Report on the Negative Air Ion Generator ("Biogun")

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INTRODUCTION

A negative air ion generator, known as the "Biogun", can deliver a concentrated stream of negative air ions. The generator acts by applying a high direct voltage to an electrode known as an emitter mounted in a probe. A stream of electrons is generated which bond to surrounding molecules of Oxygen forming the hydrated superoxide anion $(O_2^-)(H_2O)_n$ where n=4-8. This is understood to act as a nucleophile on the phospholipid bilayer which causes a de-esterification of the fatty acids and weakening the membrane of unicellular micro-organisms (Kellogg et al. 1979).

The negative air ion system has been shown to be effective in inhibiting the growth of micro-organisms implicated in the aetiology of dental caries and primary root caries in particular (Cousins et al. 1991, Burke et al. 1995a, Burke et al. 1995b) *in vitro*. Its efficacy has yet to be assessed *in vivo*.

AIM

The aim of this study was to determine the efficacy of negative air ions to kill the micro-organisms implicated in the aetiology of primary root caries *in vivo* and to evaluate the clinical effects of exposure to negative air ions.

MATERIALS AND METHODS

Study Population

The study population was selected from regular dental patients of the St Bartholomew's and the Royal London School of Medicine and Dentistry. Patients with primary root caries lesions were selected. The procedure to be carried out was explained and written consent obtained.

The criteria for inclusion in the study are that the participants were:

(a) be aged over 35 years

(b) have one primary root caries lesion

(c) be considered reliable for attendance for treatment

(d) have given written, informed consent

Ethical Approval

Consent was sought and received from the ethics committee City and East London Health Authority to carry out an *in vivo* study involving the NAI system.

Withdrawal or exclusion from the study

Patients may withdraw or be withdrawn from the study at any time for the following reasons:

(a) at the expressed wish of the participant who will not be required to give a reason.

(b) as a result of changes in the patient's domestic situation which renders impractical attendance for treatment.

(c) taking of an antibiotic or an antimicrobial mouthwash in the four weeks preceding the study.

Diagnostic Criteria

The following parameters was recorded at the dental examination:

1) Coronal caries (DMFT) according to WHO criteria (1987)

2) Partial denture wearing status.

3) Colour: Photographs of primary root caries were obtained and a four shade guide was developed. The colours were; yellow, light brown, dark brown and black.

They were used as the standard when determining the colour of each lesion under investigation.

4) Dimensions: A periodontal probe marked at 1 mm intervals was used to determine the dimensions of each lesion. The maximum mesio/distal or bucco/palatal (width) and occluso-gingival (height) dimension was assessed. The product of these two values was used as an indicator of the size of the lesion. Furthermore the minimum distance from the gingival margin of the lesion and the crest of the gingiva itself was measured.

An estimate of the amount of dentine that has been lost, cavitation or maximum loss of surface contour, was made by recording the greatest distance between the existing surface of the lesion and what was judged to have been the original root surface.

5)Texture: Three categories for the texture of each carious lesion were defined:

'Hard' the caries was comparable to the remaining sound root dentine.

'Leathery' caries will permit the penetration by a new No. 6 probe (Claudius Ash Sons & Co Limited, Potters Bar, Herts, UK) of the root tissue under moderate pressure and there was some resistance to withdrawal.

'Soft' lesions will permit a new No. 6 probe to penetrate the root tissue with ease and with no resistance to withdrawal.

6) Perceived Treatment Need: This was defined relative to their clinical signs (Beighton et al 1993):

Leathery Debride: Leathery lesions that were judged to be shallow and the surface of the exposed sound dentine easily maintained plaque free.
Leathery Restore: all leathery lesions judged to be on root surfaces that were difficult to maintain plaque free.
Soft Restore: All soft lesions.

Samples for Microbial Analysis

The primary root caries lesions were sampled before and after application of the negative air ions. The primary root caries lesions were first be cleansed to remove plaque and any other material that might contaminate the sample of carious dentine. A hand held standard fine nylon fibre sterile toothbrush was used with sterile water as a lubricant to cleanse the surface but to avoid the removal of any carious surface dentine the surface were dried using a 3-in-1 syringe. A preimpression sample was taken. An impression of each lesion will then be taken using an addition-cured silicone (Extrude putty, Kerr, USA) by the standardised method which we have developed. The tooth will then be isolated using sterile cotton wool rolls and dried using a 3-in-1 syringe and a dry sterile cotton wool roll. A new size 2 stainless steel rosehead bur in a ten-in-one reduction head in a slow speed handpiece was used to remove the sample of carious dentine. Pressure comparable with that used in normal clinical practice was used. Each biopsy was then immediately placed in 1 ml of Fastidious Anaerobe Broth (FAB) (LabM, Bury Lancs, UK) and forwarded to the laboratory at King's College immediately for bacteriological investigation.

The Negative Air Ion System

A negative air ion generator, known as the "Biogun", can deliver a concentrated stream of negative air ions. The generator acts by applying a high direct voltage to an electrode known as an emitter mounted in a probe. The negative air ions have been shown to inhibit the growth of micro-organisms.

The negative air ion generator generates up to 13,000 volts, but the current generated is 80 μA. This is less than one 150th of the lower limit set by the British Standards Institution below which no harmful effects on the

human body have been detected. A further safety factor is that the output voltage of the device falls sharply when significant current is drawn. Applying the emitter directly to the skin causes the voltage to drop almost instantaneously to zero.

The negative air ion system has been tested by the British Chiropody Association. No adverse effects have been reported and it has been shown to be effective in the management of verruccae, athlete's foot and hypergranulation (Lyall 1992, Stephens 1993).

A stream of negative air ions was applied to each primary root caries lesion. The patient was earthed by wearing a wrist band with a metal button. The tip of the negative air ion generator was held at a distance of 5 mm from the surface of the lesion and a current of 60 μA applied for two minutes. The tip was moved slowly backwards and forwards over the area of the carious lesion to be treated. A further sample will then be taken from using the same technique as before the application of the negative air ions. The sample site was from an area distal to the initial sample site. The negative air ions were applied for a further two minutes and another sample was taken from another site distal to the previous sample site.

Each sample was then immediately placed in 1 ml of Fastidious Anaerobe Broth (FAB) (LabM, Bury Lancs, UK) and forwarded to the laboratory within 30 minutes for bacteriological investigation. A further impression of each lesion was taken using an addition-cured silicone (Extrude putty, Kerr, USA). Given the sequence of sampling the pre-treatment site was the most mesially located site and the site treated for four minutes was the one located furthest distally.

Laboratory Methods

Sample Processing: To each 1 ml of FAB containing a biopsy of carious or negative air ion treated carious dentine sterile 3.5 to 4.5 mm diameter glass beads (BDH Limited, Poole, Dorset, UK) was added. They were vortexed for 15 seconds to facilitate the extraction of any micro-organisms from the carious dentine and disperse any aggregates. Then decimal dilution with FAB, 100 ml aliquots of these being spread as appropriate onto a range of culture media in plates.

Mutans Streptococci: (*S mutans* and *S sobrinus*) were enumerated on Mitis-Salivarius Agar (Difco Laboratories, Teddington, Surrey, UK) supplemented with 0.2 units per ml bacitracin and 15% (W/V) sucrose, MSB (Gold et al. 1973) in an anaerobic chamber (Don Whitley, Shipley, West Yorkshire, UK) at 37^{O}C for three days. The total number of organisms in each sample were determined by counting the colonies identified on each plate. Their identities were confirmed by subjecting up to five of these colonies to further examination by using a set of fermentation and enzymic tests. Tests for the production of acid from N-acetylglucosamine, arbutin and melibose, as well as the presence of α-galactosidase and α-glucosidase activities were useful in differentiating these species (Beighton et al. 1991).

Lactobacilli: were grown on Lactobacillus Selective Agar LBS (Oxoid Limited, Basingstoke, Hampshire, UK) in an anaerobic chamber (Don Whitley, Shipley, West Yorkshire, UK) at 37^{O}C for three days. The total number of organisms in each sample was determined by counting the typical colonies grown. They were confirmed as Gram-positive, catalase-negative and unbranched rods (Beighton et al. 1991b).

Yeasts: were grown on Sabouraud Dextrose Agar (Oxoid Limited, Basingstoke, Hampshire, UK) incubated in air at 37^{O}C for two days. The number of organisms were determined by counting the number of typical colonies on each plate. They were confirmed as large, ovoid Gram-positive, catalase positive cells (Beighton et al. 1991b).

Gram-positive pleomorphic rods (GPPRs): were grown of Fastidious Anaerobic Agar (LabM, Bury, Lancs, UK) supplemented with 5% (V/V) horse

blood (FAA, LabM) in an anaerobic chamber (Don Whitley, Shipley, West Yorkshire, UK) at 37^{o}C for seven days. The number of organisms including *Actinomyces spp* were confirmed by examination of the Gram-stained smears of colonies on the Fastidious Anaerobic Agar plates. The number of each colony type was counted and representatives of each examined so that the numbers of Gram-positive pleomorphic rods in each sample could be calculated. Because of the difficulties associated with the speciation of the Gram-positive pleomorphic rods, especially the identification of *Actinomyces spp* (Johnson et al. 1990) all Gram-positive pleomorphic rods were grouped together as a single taxon. From these plates the total numbers of all colony forming units (cfu) were also determined.

The detection limit for mutans streptococci, yeasts and lactobacilli is 10 cfu per sample, and for the GPPR it is approximately 0.2% of the number of cfu present in a sample. If no colonies of a given taxon were recovered from a sample a value of zero was included in the analysis.

Sample Volume

The impressions of the sample sites were scanned using a co-ordinate measuring machine. It was possible to superimpose the scans of the lesions before and after sample taking and so determine the volume, site and dimensions of the samples taken.

Data Analysis

The total number of colony forming units (cfu) per sample biopsy were determined by translating the numbers of cfu grown on the FAA plates through the dilutions that were deployed to relate them to the total sample. These were transformed to $\log_{10}$(colony count +1) to normalise the distributions of the individual colony counts. The numbers of each of the various categories of organisms per sample were determined in the same way and the proportions of all organisms in each sample also expressed as a percentage of the total colony count on the FAA plates. Means and standard errors of values will also be calculated, mean values being calculated by one way analysis of variance using Duncan's multiple range test, and distributions

were analysed by the Chi squared statistic(s). The frequency of isolation of individual taxa from each primary root caries lesion were recorded as zero if fewer than 10 cfu of that particular taxon are recovered from the biopsy. All statistical analyses were performed with the statistical suite of programmes: SPSS/PC + V3.0 (SPSS Inc., Chicago, Illinois, USA)

RESULTS

The demographic data pertaining to the subjects enrolled in the study and those representing for examination at 3/12 or 6/12 months are shown in Table 1. The distribution of the types of surfaces sampled in the Test and Control groups is shown in Table 2. While the proportion of lesions examined in each group categorised as to whether they were abutment lesions is shown in Table 3. The two groups for these data are overall very similar expect that the proportion of abutment lesions was greater in the Test group than in the Control group. However this would exacerbate against demonstrating a positive effect with the Biogun

Sample Dimensions

The samples of carious tissue were taken from the gingival margin of the primary root carious lesions with the first sample being the most mesially located with further samples being located progressively further distally. The average dimensions of the samples of carious tissue were 0.583 mm width, 0.794 mm length and 0.152 mm depth. The average volume was 0.137 mm^3. The consistency of the volumes of dentine removed indicate that the standardised sampling procedure enabled the removal of very similar volumes of dentine and confirm that, overall, the sampling procedure was valid.

Table 1. Demographic profile of study group

Group	Lesions	Patients	Gender	Age	
				Mean	**(Range)**
Control	54	16	M 11	58.7	(20-75)
			F 5	60.6	(46-69)
			All	59.4	(20-75)
Control 3/12	34	12	M 9	61.7	(58-75)
			F 3	62.7	(59-67)
			All	61.9	(58-75)
Control 6/12	15	5	M 3	63.7	(58-75)
			F 2	60.5	(59-62)
			All	62.4	(58-75)
Test	50	23	M 8	64.3	(58-72)
			F 15	59.9	(39-85)
			All	61.4	(39-85)
Test 3/12	36	17	M 6	64.5	(65-72)
			F 11	65.8	(47-85)
			All	65.4	(47-85)
Test 6/12	30	12	M 5	65.2	(58-72)
			F 7	68.7	(46-82)
			All	67.3	(46-82)

Table 2 Surfaces Studied (%)

	Control	Test
Buccal	19 (37)	10 (20)
Distal	7 (14)	13 (26)
Labial	4 (8)	8 (16)
Lingual	9 (18)	6 (12)
Mesial	10 (20)	6 (12)
Palatal	2 (4)	7 (14)

Table 3 Abutment Lesions (%)

	Control	Test
Number of subjects	20 (40)	31 (62)

Microbial data.

The microbiological data are summarised in Table 4. The reductions in the numbers of yeasts, lactobacilli, mutans streptococci, Actinomyces (GPPR) and total microbial count were generally similar in both groups and after 3 and 6 months the microbial recoveries were not different from the samples taken at baseline.

Table 4. Microbial data

The data are for mean $Log_{10}(CFU+1)$ ±(SE) of the microflora involved.

	Baseline	2 mins NAI	4 mins NAI	3 month	6 month
Yeast					
Control	1.69 (0.29)	0.53 (0.27)	0.78 (0.38)	2.24 (0.20)	1.18 (0.14)
Test	2.54 (0.16)	1.50 (0.24)	0.94 (0.22)	1.86 (0.03)	1.56 (0.42)
Lactobacilli					
Control	2.61 (0.24)	1.97 (0.34)	1.66 (0.35)	2.29 (0.49)	3.18 (0.76)
Test	2.98 (0.26)	1.75 (0.31)	1.20 (0.29)	2.27 (0.42)	1.51 (0.37)
Mutans streptococci					
Control	2.81 (0.20)	1.71 (0.33)	1.12 (0.29)	3.35 (0.34)	2.36 (0.32)
Test	3.13 (0.23)	1.93 (0.30)	1.23 (0.27)	2.22 (0.32)	2.60 (0.40)
Actinomyces					
Control	2.59 (0.22)	1.46 (0.32)	0.93 (0.31)	2.43 (0.19)	1.68 (0.20)
Test	3.65 (0.23)	2.25 (0.29)	1.36 (0.28)	2.25 (0.53)	1.60 (0.49)
Total M-O					
Control	4.37 (0.21)	3.85 (0.23)	3.56 (0.26)	5.23 (0.18)	4.84 (0.28)
Test	5.27 (0.19)	4.15 (0.23)	3.25 (0.26)	5.56 (0.18)	4.57 (0.31)

Clinical Effect of Treatment

The carious lesions were categorised according to the Perceived Treatment Need and the distribution of these lesion types in both groups after 3 months (3/12) and 6 months (6/12) are shown in Table 5. The distribution of lesion types between groups was such that there were slightly more soft restore lesions in the Test group.

Table 5. Treatment need categorisation of primary root carious lesions

Control

	Baseline	3/12	6/12
Leathery Debride	26	15	6
Leathery Restore	16	13	6
Soft Restore	8	6	3

Test

	Baseline	3/12	6/12
Leathery Debride	17	15	12
Leathery Restore	17	10	8
Soft Restore	16	11	10

The changes in the clinical status of the perceived treatment needs of the lesions in the study is shown in Table 6. There was a significantly better clinical improvement in the test group compared to the control group. It can be seen that after three months while three of the lesions in the control group (8.8%) improved clinically fifteen of the lesions in the test group (41.7%) improved. Ten of the lesions in the control group (29.4%) got worse clinically after three months while only two of the test lesions (5.6%) disimproved.

After six months while two of the lesions in the control group (13.3%) improved clinically fifteen of the lesions in the test group (50.0%) improved. Seven of the lesions in the control group (46.7%) got worse clinically after three months while only three of the test lesions (10.0%) got worse.

Table 6. Change in treatment need of primary root carious lesions

	Control	**Test**				
	Improved		**Same**		**Worse**	
3 Months						
Leathery Debride	**-**	**-**	8	**13**	7	**2**
Leathery Restore	2	**5**	8	**5**	3	**0**
Soft Restore	1	**10**	5	**1**		
6 Months						
Leathery Debride	**-**	**-**	1	**10**	5	**2**
Leathery Restore	1	**5**	3	**2**	2	**1**
Soft Restore	1	**10**	2	**0**		

Analysis of the data using Chi^2 analysis demonstrated that there was some significant improvement in the Test group compared to the control group at 3 months and also at 6 months, particularly in the lesions designated soft restore. Comparison of the leathery lesions indicated no significant difference at 3 months ($\chi^2 = 2.54$; ns) but at 6 months the difference was significant ($\chi^2 = 4.94$; p=0.026). For the leathery restore lesions the differences at 3 and 6 months were not significantly different ($\chi^2 = 4.67$ and $\chi^2 = 2.97$, respectively).

However for the soft restore lesions there was a significant difference between test and control at 3 months (χ^2 = 6.4; p=0.011) while at 6 months the difference approached significance (χ^2 = 3.59; p=0.058).

Consideration of the treatment data as a whole indicates that 15 of 36 lesions in the Test group improved while 3 of 34 lesions in the Control group improved at 3 months while at 6 months 15 of 30 lesions in the test group exhibited improvement and only 2 of 15 in the Control group. Analysis of the distribution of these data indicate that the effect of exposure to the negative air ions was significant: (χ^2 = 13.29; p=0.0012 and χ^2 = 9.61; p=0.0082 at the 3 and 6 month interval, respectively).

Effect of NAI on the Colour of Lesions.

The colour of the lesions included in the study at baseline and at the subsequent 3 and 6 months recall are shown in Table 7. The changes in colour; lesions getting lighter or lesions getting darker are shown in Table 8.

Table 7. Colour of primary root carious lesions throughout the study period.

Control

	Baseline	**3/12**	**6/12**
Black	16	3	0
Dark Brown	21	16	8
Light Brown	14	14	7
Yellow	3	1	1

Test

	Baseline	**3/12**	**6/12**
Black	14	16	3
Dark Brown	14	13	13
Light Brown	20	14	14
Yellow	2	3	0

Table 8. Change in colour of primary root carious lesions

	Control	**Test**				
Initial colour	**Lighter**		**Same**		**Darker**	
3 Months						
Black	8	**3**	1	**5**	0	**0**
Dark Brown	5	**3**	10	**6**	0	**1**
Light Brown	0	**2**	7	**11**	1	**3**
Yellow	0	**0**	1	**1**	1	**0**
6 Months						
Black	2	**4**	0	**3**	0	**0**
Dark Brown	3	**2**	5	**7**	0	**0**
Light Brown	0	**0**	5	**11**	1	**1**
Yellow	0	**0**	0	**0**	2	**2**

There was no significant change in the appearance of the lesions when colour alone was considered.

Influence of NAI on the size of root caries lesions.

The size of the lesions at baseline and at the subsequent sampling times are shown in Table 9 while the changes in the size distribution of the lesions are shown in Table 10.

Table 9. Size of primary root carious lesions (mm^2) at baseline, 3 and 6 months following exposure to negative air ions.

Control

	Baseline	**3/12**	**6/12**
<4	16	10	5
4-7	19	12	3
7>	19	12	7

Test

	Baseline	**3/12**	**6/12**
<4	2	8	9
4-7	21	19	12
7>	24	9	8

Table 10. Change in size of primary root carious lesions

	Control **Smaller**	**Test**	Control **Same**	**Test**	Control **Larger**	**Test**
3 Months	6	**17**	15	**7**	13	**12**
6 Months	4	**17**	5	**9**	6	**4**

A significantly greater proportion of the Test lesions exhibited a decrease in size compared to the control group at 3 months (χ^2 = 8.16; p=0.0169) but this difference was no longer demonstrable after 6 months (χ^2 = 5.16; p=0.076)

Influence of the NAI on the cavitation of root caries lesions.

The cavitation of lesions at baseline, 3 and 6 months following exposure to the negative air ions is shown in Table 11 while the changes in the measured cavitation of lesions is shown in Table 12.

Table 11. Cavitation of primary root carious lesions (mm)

Control

	Baseline	**3/12**	**6/12**
<0.5	16	4	0
0.5-<1.5	34	30	9
1.5-<2.5	3	1	2
2.5>	1	1	2

Test

	Baseline	**3/12**	**6/12**
<0.5	6	4	6
0.5-<1.5	36	28	22
1.5-<2.5	6	3	2
2.5>	2	1	0

Table 12. Change in cavitation of primary root carious lesions

	Control	**Test**				
	Shallower		**Same**		**Deeper**	
3 Months	4	**12**	22	**16**	10	**8**
6 Months	1	**9**	4	**14**	8	**7**

At 3 months the changes in the cavitation approached significance (χ^2 = 5.17; p=0.07) but was not significant after 6 months. Comparing the data at 3 months as those lesions which became shallower with the number that remained the same or got deeper the Test group was significantly better (χ^2 = 3.94; p=0.047)

Influence of Negative air ions on the distance of the lesions from the gingival margin.

These data are summarised in Table 13 and the change in the distance of lesions is shown in Table 14.

There was no significant effect of exposure of the negative air ions on the distance of the lesions from the gingival margin after 3 and 6 months following treatment. The χ^2 values were 3.56 (p=0.169) and 0.19 (p=0.91), respectively.

Table 13. Distance of primary root carious lesion relative to gingival margin (mm)

Control

	Baseline	**3/12**	**6/12**
0.0	18	12	2
0.5-1.0	14	7	2
1.5-2.0	13	10	4
2.5-3.0	7	2	3
3.5>	2	3	4

Test

	Baseline	**3/12**	**6/12**
0.0	13	11	5
0.5-1.0	23	11	13
1.5-2.0	10	12	9
2.5-3.0	4	1	1
3.5>	0	1	1

Table 14. Change in distance of primary root carious lesion relative to gingival margin

	Control	**Test**					
	Closer		**Same**		**Further**		
3 Months	8	**3**	21	**24**	5	**9**	
6 Months	3	**5**	6	**14**	6	**11**	

Current Applied

The current applied was determined by the patients themselves. The emitter tip was held at approximately 5 mm from the surface of the lesion and the patient advised to raise their hand if they felt any discomfort. The emitter tip was moved accordingly. The average current applied for the lesions in the test group was 41.7 μA, (range 24-80μA). The patients in the control group experienced a current of 0.0 μA. The fact that the patients could control the degree of sensation they felt enhanced their sense of empowerment and confidence in the system.

Patients Perception of discomfort with NAI treatment

A total of 43 patients were deemed to be suitable for the study, 39 agreed to participate, 3 declined to participate and one failed to participate by not attending for his appointments.

Of the patients who under took the study two patients, one in each the test and control groups requested that local anaesthetic be administered during the course of the procedure.

A ten point pain scale was developed with 1 being described as “”very comfortable” and 10 being ”extremely painful”. The patients were asked to score the procedure on this scale. The results are shown in Table 15.

Table 15. Pain perception with "Biogun"

	Pain Score			
Group	Mean	S.D.	Range	Frequency of 1 (%)
Control	1.39	1.35	1-7	91
Test	2.14	1.52	1-7	50

Clearly patients on whom the Biogun was used found the experience slightly more uncomfortable than the patients in the Control group. However the patients accepted the Biogun and did not find the use of the Biogun too disagreeable.

General comment regarding the use of the "Biogun"

The "Biogun" proved to be a durable instrument. Over the period of the study the emitter probe handle became detached from lead the once. The emitter tips were autoclaved repeatedly without any obvious deterioration. The emitter probe did have a propensity to roll around due to the circular shape of the collar, it was suggested that a "D" shaped collar would be more stable. It proved to be very easy to transport the "Biogun" to different clinics in the hospital. A carrying pouch or case for the wrist bands and leads, emitter probe and tips would have been of use.

Safety

Work on the effect of the "Biogun" on cells and skin tissue has been carried out by Fitzgeorge and Shakespeare however this has not been published. In order to allay potential anxieties regarding the systemic safety of the "Biogun" it would be of benefit if this work was followed up and published. The possible generation of ozone by the "Biogun" as demonstrated Shargawi et al. (1995, 1996) may warrant further investigation.

Ozone Detection

A "Gastec" gas detector was used to determine increase in levels of ozone above ambient generated by the "Biogun". It was held at a distance of 20 mm for the "Biogun" tip while it was activated for up to 65 minutes continuously *in vitro* on three occasions. On the first occasion, Test 1, there was some

ventilation as the window of the laboratory was open some two meters away. Over 65 minutes no ozone was detected. On the other two occasions there was no ventilation and the values found are given in Table 16.

Table 16. Changes in the ozone levels above ambient during prolonged usage of the Biogun

Time (min)	Ozone Increase (ppm)	
	Test 2	Test 3
0	0.0	0.0
5	0.0	0.05
10	0.0	0.05
15	0.05	0.05
20	0.0	0.05
25	0.05	0.05
30	0.1	0.0
35	0.1	0.0
40	0.05	0.0
45		0.05
50	0.1	0.0
55	0.05	0.0
60	0.1	
65	0.0	

The UK safety limits for ozone is 0.1 ppm over 8 hours or 0.3 ppm over 15 minutes.

CONCLUSIONS

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.

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Appendix

Research Presentations on the "Biogun" 1995-96

International Association for Dental Research Presentation, San Francisco, USA, March 1996

Negative air ion effect on Mutans streptococci in primary root-caries. Burke F.M.*, Lynch E., Beighton D.[1], Ludford R.[1]. Department of Conservative Dentistry, The London Hospital Medical College, [1]Oral Microbiology, RCS, KCSMD, London, UK.

Various methods of non-restorative management of root caries are being evaluated . This study has determined the ability of a negative air ion (NAI) generator (Dentron Ltd, 55 lower Ashley Road, New Milton, Hampshire BN25 5QF, UK) to kill Mutans streptococci from active primary root caries *in vivo*.

Samples were taken from primary root caries lesions deemed to require treatment. Each lesion was sampled and NAI were applied to each lesion for 2 minutes and the lesions were again sampled. The NAI were then applied for a further 2 minutes and another sample was taken subsequently. All samples were stored in fastidious anaerobic broth prior to culturing on Mitis-Salivarius Agar supplemented with 0.2 units per ml bacitracin and 15% (W/V) sucrose in an anaerobic chamber at 37^{o}C for three days. Fifty lesions were sampled from 23 patients. The resulting mean colony forming units (cfu) were (S.E.);

	before treatment	after 2 mins	after 4 mins
Mutans streptococci	1.8×10^4 (1.4×10^4)	5.4×10^2 (2.8×10^2)	1.6×10^2 (1.2×10^2)

ANOVA showed that NAI had a highly significant ($p<0.001$) effect on Mutans streptococci after 2 minutes. Prolonging the period of NAI application to 4 mins was significantly more effective ($p<0.001$) than 2 minutes.

The negative air ion system is an effective agent at killing Mutans streptococci involved in primary root caries and this effect appears to be time-dependent.

This work arises from an invention by J H L Copus. The financial support from Dentron Ltd is also gratefully acknowledged.

Presented at British Society for Dental Research Meeting,

Bristol, England, April 1996

Negative air ion effect on lactobacilli from root caries lesions *in vivo*.

F M BURKE*, E LYNCH, R LUDFORD[1] and D BEIGHTON[1] (Depts. of Conservative Dentistry, LHMC, [1]Oral Microbiology, KCSMD, UK):

The ability of a negative air ion (NAI) generator[1] to kill lactobacilli from active primary root caries *in vivo* has been determined. Standardised samples were taken from 50 primary root carious lesions deemed to require treatment using a sterile number 2 rosehead bur. NAI were applied to each lesion for 2 minutes and the lesions were again sampled. The NAI were then applied for a further 2 minutes and another sample was taken. All samples were stored in fastidious anaerobic broth prior to culturing for lactobacilli. Lactobacilli were grown on Lactobacillus Selective Agar LBS oxoid in an anaerobic chamber at 37^{o}C for three days. Lactobacilli were isolated from 64% of the lesions at baseline. The total number of lactobacilli in each sample was determined by counting the number of typical colonies grown. They were confirmed as Gram-positive, catalase-negative and unbranched rods. The resulting means as $\log_{10}$(cfu+1) ($\pm$S.E.) per sample was;

	before treatment	after 2 mins	after 4 mins
lactobacilli	2.03 (0.25)	1.15 (0.24)	0.78 (0.21)

Anova revealed these values are significantly different ($p<0.01$).

The negative air ion system is an effective agent at killing lactobacilli involved in primary root caries and the effect is time-dependent.

This work arises from an invention by J H L Copus. The financial support from Dentron Ltd is also gratefully acknowledged.

Irish Division of International Association for Dental Research,
Sligo, Ireland, May 1996

Negative air ion effect on *Candida albicans* from root-carious lesions *in vivo*. F M Burke*, E Lynch, R Ludford[1] and D Beighton[1] (Depts. of Conservative Dentistry, St Bartholomew's and The Royal London School of Dentistry, [1]Oral Microbiology, KCSMD, UK).

The ability of a negative air ion (NAI) generator[1] to kill *Candida albicans* from active primary root caries *in vivo* has been determined. Standardised samples were taken from 50 primary root carious lesions deemed to require restorative treatment. NAI were applied to each lesion for 2 minutes and the lesions were again sampled. The NAI were then applied for a further 2 minutes and another sample was taken. All samples were stored in Fastidious Anaerobic Broth prior to culturing for *Candida albicans*. *Candida albicans* were grown on Sabouraud Dextrose Agar in air at 37^{o}C for two days. *Candida albicans* were isolated from 54% of the lesions at baseline. The total number of *Candida albicans* in each sample was determined by counting the number of typical colonies grown. They were confirmed as Gram-positive, catalase-positive cells. The resulting means of $\log_{10}$(cfu+1) ($\pm$S.E.) per sample were;

	before treatment	after 2 mins	after 4 mins
Candida albicans	1.59 (0.15)	0.88 (0.17)	0.52 (0.14)

ANOVA revealed these values are significantly different ($p<0.01$).

The negative air ion system is an effective agent at killing *Candida albicans* involved in primary root caries and the effect is time-dependent.

This work arises from an invention by J H L Copus. The financial support from Dentron Ltd is also gratefully acknowledged.

ORCA Congress, Aarhus, Denmark, July 1996

Negative Air Ion effect on the viability of mutans streptococci isolated from active primary root-caries

F.M. Burke[a*], *E. Lynch*[a], *R. Ludford*[b], *D. Beighton*[b]

[a] Department of Conservative Dentistry, The London Hospital Medical College;

[b] Oral Microbiology, RCS Department of Dental Sciences, KCSMD, London, UK

The surgical management of active root caries entails the removal of infected and dentine as well as usually the placement of a restorative material. The development of a therapeutic, non-invasive treatment modality would minimise tooth destruction and the requirement for restorative material placement. We have therefore investigated the ability of negative air ions (NAI) to kill mutans streptococci (m-s) from active primary root carious lesions *in vivo*. The NAI were produced by an air ion generator (J. Copus, Dentron Ltd, U.K.). Standardised samples were taken from 35 primary root caries lesions deemed to require treatment. NAI were applied to each lesion for 2 minutes and the lesions were again sampled. NAI were then applied for a further 2 minutes and another sample was taken. The lesions were also sampled 3 months after application of the NAI. All samples were stored in fastidious anaerobic broth prior to culturing for m-s on Mitis-Salivarius Agar supplemented with bacitracin in an anaerobic chamber at 37^{o}C for three days. The total number of m-s in each sample were determined by counting the pleomorphic colonies identified on each plate. The resulting mean per sample $\log_{10}$(cfu+1) were ($\pm$S.E.);

before treatment	2 mins	4 mins	3 months
2.15 (0.27)	1.28 (0.29)	0.77 (0.23)	1.44 (0.25)

Anova revealed the NAI system is an effective method for killing m-s for at least three months ($p<0.005$).

This work arises from an invention by J H L Copus. The financial support from Dentron Ltd is also gratefully acknowledged.

European College of Gerodontology and Scandinavian and Central European Division of International Association for Dental Research, Berlin, Germany, September 1996

Prolonged negative air ion effect on *Candida albicans* from root-caries. F M BURKE*, E LYNCH, R LUDFORD[1] and D BEIGHTON[1] (Dept. of Conservative Dentistry, St Bartholomew's and The Royal London School of Dentistry, [1]Oral Microbiology, KCSMD, UK).

The ability of a negative air ion (NAI) generator[1] to kill *Candida albicans* from active primary root caries *in vivo* has been determined. Standardised samples were taken from 50 primary root carious lesions deemed to require restorative treatment. NAI were applied to each lesion for 2 minutes and the lesions were again sampled. The NAI were then applied for a further 2 minutes and another sample was taken. Further samples were taken after three months. All samples were stored in Fastidious Anaerobic Broth prior to culturing for *Candida albicans*. *Candida albicans* were grown on Sabouraud Dextrose Agar in air at 37^{o}C for two days. *Candida albicans* were isolated from 54% of the lesions at baseline. The total number of *Candida albicans* in each sample was determined by counting the number of typical colonies grown. They were confirmed as Gram-positive, catalase-positive cells. The resulting means of $\log_{10}$(cfu+1) ($\pm$S.E.) per sample were;

	before NAI	2 mins NAI	4 mins NAI	3 months
Candida albicans	1.59 (0.15)	0.88 (0.17)	0.52 (0.14)	0.55 (0.27)

ANOVA shows the immediate follow up values are highly significantly different ($p<0.01$) and the three month follow up value is significantly different ($p<0.05$) from baseline.

The negative air ion system is an effective agent at killing *Candida albicans* involved in primary root caries and it appears that the effect lasts for at least three months.

This work arises from an invention by J H L Copus. The financial support from Dentron Ltd is gratefully acknowledged.