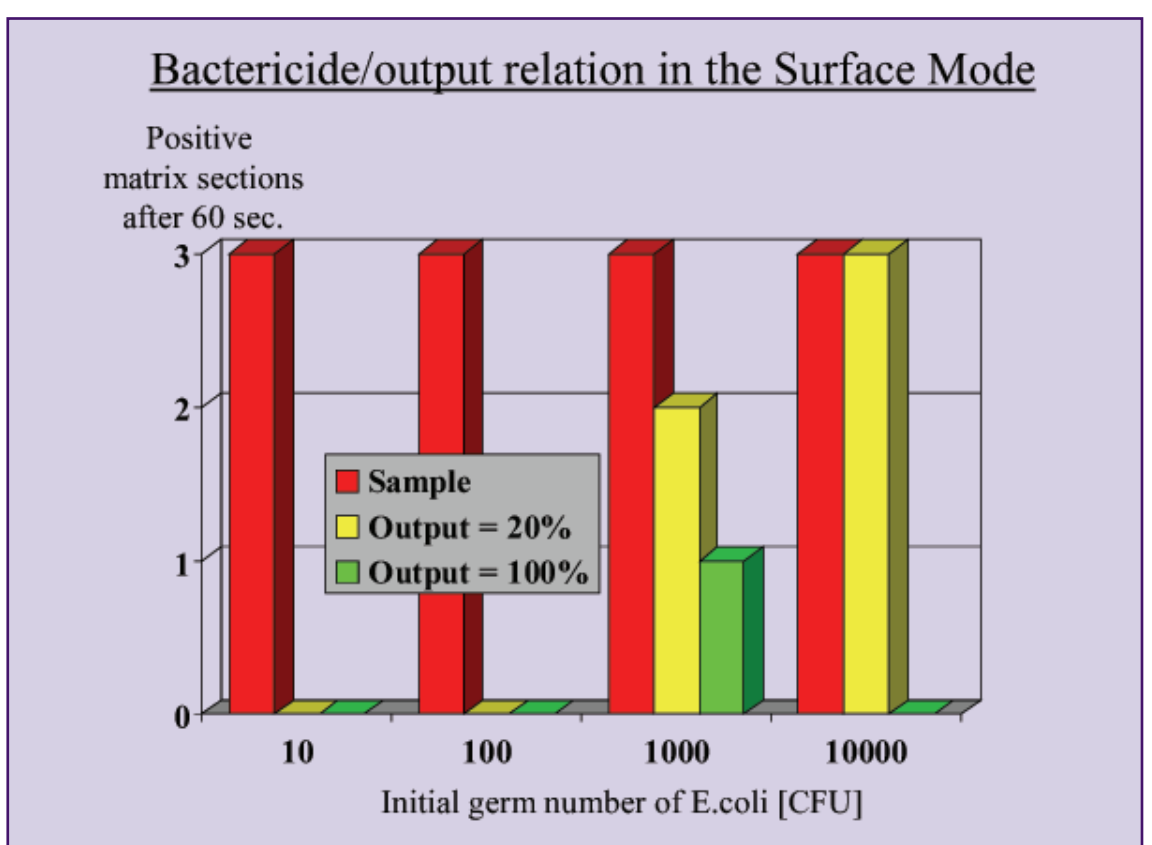


Figure 6. In the Surface Mode, there appears to be a dependence on the output selected at the device. At a selected output of 20%, bacteria samples with low bacteria count were killed whereas bacteria samples with a high bacteria count survived (yellow). At a selected output of 100%, the bacteria samples with a high bacteria count were also killed (green).

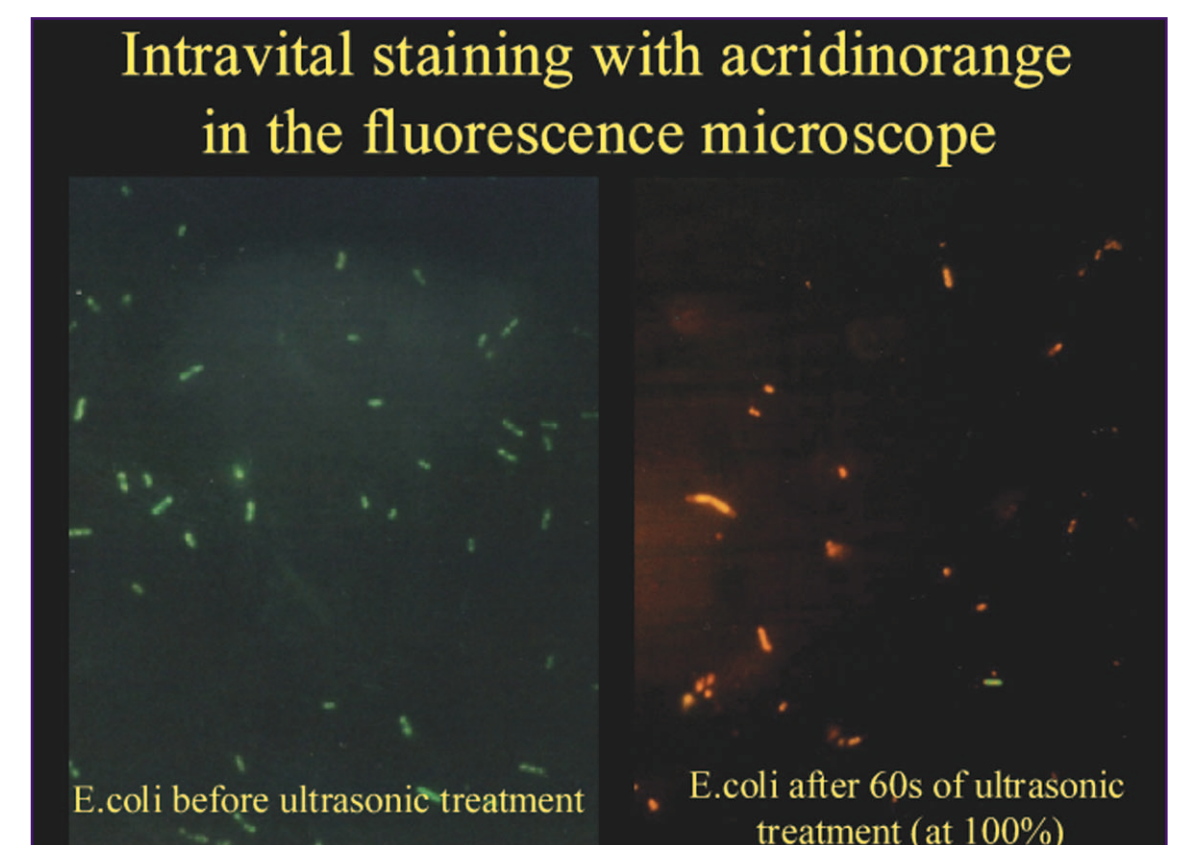


Figure 7. Intravital staining with acridinorange under fluorescent light impressively demonstrates the bactericidal effect of ultrasonic treatment using the Sonoca 180. Using this staining technique, vital bacteria are fluorescing in green whereas bacteria appearing in red are dead. Many bacteria are torn into small particles by the ultrasonic treatment.