An investigation into the effectiveness of the Dentron Biogun® in killing strains of Methicillin Resistant Staphylococcus aureus (MRSA) in a controlled laboratory environment.

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Introduction

The Dentron Biogun is an interesting piece of equipment designed to help reduce the numbers of microorganisms in dental caries (Burke et al. 1995). Some chiropodists have also found a use for the Biogun in the treatment of fungal skin & nail infections.

The Biogun achieves its effect by delivering a stream of electrons which bind to the surrounding molecules of oxygen, forming the hydrated superoxide anion, $(O_2\text{-})(H_2O)n$ where n is between 4 and 8. This anion radical is thought to act as a nucleophile on the phospholipid bi-layer of micro-organisms, causing de-esterification of fatty acids and weakening the cell membrane (Kellogg et al. 1979)

Much work has been performed in establishing the bacteriocidal effect of the Biogun on populations of micro-organisms both in-vitro and in-vivo, including Streptococcus spp, lactobacilli, Actinomyces spp, Candida albicans and coagulase negative staphylococci (Shargawi et al. 1999, Kellogg et al. 1979, Rosenthal B. et al. 1979, Burke F.M. et al. 1995). No data are available, however, to show the efficacy of the Biogun in killing populations of Methicillin Resistant Staphylococcus aureus (MRSA). This study aims to investigate the effect of the Biogun in-vitro when applied to different strains of MRSA.

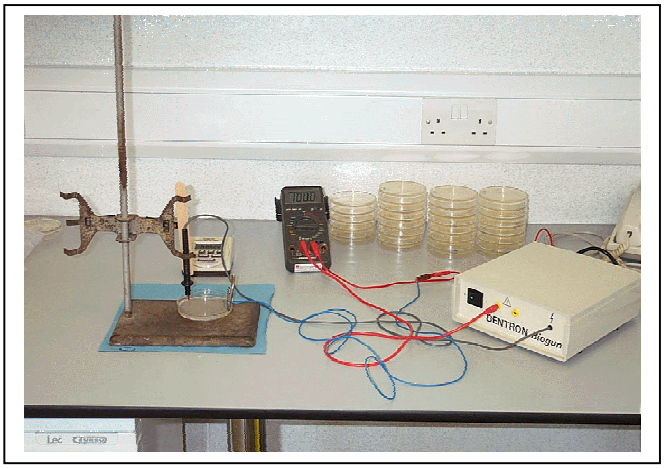
MRSA is a term applied to strains of Staphylococcus aureus, which have become resistant to a range of antibiotics including all of the beta-lactam agents (penicillins, cephalosporins and carbapenems). MRSA are also resistant to the beta-lactamase / beta-lactam combinations such as co-amoxiclavulanate and piperacillin-tazobactam. Staphylococcus aureus is commonly isolated from infected wounds and abscesses. MRSA strains are now widely distributed in UK hospitals.

Materials & Methods

The Biogun was supplied by the manufacturer, Dentron Ltd, and set up as recommended. When in use clinically, the patient and operator are 'earthed' by connecting them using metal wristbands wired to the generator. For these experiments, the agar surfaces being treated were similarly 'earthed' using a wire connector embedded in the agar of each treated plate.

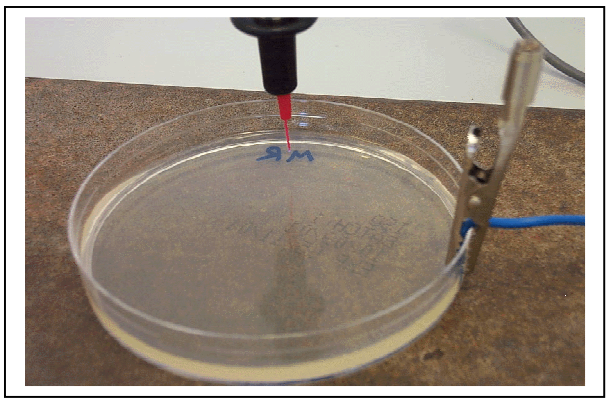
The equipment is shown in photo 1 to the right. The hand piece was held in a clamp, and the distance between each agar surface being treated and the Biogun emitter was measured and set to 6mm, as shown in detail in photo 2. This distance was determined to be optimal, reflecting actual use, where the emitter to surface recommended distance is between 2 – 10 mm.

Photo 1



An ammeter was connected into the circuitry to ensure that a good earth connection had been established with the agar, and that the current was regulated to a steady 100µA.

Photo 2



The agar plates (Oxoid Isosensitest, code CM471) were seeded with four strains of Staphylococcus aureus, as detailed in table A. Two of these were National Type Culture strains and the other two were wild strains isolated from hospital patients. These were grown up overnight in nutrient broth (Oxoid, code CM1), and diluted to give an opacity

equal to a 0.5 McFarland standard. These suspensions were then diluted a further 100-fold to give the working dilutions. Agar plates were dried at room temperature and then seeded with the working dilutions of test organisms using sterile cotton tipped applicator swabs and a rotary plater.

Table 1

A	Staphylococcus aureus	NCTC 6571	(Methicillin sensitive)
B	Staphylococcus aureus	NCTC 10442	(Methicillin resistant)
C	Staphylococcus aureus	Hospital isolate Z26193	(MRSA)
D	Staphylococcus aureus	Hospital isolate Z25806	(MRSA)

The seeded plates were allowed to dry at room temperature for a further 90 minutes before being treated with the Biogun. Each culture plate was then subjected to a timed burst of ionised air , and the test performed in triplicate on three separate areas of the each agar surface. Intervals of 30, 60, 120 and 240 seconds treatment were used in different experiments to gauge how time dependant the effects would be.

After treatment, all plates were incubated at 30^0C for 24 hours to allow the viable organisms to grow, and plates read with a plate microscope to quantify the results.

Results

After incubation, all culture plates showed a semi-confluent growth of organisms as expected. It was evident that after only 30 seconds of treatment with the Biogun some reduction of the numbers of organisms had been achieved, and that number of cells killed increased with time exposure to the negative air ions. Using a plate microscope and a 10mm diameter circular template, the numbers of colonies for each organism were counted within the template area on areas of untreated agar surface, and then each treated area was counted using the same template to determine the effect of the Biogun. Photo 3 shows the three areas of treatment where the bacterial cells have been killed and failed to grow during the post-treatment incubation period.

Photo 3

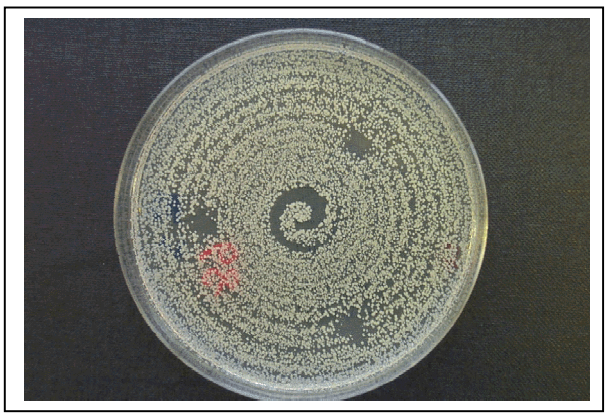


Table 2 gives the actual counts of the numbers of surviving organisms in the treated areas for each time band of treatment.

Table 2

Organism	Inoculum (cfu/template)	Survivors within template after treatment					% reduction
		Test :	1	2	3	Ave.	
A Staphylococus aureus	**330**	30 sec	185	194	201	193	41.5
NCTC ZZ		60 sec	156	147	172	158	52.1
(not MRSA)		120 sec	94	92	108	98	70.3
		240 sec	38	45	46	43	87.0
B Staphylococus aureus	**365**	30 sec	205	226	229	220	39.7
NCTC ZZ		60 sec	177	170	158	168	54.0
(MRSA NCTC strain)		120 sec	111	131	108	117	68.0
		240 sec	52	52	61	55	84.9
C Staphylococus aureus	**425**	30 sec	233	237	242	237	44.2
Wild Hospital strain		60 sec	204	184	200	196	53.9
(MRSA Z26193)		120 sec	136	155	128	140	67.1
		240 sec	48	41	37	42	90.1
D Staphylococus aureus	**405**	30 sec	238	250	260	249	38.5
Wild Hospital strain		60 sec	188	239	219	215	46.9
(MRSA Z25806)		120 sec	165	120	134	140	65.5
		240 sec	47	53	53	51	87.4

Discussion

From the results in table 2, and as has been shown in several other studies, the Biogun is capable of killing bacterial cells within a fairly short exposure time when used under controlled conditions.

Another way of looking at the direct killing effect of the negative air ions is to look at relative differences in total eradication of the staphylococci on each plate in the test area. Using this criterion, all four strains showed very similar results in that after 30 seconds exposure there was no clear zone of total eradication in the treatment area. After 60 seconds exposure, a clear zone of 1.5mm was evident. After 120 seconds this zone had increased to 4mm and after 240 seconds there was a 6mm zone of sterile agar, where all the colony forming units had been rendered incapable of multiplying.

These data highlight the fact that the three strains of MRSA tested behaved in the same manner as the Methicillin sensitive strain of Staphylococcus aureus. This also is in agreement with work on other bacterial species, demonstrating that the effect of the Biogun is seen against a number of different species and genera.

References

Burke F.M. et al. (1995) Use of negative air ion treatment on carious micro-flora. Journal of Dental Research 74, 952.

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Kellogg E.W. et al. (1979) Superoxide involvement in the bactericidal effects of negative air ions on Staphylococcus albus. Nature 281, 400-401

Rosenthal B. et al. (1979) Effects of negative-charged atmosphere on micro-Organisms. J. Applied Bacteriology 46, 451-454.

Shargawi et al. (1999) Negative air ions and Candida albicans. J.App. Microbiol 87, 89-897