CRITICAL REVIEW

completed by Jonathan Copus MA LGSM FInstPI on 15th August, 2002 and updated on 11th February, 2011

Overview

The bactericidal action of negative air ions on Staphylococci and *Escherichia coli* was established by Kreuger *et al* as long ago as 1957[1], and the subsequent relevant literature is extensive. Since 1991, Dentron Limited has commissioned a number of *in vitro* and clinical studies specifically on the Biogun, which demonstrate that the device destroys bacteria (including the 'superbug' MRSA), yeasts and virally-invaded skin cells. In all, 44 strains of micro-organism have been involved in the laboratory experiments and clinical trials, chosen to represent microbes with differing characteristics such as Gram-positive and Gram-negative bacteria, aerobic and anaerobic spore-forming bacteria, yeasts and pseudomonads. The micro-organisms were also selected for their known involvement in a range of dental and dermatological conditions or in food spoilage or food poisoning. Much of this work was supported by a succession of awards from the Department of Trade and Industry, and all of it was carried out in British universities, teaching hospitals or internationally-recognised research institutions. Experiments were also carried out by independent bodies to establish various aspects of the device concerning safety, including electrical safety, ozone emissions and the absence of effect on healthy skin cells, both with regard to their viability and, by Comet assay, to cellular damage. Institutions involved in this research include:

BIBRA International, Carshalton
Institute of Dental Research, London
Institute of Food Research, Reading
Laing Laboratory, Odstock Hospital, Wiltshire
Manchester Royal Infirmary
Public Health Laboratory, Carmarthen
Public Health Laboratory, Porton Down, Wiltshire
Royal College of Surgeons, King's College School of Medicine and Dentistry, London
SGS United Kingdom, Durham
Saint Bartholomew's and The Royal London School of Medicine and Dentistry, London
Southern Counties Scientific Services, Southampton
Surgical Materials Testing Laboratory, Bridgend
University of Manchester

Use of the Biogun in clinical practice is not new. The device has been used by chiropodists, dentists and dermatologists since 1991: reports of success in a wide variety of conditions have been received, and no adverse incidents have been reported.

Claims of efficacy are based on one or more of the following criteria:

a two-year clinical trial on the treatment of dental caries;
three independent clinical studies on the treatment of *verruca pedis*;
a multi-centre clinical study on the treatment of a variety of chiropodial conditions;
two sets of *in vitro* tests on Methicillin resistant *Staphylococcus aureus* (MRSA)*;*
a clinical study using the Biogun to eradicate MRSA from diabetic foot ulcers;
extrapolation from *in vitro* tests coupled with *in vivo* results with similar micro-organisms.

[1] Kreuger AP, Smith RF & Gan Go, I: The action of air ions on bacteria. Journal of General Physiology 1957;**41**:359-381

Literature review: pre-1991

Before Dentron Limited commissioned research specifically on the Biogun, the basic principles of the technology were already well established. Staphylococci and *Escherichia coli* were shown to be susceptible to killing by both negative air ions (NAI) and positive air ions (PAI) by Kreuger *et al,* 1957[1]; Kellogg *et al,* 1979[2]; Kingdon, 1960[3] and Marin *et al,* 1989[4]. Phillips *et al* reported that *Serratia marescens* was killed by NAI but not by PAI[5]; while Pratt and Barnard found that both NAI and PAI had an effect on *Penicillium notatum*[6]. By 1976 a considerable body of literature on the subject had accumulated, which was reviewed by Kreuger and Reed[7]. Although differences between the various series of experiments both in technique and in the parameters measured make direct quantitative comparisons between them difficult, there is sufficient common ground to assert that by 1991 the microbicidal effect of negative air ions was an accepted datum.

Research sponsored by Dentron Limited

In 1991, Dentron Limited began sponsoring a series of laboratory experiments and clinical trials to determine more precisely the effects of a concentrated stream on negatively-charged air particles on specific strains of micro-organism and on specific medical and dental conditions. Sufficient control was thus achieved over the experimental protocols (including the variety of parameters measured) to ensure that reliable comparisons could be made between different series of experiments, while scientific integrity and independence were ensured by engaging the services of institutions and experimenters of recognised standing to draw up the protocols and conduct the experiments.

Research into bacterial killing in animal skin

In two series of experiments conducted by Dr Roy Fitzgeorge of the Public Health Laboratory, Porton Down, Wiltshire, the effect of negative air ion streams produced by the Biogun was investigated using skin from a baboon's outer ear.

In the first series, the skin was smeared with 0.01 ml of an overnight culture of *Staphylococcus aureus.* a circle of skin 1 cm in diameter was cut and attached to a saline-damped card electrically connected to the positive pole of the Biogun. The ion emitter was held at approximately 7 mm from the skin specimen with a current of 40 μA for 7 minutes. The skin was then macerated and the suspension diluted, and viable counts made on blood agar medium. Untreated but similarly infected control specimens were likewise prepared.

In the second series, the same experiments were conducted but the inoculum was injected intradermally into the skin specimens instead of being smeared on the surface.

When the results were compared, the injected specimens showed no significant effect of ion exposure on bacterial count, but negative ion treatment markedly reduced numbers of *Staphylococcus aureus* when these were on the skin surface, resulting in a reduction of approximately 100 times.

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[2] Kellogg EW, Yost MG, Barthakur N & Kreuger, AP: Superoxide involvement in the bactericidal effects of negative air ions on *Staphylococcus albus.* Nature 1979;**281**:400-401

[3] Kingdon KH: Interaction of atmospheric ions with biological material. Physics in Medicine and Biology 1960;**5**:1-10

[4] Marin V, Moretti G & Rassu, M: Effetti della ionizzazione dell' aria su alcuni ceppi batterici. Annali di Igiene, Medicina Preventiva e di Communità 1989;**I**:1491-1501

[5] Phillips G, Harris GJ & Jones MW: Effect of air ions on bacterial aerosols. International Journal of Biometeorology 1960;**8**:27-37

[6] Pratt R & Barnard RW: Some effects of ionised air on *Penicillium notatum.* Journal of the American Pharmaceutical Association 1960;**49**:643-646

[7] Kreuger AP & Reed EJ: Biological impact of small air ions. Science 1976;**193**:1209-1213

Research into safety aspects

If negative air ion streams are lethal to micro-organisms and to virally-invaded skin cells, what is their effect on healthy tissue? This question was investigated by Dr Peter Shakespeare of the Laing Laboratory, Odstock Hospital, Salisbury, and Dr Roy Fitzgeorge of the Public Health Laboratory, Porton Down, Wiltshire. Shakespeare cultured human keratinocytes in 75 cm tissue culture dishes until about half confluent. The medium was decanted and the dishes drained on tissues. A nickel wire was placed in contact with the film of liquid on the surface of the dish and electrically connected to the positive pole of a Biogun, while the negative ion emitter was scanned across the surface at a distance of between 2 and 3 mm. The current was held at 50μA ± 20% and the dishes scanned for 2 minutes and 4 minutes. The cultures were then tested with Trypan blue and showed no uptake, indicating that the exposure to the negative air ions had no effect on the viability of the cells. The dishes were rinsed, re-fed with medium and replaced in the incubator. Growth rate was then assessed in comparison with an untreated control sample: no effect on growth was apparent. The results of these experiments appear elsewhere in the Biogun Technical Construction File.

In the experiments conducted by Dr Roy Fitzgeorge, samples of abdominal human skin were obtained from the Plastic Surgery Unit, Odstock Hospital, Salisbury and cut into circular specimens 5 mm in diameter. These were attached to saline-damped card connected to the positive pole of the Biogun. The ion emitter was scanned across the surface of the specimen at a distance of approximately 7 mm, the current being maintained at 40 μA. The scanning was maintained for 30 seconds in one series and for 7 minutes in a second series. In two further series of experiments, the ion emitter was brought close enough to the skin specimen to form an arc. The current in this case was 60 μA, and the scansion times again 30 seconds and 7 minutes. After treatment, all the skin specimens were placed in neutral Formal Saline and after fixation were processed by normal paraffin wax methodology. Sections cut at 5 μm thickness were mounted on microscope slides and stained by haematoxylin and eosin. Microscopical examination did not reveal any differences between treated and untreated tissue. The results of these experiments appear elsewhere in the Biogun Technical Construction File.

The possibility of more subtle damage to healthy skin cells was investigated in a two-series Comet assay directed by Dr Diana Anderson of BIBRA International at their laboratories in Surrey. Cells were collected from the buccal mucosa of a volunteer by scraping with a wooden spatula from four areas in the mouth, the first being used as the source of untreated, control cells (negative and positive) and the other three providing cells after exposure *in situ* to the Biogun for 2, 4 and 8 minutes. Cells used as the positive control were treated with 20 μM hydrogen peroxide for 30 minutes. The cell samples were tested using a Comet assay with the incorporation of proteinase K (0.5 mg/ml) into the lysing solution to digest the structural proteins surrounding the cells and enabling the release of the DNA. A computerized image analysis system was used to analyse the tail moments and tail lengths of the comets produced. No biologically significant increases were observed at any of the exposure times by comparison with the negative control values under the experimental conditions used.

The results of tests regarding electrical safety, electromagnetic compatibility and ozone emissions appear elsewhere in the Biogun Technical Construction File.

Dental Research

The first work to be done on the Biogun with reference to micro-organisms of dental interest was a series of *in vitro* experiments conducted at the Institute of Dental Research in London under the direction of the Head of the Department of Clinical Pathology and Immunology, Dr (now Professor) Michael Wilson. The conclusion was that a variety of microbes was found to be killed by negative air ions generated by the Biogun following exposure for periods as short as 30 seconds. Susceptible organisms included Gram-positive and Gram-negative bacteria, a pseudomonad, aerobic and anaerobic spore-forming bacilli and *Candida albicans*. The microbicidal effect was

dose-dependent and the extent of killing could be altered by varying the time of exposure, the distance between the microbes and the ion source and the potential difference between the microbes and the negative ion generator. The conclusion was that "bombardment of microbes by negative air ions could prove to be an effective means of treating topical infections and may be useful for disinfecting or sterilising contaminated surfaces". The microbes involved were *Escherichia coli, Staphylococcus aureus, Streptococcus sanguis, Streptococcus mutans, Clostridium perfringens, Actinobacillus, Actinomycetemcomitans, Candida albicans, Pseudomonas aeruginosa,* the *Bacillus* sp. and the *Proteus* sp. These micro-organisms are associated with tooth decay, gum disease, thrush infestations (including angular cheilitis), gangrene and infected burns, bedsores and ulcers. The paper resulting from this research is appended[8], as is the abstract which was published in the Journal of Dental Research[9].

Another centre to undertake research into the dental applications of the Biogun was the Department of Dental Medicine and Surgery, University of Manchester. Here, the work aimed to determine the influence of variation in experimental parameters on the sensitivity of *C. Albicans* to negative air ions, and the role of ozone in this process. Ten strains of *C. Albicans* were exposed to NAIs generated at different emitter distances, exposure times relative humidities and under aerobic and oxygen-free conditions, including an atmosphere of 99.999% pure nitrogen. Significant zones of inhibition were observed in all the resulting cultures, though they were much larger after exposure in air than in nitrogen. The results were published in the Journal of Applied Microbiology[10] and the Journal of Dental Research[11].

The main body of dental research, however, was a two-year study including both *in vitro* experiments and a clinical trial directed by Dr E Lynch, Senior Lecturer in the Department of Conservative Dentistry at Saint Bartholomew's and the Royal London School of Medicine and Dentistry. The microbiological elements were carried out under the direction of Professor D Beighton, Professor of Oral Microbiology, Royal College of Surgeons, Kings College School of Medicine and Dentistry, London. The work was supported by two SMART awards from the Department of Trade and Industry. The conclusion was: "The negative air ion generator (Biogun) exerted a significant effect on the perceived treatment needs of primary root caries lesions. This effect was manifest after 3 and 6 months to a significantly greater extent in the test group compared with the control group."[12] The report itself was not published but several papers concerning the results appeared in a variety of journals[13,14,15,16,17,18,19,20,21,22,23,24].

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[8] Copus J, Cousins D and Wilson M: Killing of micro-organisms by negative air ions (unpublished) 1991.
[9] Cousins D, Copus J & Wilson M: Microbicidal effects of negative air ions. J Dent Res 1991;**70**:709,315
[10] Shargawi JM, Theaker ED, Drucker DB, MacFarlane T and Duxbury AJ: Sensitivity of *Candida Albicans* to negative air ion streams
[11] Shargawi JM, Drucker DB & Duxbury AJ: Effect of negative air ion (NAI) streams on *Candida albicans.* J Dent Res 1995; **74(3)**:887
[12] Lynch E & Beighton D: Report on the negative air ion generator ("Biogun") (unpublished)
[13] Burke FM, Lynch E, Beighton D & Ludford R: Effect of negative air ions on *Actinomyces naeslundii.* Caries Research 1995;**29**:296,16
[14] Burke FM, Johnson ND, Samarawickrama DYD, Beighton D & Lynch E: Use of negative air ion treatment on dentine caries microflora. Presentation, Irish Division of the International Association for Dental Research, Dublin, May 1994
[15] Burke FM, Lynch E, Ludford R and Beighton D: Negative air ion effect on *Candida albicans* from root-carious lesions *in vivo.* Presentation, Irish Division of the International Association for Dental Research, Sligo, Ireland, May 1996
[16] Burke FM, Samarawickrama, DYD, Johnson ND, Beighton D and Lynch E: Use of negative air ion treatment on carious microflora. J Dent Res 1995;**74**:952,#28
[17] Burke FM, Lynch E, Beighton D & Ludford R: Effect of negative air ions on the viability of *Candida albicans* from primary root caries lesions. Abstract #5, Irish Division of the International Association for Dental Research, Belfast, June 1995
[18] Burke FM, Lynch E, Beighton D & Ludford R: Effect of negative air ions on *Actinomyces naeslundii.* Gerodontology 1995;**12**;113,#15

Research into chiropodial/podiatric applications

Three separate clinical trials have been conducted by qualified chiropodists into the effectiveness of the Biogun in treating verrucae and a broad-spectrum multi-centre study has been carried out of the technique's efficacy in a variety of chiropodial conditions including verrucae.

The first trial[25], conducted in 1993 by Derrick Stephens, MSSCh, MBChA, MRPhamS, Dip Cryosurg, involved 30 patients with a total of 43 verrucae (excluding two patients who attended only once). The purpose of the study was to investigate the synergistic action of the Biogun with a variety of traditional chemical treatments. Of the 43 verrucae, 36 were cured, 1 improved, and the treatment failed in 6 cases (cured: 83.72%; improved: 2.33%; failed: 13.95%). The full text of the paper is given elsewhere in the Biogun Technical Construction File.

Two years later a second trial[26] was conducted by Clive Vernon, MSSCh, MBChA involving 207 patients, using the Biogun in conjunction with a keratolytic. Excluding the 3 patients who did not complete treatment the remainder were all reported cured. The resulting paper is reproduced in full elsewhere in the Biogun Technical Construction File.

The same researcher conducted another study in 2000[27], again using his preferred method of combining Biogun treatment of *verruca pedis* with the use of a keratolytic to disperse the resultant hard tissue. Of the 121 patients involved in the trial, all were cured. The results appear elsewhere in the Biogun Technical Construction File.

Dentron itself began collating case studies from practising chiropodists soon after the launch of the Biogun in 1991. The results of this multi-centre clinical study on the treatment of a variety of chiropodial conditions are given in full elsewhere in the Biogun Technical Construction File. In summary, however, the Biogun achieved a 100% cure rate in cases of *verruca pedis,* other warts, *tinea pedis,* fungal nail infections, inflamed onychocryptosis, onycholysis, septic heloma, streptococcal infection of the foot, infected bedsores, and acne, a 75% success rate against onychomycosis and a 60% success rate against leg ulcers. The results from the multi-centre clinical study were assessed by a senior chiropodist, namely Mr Michael Paynton, Chairman of the Council of the British Chiropody Association.

Since the last edition of this Review, work has been carried out using the Biogun against Methicillin resistant *Staphylococcus aureus* (MRSA), both in Petri dishes and in diabetic foot ulcers. The laboratory work was conducted by the Public Health Laboratory Service in Carmarthen and at the Surgical Materials Testing Laboratory, Princess of Wales Hospital, Bridgend, and the

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[19] Burke FM, Lynch E, Ludford R & Beighton D: Negative air ion effect on the viability of mutans streptococci isolated from active primary root-caries. Presentation, ORCA Congress, Aarhus, Denmark, July 1996

[20] Burke FM, Lynch E, Ludford R & Beighton D: Prolonged negative air ion effect on *Candida albicans* from root-caries. Presentation, European College of Gerodontology and Scandinavian and Central European Division of the International Association for Dental Research, Berlin, Germany, September 1996

[21] Burke FM, Lynch E, Beighton D & Ludford R: Negative air ion effect on micro-organisms from root caries lesions *in vivo*. Poster presentation, British Association of Teachers of Conservative Dentistry, Belfast, July 1995

[22] Burke FM, Lynch E, Beighton D & Ludford R: Negative air ion effect on mutans streptococci in primary root-caries. Presentation, International Association for Dental Research, San Francisco, USA, March 1996

[23] Burke FM, Lynch E, Ludford R & Beighton D: Negative air ion effect on lactobacilli from root caries lesions *in vivo.* Presentation, British Society for Dental Research, Bristol, England, April 1996

[24] Burke FM, Lynch E, Beighton D & Ludford R: Effect of negative air ions on the viability of *Actinomyces naeslundii* genospecies 1 from primary root caries lesions. Poster presentation, ORCA meeting, Noordwijkerhout, the Netherlands, July 1995

[25] Stephens DG: Biogun versus verruca. SMAE Journal, Winter 1993: 10-13

[26] Vernon CJ: Three years' experience with the Biogun. SMAE Journal, Winter 1995

[27] Vernon CJ: Long term experience of the Dentron Biogun in *Verruca pedis,* mosaic verrucae and hand warts.

clinical study was conducted at Manchester Royal Infirmary. Reports of all three activities is included in this Technical Construction File, but the results may be summarised as follows: the Biogun was successful in killing MRSA both in the laboratory, where clear zones of inhibition were consistently obtained with exposures ≥ 60 seconds, and in small diabetic foot ulcers (average diameter 19 mm), although eradication of MRSA was less successful in larger diabetic foot ulcers (average diameter 33 mm).

Research into Food Preservation

Dentron Limited received two SMART awards from the Department of Trade and Industry and one from the Welsh Development Agency for developing the Biogun as a medical device, and a third SMART award for investigating the potential of the technology in the field of food preservation. Because this research, carried out at the Institute of Food Research in Reading, revealed further micro-organisms susceptible to Biogun action (including *E Coli* H:O157) and also confirmed that the Biogun was effective on a wide range of electrically-conductive physiological tissue by conducting experiments on sliced poultry. The final report of this work is reproduced elsewhere in the Biogun Technical Construction File.

Conclusion

There is thus abundant clinical evidence of the efficacy of the Biogun involving a variety of sites and types of condition, from primary root caries in the mouth to verrucae on the foot. There is also a wealth of *in vitro* evidence of the ability of the device to destroy a wide range of micro-organisms of varying types, largely chosen for their representative nature (see below). No resistant strains have been found, and in the light of the physical nature of the mechanism it is inherently unlikely that resistance could develop. A knowledge of the physics involved suggests that the method is unlikely to be effective in the case of surfaces which are electrically non-conductive; but it is reasonable to conclude from the evidence that it is likely to succeed in the case of electrically-conductive surfaces, a class which includes skin and most other mammalian physiological tissue in the living state. But caution is still necessary because of the varying nature of the individual sites (primarily in terms of the degree of convolution) and the behaviour of the organism concerned: for instance, it would be unreasonable to predict success in the case of an organism known to prefer an intradermal habitat, in the light of the Fitzgeorge experiments with *S aureus* injected into skin from a baboon's ear. There are circumstances, though, in which projection of success can be justified. For example, it is known that the Biogun kills *Pseudomonas aeruginosa* when the organism is exposed *in vitro. P aeruginosa* is the predominant micro-organism infecting bedsores. So far, the evidence is not of itself sufficient to justify a claim that the device will treat infected bedsores; but when the fact is added that infected leg ulcers, an example of a very similar site, have been successfully treated, it is valid to conclude that success in the treatment of infected bedsores may reasonably be expected. Similar extrapolations are commonplace in drug testing. A wide-spectrum antibiotic, for instance, is not tested on every individual member of the world's population against every known microbe; it is tested against a range of representative micro-organisms and using a variety of typical patients, and from these data conclusions are drawn about the drug's performance against similarly-classified micro-organisms in patients of similar sex, age and physical condition.

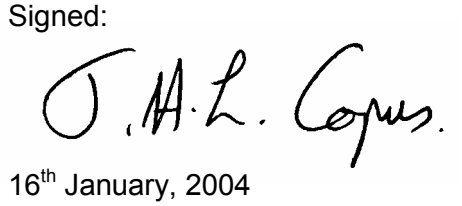
From the foregoing evidence, therefore, it is valid to conclude in general that the Biogun is an effective microbicide when used on electrically-conductive surfaces, and more particularly has a wide-spectrum biocidal effect in the case of a large variety of skin infections. Thus while its efficacy is demonstrable in those conditions where specific clinical data are available (most especially in the case of primary dental root caries) its potential applications include all areas involving surface infections, where its success may be predicted with an evidentially based degree of certainty.

The Biogun is truly at the cutting edge of many aspects of medicine and dentistry; but the underlying science dates back at least to 1957, and the application in its present form has been in clinical use since 1991. Claims of efficacy in dentistry are backed by full-scale clinical trials, in

chiropody/podiatry by clinical trails and a longitudinal multi-centre study, and in other areas of medicine by a combination of *in vivo* and *in vitro* data. The latest news that the Biogun is effective against resistant strains of *Staphylococcus aureus* both *in vitro* an *in vivo,* though not yet fully supported by full-scale clinical trials, nevertheless provides a firm foundation for confidence that the electronic microbicide will earn its place among the most significant advances of the twenty-first century.

Between 20th October, 1993 and 14th August, 2002, 420 Bioguns were sold for clinical use.

Signed:



16th January, 2004

CONDITIONS AGAINST WHICH EFFICACY IS CLAIMED

carious dental lesions
periodontitis
gingivitis
mouth ulcers
candidiasis, including angular cheilitis
verrucae
athlete's foot
Infected leg ulcers
hypergranulation
porokeratosis
infected onychocryptosis
onychomycosis
hand warts
exudative venous ulcers

MICRO-ORGANISMS KILLED BY THE BIOGUN *IN VITRO*

Acinetobacter sp.
Actinomyces actinomycetemcomitans
Actinomyces naeslundii genospecies 1
Actinomyces naeslundii genospecies 2
Actinomyces georgiae
Actinomyces gerensceriae
Actinomyces isrealii
Bacillus cereus
Bacillus sp.
Brochothrix thermosphacta
Campylobacter jejuni
Candida albicans
Carnobacterium piscicola
Clostridium perfringens
Clostridium sporogenes
Enterobacter agglomerans

Escherichia coli (non-pathogenic)
Escherichia coli O157:H7
Klebsiella pneumoniae
Lactobacillus sp.
Leuconostoc gelidum
Listeria monocytogenes
Methicillin resistant Staphylococcus aureus
Morganella morganii
Pichia membranaefaciens
Pichia anomala
Proteus sp.
Pseudomonas aeruginosa
Pseudomonas putida
Psychrobacter immobilis
Saccharomyces cerevisiae
Saccharomyces exogenes
Saccharomyces oviformis
Salmonella
Salmonella enteriditis
Shewanella putrefaciens
Staphylococcus aureus
Staphylococcus sanguis
Streptococcus mutans
Tinea
Zygosaccharomyces baillii
Zygosaccharomyces rouxii

Research supporting these findings was carried out at:

Institute of Dental Research, London
Institute of Food Research, Reading
Public Health Laboratory, Carmarthen
Public Health Laboratory, Porton Down, Wiltshire
Royal College of Surgeons, King's College School of Medicine and Dentistry, London
Saint Bartholomew's and The Royal London School of Medicine and Dentistry, London
Surgical Materials Testing Laboratory, Bridgend