ELSEVIER

Biomaterials 22 (2001) 1683–1687

Biomaterials

www.elsevier.com/locate/biomaterials

Antibacterial activity of particulate Bioglass® against supra- and subgingival bacteria

I. Allan[a,*], H. Newman[b], M. Wilson[a]

[a]Department of Microbiology, Eastman Dental Institute, University College London, 256 Gray's Inn Road, London WC1X 8LD, UK
[b]Clinical Research Centre, International Centre for Excellence in Dentistry, London, UK

Received 6 July 2000; accepted 10 October 2000

Abstract

Particulate Bioglass® is a bioactive material used in the repair of periodontal defects. This material undergoes a series of surface reactions in an aqueous environment which lead to osseointegration. The aim of this study was to determine whether these reactions exerted an antibacterial effect on a range of oral bacteria. *Streptococcus sanguis*, *Streptococcus mutans* and *Actinomyces viscosus* were suspended in nutrient broth (NB), artificial saliva (AS) or Dulbecco's modified eagle medium plus 10% foetal calf serum (DMEM + 10%FCS), with or without particulate Bioglass®. All bacteria showed reduced viability following exposure to Bioglass® in all the media after 1 h. This antibacterial effect increased after 3 h. *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Prevotella intermedia* and *Actinobacillus actinomycetemcomitans* were suspended in either BM broth or 40% horse serum (HS) in RPMI. A considerable reduction in viability was observed with all bacteria tested, in both media, compared to inert glass controls. In further experiments it was found that the viability of *S. sanguis* was significantly reduced following exposure to NB pre-incubated with Bioglass®. Additionally, it was found that neutralisation of this highly alkaline solution eliminated the antibacterial effect. Moreover, a solution of NB and NaOH (of equivalent pH) exerted an antibacterial effect of similar magnitude to that of the solution pre-incubated with Bioglass®. Thus, particulate Bioglass® exerts an antibacterial effect on certain oral bacteria, possibly by virtue of the alkaline nature of its surface reactions. This may reduce bacterial colonisation of its surface in vivo.

Keywords: Bioactive glass; Implant; Periodontal; Antibacterial

1. Introduction

45S5 Bioglass® is a bioactive implant material that stimulates bone repair. In an aqueous environment, this material undergoes a series of surface reactions resulting in the formation of a hydroxycarbonate apatite (HCA) surface layer [1]. It is the formation of this layer, together with the release of soluble silica, that leads to the attachment and proliferation of osteoblasts on the glass surface and osseointegration [2].

Inflammatory periodontal disease can result in the creation of periodontal bone defects [3]. Such defects have been treated with autogenous bone grafts and allografts, guided tissue regeneration and a combination of guided tissue regeneration and decalcified freeze-dried bone [4]. However, the use of autogenous bone requires a second surgical site, allografts have been found to be poor at inducing bone growth, and the outcome of guided tissue regeneration may be unpredictable [4]. Bioglass®, in its particulate form, is used in the treatment of periodontal defects [5] and this preparation is known commercially as Perioglas®. A 1 year clinical trial showed a greater degree of periodontal defect infill by application of particulate Bioglass® following debridement (root planing and removal of chronic inflammatory tissue), than with debridement alone [4].

The use of implants in the body is associated with a risk of bacterial colonisation of these materials and subsequent failure of the implant [6]. Moreover, those bacteria associated with periodontal disease have also been associated with dental implant failure [7]. However, certain bioactive glasses have been shown to exert an antibacterial effect when challenged with bacteria [8].

* Corresponding author. Tel.: +44-207-915-1016; fax: +44-207-915-1127.
E-mail address: iallan@eastman.ucl.ac.uk (I. Allan).

0142-9612/01/$ - see front matter
PII: S0142-9612(00)00330-6

This antibacterial effect may result from one of the surface reactions undergone by these glasses.

The purpose of this study was to determine the effect of exposure to particulate 45S5 Bioglass® on the viability of a range of oral bacteria.

2. Materials and methods

2.1. Particulates

Particulate 45S5 Bioglass® with grain size ranges of 90–710 μm and 355–500 μm (US Biomaterials, FL, USA) was used for viability studies on typical supragingival and subgingival microorganisms, respectively. For the subgingival studies, particulate glass of grain sizes 455–600 μm (Sigma, Poole, UK) was used as an additional control.

2.2. Bacterial cultivation

In the studies with typical supragingival bacteria, *S. sanguis* NCTC 10904, *S. mutans* NCTC 10449 and *A. viscosus* NCTC 10951 were grown aerobically for 24 h in NB (Oxoid, Basingstoke, UK) at 37°C to 10^6–10^7 CFU/ml. One ml volumes of the cultures were then centrifuged for 10 min, washed once and resuspended in 1 ml NB, AS or DMEM (with sodium glutamate, without L-glutamine) + 10%FCS (GibcoBrl, Paisley, UK).

In the studies with typical subgingival bacteria, *A. actinomycetemcomitans* NCTC 9710, *P. gingivalis* W50, *F. nucleatum* ATCC 51190 and *P. intermedia* (clinical isolate, strain DS102) were grown in BM broth anaerobically at 37°C for 48 h. The cultures were centrifuged for 10 min, washed once and resuspended in 1 ml 40% HS (Oxoid) in RPMI (without phenol red, with L-glutamine) (Sigma). The pH's of the suspensions were measured using a pH meter (pH Boy-P2, Camlab, Cambridge, UK).

2.3. Incubation of cultures with bioactive glass

2.3.1. Experiments with supragingival bacteria

The cultures in NB, AS or DMEM + 10% FCS were dispensed in 50 μl volumes into cryotubes containing 0.05 g particulate Bioglass® (90–710 μm). Control tubes contained no particulates. The cultures were then incubated aerobically for 1 or 3 h at 37°C. Following incubation, 950 μl of sterile phosphate buffered saline (PBS) (Oxoid) was added to each tube to facilitate viable counting. The samples were then vortexed for 1 min to dislodge adherent bacteria. Viable counts were performed by serial dilution in PBS and plating onto Tryptone Soya Agar (TSA) (Oxoid) plates with anaerobic incubation for 48 h at 37°C. The resultant colonies were counted and the percentage kills relative to controls without Bioglass®, calculated.

2.4. Incubation of cultures with bioactive glass

2.4.1. Experiments with subgingival bacteria

The cultures in BM broth, or 40% HS in RPMI, were dispensed in 50 μl volumes into cryotubes containing 0.1 g particulate Bioglass® (355–500 μm), or 0.1 g glass beads (455–600 μm), or no beads. The cultures were then incubated anaerobically for 1 h at 37°C. Samples were vortexed, as above, and serially diluted in BM broth before plating onto blood agar plates (Blood Agar No. 2, Oxoid, 7% defibrinated horse blood, Oxoid). Plates were incubated anaerobically for 10 days (2 days for *A. actinomycetemcomitans*) at 37°C. Resultant colonies were counted and the percentage kills relative to controls without Bioglass® or glass beads, calculated.

2.5. The antibacterial properties of Bioglass® supernates

Universals contained 5 g of particulate Bioglass® (355–500 μm), 5 g glass particulates (455–600 μm) or no particulates. Sterile NB (10 ml) was then added and the contents mixed on a multi-axle rotator for 1 h at 37°C. The choice of 5 g and 10 ml for particulates and media, respectively, was to allow recovery of adequate volumes of supernates after mixing. Following the 1 h incubation period, 950 μl volumes of the supernates were removed and added to tubes containing 50 μl volumes of an overnight culture of *S. sanguis*. The Bioglass® supernate-containing cultures were then incubated aerobically for 1 h at 37°C. The cultures were then serially diluted in NB, plated onto TSA plates and incubated aerobically at 37°C for 48 h. The resultant colonies were counted and expressed as colony-forming units (CFU)/ml.

2.6. The effect of pH adjustment on the antibacterial properties of Bioglass® supernates

Bioglass® supernates were produced as above. The pH's of the supernates were measured and, on being found to be a constant 9.8, pooled into one universal. One milliliter volumes were then dispensed into sterile plastic universals. The pH was reduced in one set of replicates by addition of 35 μl of 1 M HCl to give final pH's of 7.2, which is the same as that of NB. To one set of Bioglass® supernates was added 35 μl of 1 M NaCl. The pH remained at 9.8. The addition of NaCl acted as a control for the addition of chloride ions (in the form of HCl) in the pH-adjusted supernates. NB was included in place of Bioglass® supernates as a negative control. As a positive control, 1 M NaOH was added to NB to a final pH of 9.8: 950 μl volumes of the solutions were then

added to cryotubes containing 50 μl of an overnight culture of *S. sanguis*, and the experiments continued as above.

3. Results

3.1. Incubation of cultures with bioactive glass

3.1.1. Experiments with supragingival bacteria

S. sanguis, *S. mutans* and *A. viscosus* incubated with particulate Bioglass® or no particulates in NB, DMEM + 10% FCS or AS for 1 or 3 h.

The particulate Bioglass® exerted an antibacterial effect against all three bacterial species in all of the suspending media. This effect was greater after 3 h exposure. After 1 h, a lesser antibacterial effect was observed against bacteria suspended in DMEM + 10% FCS compared to the other media, however, the viability of the bacteria further decreased after 3 h. No decrease in the viability of the controls without particulates was observed after 1 or 3 h. Table 1 shows the percentage kills after 1 and 3 h. After 1 h, the mean percentage kills of all three bacterial species were approximately: 93% in NB, 85% in AS and 65% in DMEM + 10% FCS. After 3 h, the approximate mean percentage kills were 99% for NB, 98% for AS and 93% for DMEM + 10% FCS.

3.2. Incubation of cultures with bioactive glass

3.2.1. Experiments with subgingival bacteria

The percentage kills of *P. gingivalis*, *F. nucleatum*, *Prev. intermedia* and *A. actinomycetemcomitans* following exposure to particulate Bioglass®, particulate glass or no particulates in BM broth are shown in Table 2.

The Bioglass® particulates demonstrated a considerable antibacterial activity against all of the subgingival bacteria tested, with 100% kill of *Prev. intermedia*. However, there was in some instances, a reduction in viability observed with the glass particulates. This was most prevalent in the tests with *P. gingivalis* and *Prev. intermedia*. It was found that the reductions in viability in BM broth were greater than those in 40% HS in RPMI.

Table 1
Percentage kill of supragingival bacteria following exposure to Bioglass® particulates

Bacterium and suspending medium	% kill	
	1 h	3 h
S. sanguis – NB	98.0	99.5
S. sanguis – DMEM + 10% FCS	71.1	99.9
S. sanguis – AS	65.8	94.4
S. mutans – NB	83.1	97.3
S. mutans – DMEM + 10% FCS	51.5	79.1
S. mutans – AS	94.4	98.4
A. viscosus – NB	97.3	99.5
A. viscosus – DMEM + 10% FCS	72.7	99.9
A. viscosus – AS	96.2	99.6

Table 2
Percentage kill of subgingival bacteria following exposure to Bioglass® particulates[a]

Bacterium	% kills			
	BM broth		40% HS in RPMI	
	Bioglass®	Glass	Bioglass®	Glass
P. gingivalis	91.2	62.3	nd	nd
F. nucleatum	95.0	14.7	85.9	0.9
Prev. intermedia	100	48.8	nd	nd
A. actinomycetemcomitans	98.6	8.0	87.9	4.8

[a]nd — no data.

Table 3
pH measurements of suspension medium + Bioglass®

Medium	Time (h)		
	0	1	3
NB	7.2	10.3	10.0
AS	6.6	10.1	10.2
BM broth	6.5	10.1	10.1
40% HS in RPMI	7.7	10.4	10.3
DMEM + 10% FCS	8.1	10.0	10.2

3.3. pH measurements

The pH values at each time interval are shown in Table 3. The values at 0 h represent the pH's of the media prior to addition of particulates.

3.4. The antibacterial properties of Bioglass® supernates

Fig. 1 shows the viability of *S. sanguis* following incubation with the supernates from mixtures of Bioglass® and glass particulates in NB. A statistically significant reduction ($p < 0.01$) in the viability of *S. sanguis* was observed after exposure to the supernates produced from Bioglass® and NB, compared to the controls. The cultures exposed to Bioglass®/NB supernates had a mean viable count of approximately $3.8 \pm 1.6 \times 10^4$ CFU/ml. The cultures exposed to glass/NB supernates and NB alone had mean viable counts of approximately $9.9 \pm 3.6 \times 10^6$ CFU/ml, and $8.2 \pm 2.8 \times 10^6$ CFU/ml. This constituted a percentage kill of *S. sanguis* of greater than 99% when exposed to the Bioglass®/NB supernates. No significant difference was found between the controls of glass particulates in NB, and NB alone.

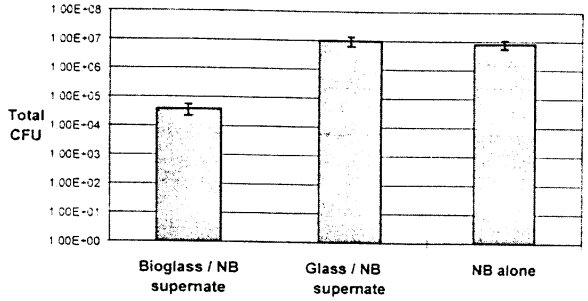

Fig. 1. Viability of *S. sanguis* following 1 h exposure to supernates from Bioglass® particulates in NB.

Table 4
The effect of pH adjustment of Bioglass® supernates on the viability of *S. sanguis*

	% kill
Untreated Bioglass®	91.1
Bioglass® + HCl	31.5
Bioglass® + NaCl	91.7
NB + NaOH	97.2

3.5. The effect of pH adjustment on the antibacterial activity of Bioglass® supernates

Table 4 shows the effect of pH adjustment of the supernates on the viability of *S. sanguis*. Reduction of the pH of the supernates to that of NB (pH 7.2) by the addition of HCl, eliminated the antibacterial activity. NB alone had a mean viable count of approximately $2.6 \pm 0.8 \times 10^7$ CFU/ml, while the Bioglass® supernates pH adjusted with HCl had a mean viable count of approximately $1.8 \pm 0.5 \times 10^7$ CFU ml. These differences were not significant ($p < 0.01$). Untreated Bioglass®/NB supernates had a mean viable count of approximately $2.3 \pm 0.6 \times 10^6$ CFU/ml. The addition of NaCl to Bioglass® supernates had no significant effect on the antibacterial activity of the Bioglass® supernates, with a mean viable count of approximately $2.2 \pm 0.4 \times 10^6$ CFU/ml. Raising the pH of NB, by the addition of NaOH, to the same as that of Bioglass® supernates, was found to produce an antibacterial effect, with a mean viable count of approximately $7.4 \pm 5.0 \times 10^5$ CFU/ml. This value was significantly lower than that found with the untreated Bioglass® supernates.

4. Discussion

These experiments have demonstrated that particulate Bioglass® has an antibacterial effect against certain supragingival and subgingival bacteria. Such an effect may be an important advantage for the successful osseointegration of Bioglass® when implanted periodontally as bacterial colonisation of implant sites has been suggested to be an important cause of implant failure [7,9,10].

The experiments examining the antibacterial properties of Bioglass® supernates demonstrated that direct contact between Bioglass® particulates and bacterial cells was not required to produce an antibacterial effect. Hence, the surface reactions of Bioglass® were found to produce a solution capable of killing the target bacteria. This may provide particulate Bioglass® with the advantage of being able to sterilise an implant site, or at least reduce the numbers of viable bacteria in the vicinity. The fact that contact between the granules and bacteria was not necessary for an antibacterial effect suggests that bacteria could be killed before they encounter the Bioglass® surface. Whether this would actually reduce bacterial accumulation on Bioglass® is uncertain. Bacterial co-aggregation, which relies on receptor–adhesin interactions, is an energy-independent process and therefore viability independent [11]. It seems likely that this is also the case for bacterial adhesion to surfaces, as this has been shown to be mediated by hydrophobic and receptor–adhesin interactions [12].

The results from the experiments examining the effect of pH adjustment on the antibacterial activity of Bioglass® supernates indicated that the antibacterial activity of Bioglass® supernates was a pH-related phenomenon. However, it was not possible to conclude from these experiments that the antibacterial activity was due to a high pH per se. A reduction in the pH of the Bioglass® supernates may have altered the solubilities of particular ions in the supernates which, at pH 9.8, could also have been responsible for the bactericidal effect. However, the antibacterial activity observed with addition of NaOH to nutrient broth was of similar magnitude to that elicited by the Bioglass® supernates. The antibacterial activity represented percentage kills of approximately 91% for the untreated Bioglass® supernates and 97% for NaOH in nutrient broth. This provided evidence that high pH alone was responsible for the antibacterial activity. High pH solutions have previously been shown to have some antibacterial activity [13]. Dentifrices of high pH have also been shown to be bactericidal [14].

While no other studies appear to have been undertaken examining the antibacterial properties of 45S5 Bioglass®, some work has been carried out examining a similar material, S53P4 (SiO_2, 53; Na_2O, 23; CaO, 20; and P_2O_5, 4; wt%). This material has been used in the treatment of hypersensitive teeth and sinusitis [15,16]. These workers found even greater antibacterial effects, although the glass surface area-to-volume (SA/V) ratio used in their experiments was between 10 and 20 times greater than the supra- and subgingival studies described in this report. Bioglass® of high SA/V ratios can generate a faster rate of pH increase in the surrounding solution

and a faster rate of ion release compared to lower SA/V ratios [17]. Such findings may explain the even greater antibacterial effects found with S53P4 compared to the materials used in the present study.

5. Conclusions

Particulate Bioglass® was shown to exert a considerable antibacterial effect against certain oral bacteria, including those associated with caries and periodontal disease. This antibacterial effect may reduce the potential for bacterial colonisation of Bioglass® when used in periodontal applications.

Acknowledgements

We would like to thank US Biomaterials Co. for funding this project.

References

[1] Cao W, Hench LL. Bioactive materials. Ceram Int 1995;22:493–507.
[2] Hench LL. Bioactive ceramics: theory and clinical applications. In: Anderson OH, Yli-Urpo A, editors. Bioceramics, vol. VII. Oxford: Butterworth-Heinemann, 1994. p. 3–14.
[3] Tanner A, Maiden MF, Macuch PJ, Murray LL, Kent Jr RL. Microbiota of health, gingivitis, and initial periodontitis. J Clin Periodontol 1998;25:85–98.
[4] Zamet JS, Darbar UR, Griffiths GS, Bulman JS, Bragger U, Burgin W, Newman HN. Particulate Bioglass® as a grafting material in the treatment of periodontal intrabony defects. J Clin Periodontol 1997;24:410–8.
[5] Wilson J, Yli-Urpo A, Happonen RP. Bioactive glasses: clinical applications. In: Hench LL, Wilson J, editors. An introduction to bioceramics. London: World Scientific, 1993. p. 63–73.
[6] Gristina AG. Biomaterial-centred infection: microbial adhesion versus tissue integration. Science 1987;237:1588–95.
[7] Rams TE, Roberst TW, Tatum H, Keyes PH. The subgingival microflora associated with human dental implants. J Prosthetic Dent 1984;51:529–34.
[8] Stoor P, Soderling E, Salonen JI. Antibacterial effects of a bioactive glass paste on oral microorganisms. Acta Odontol Scand 1998;56:161–5.
[9] Mombelli A, van Oosten MAC, Schurch E, Lang NP. The microbiota associated with successful or failing osseointegrated titanium implants. Oral Microbiol Immunol 1987;2:145–51.
[10] Sbordone L, Barone A, Ramaglia L, Ciaglia RN, Iacono VJ. Antimicrobial susceptibility of periodontopathic bacteria associated with failing implants. J Periodontol 1995;66:69–74.
[11] Kolenbrander PE, London J. Adhere today, here tomorrow: oral bacterial adherence. J Bacteriol 1993;175:3247–52.
[12] Busscher HJ, van der Mei. Physico-chemical interactions in initial microbial adhesion and relevance for biofilm formation. Adv Dent Res 1997;11:24–32.
[13] Barbosa SV, Spangberg SW, Almedia D. Low surface tension calcium hydroxide solution is an effective antiseptic. Int Endodontics J 1994;27:6–10.
[14] Drake DR, Vargas K, Cardenzana A, Srikantha R. Enhanced bactericidal activity of Arm and Hammer Dental Care. Am J Dent 1995;8:308–12.
[15] Salonen J, Tuomine U, Andersson OH. Mineralisation of dentin by making use of bioactive glass S53P4. In: Anderson OH, Salonen J, Yli-Urpo A, editors. Biomaterials today and tomorrow. Proceeding of the Finnish Dental Society, Turku Centre for Biomaterials, 1996. p. 25–6.
[16] Aitasalo K, Peltola M, Suonpaa J, Yli-Urpo A, Andersson OH, Varpula M, Happonen RP. In: Anderson OH, Yli-Urpo A, editors. Bioceramics, vol. 7. Oxford: Butterworth-Heinemann Ltd, 1994. p. 409–14.
[17] Greenspan DC, Zhong JP, LaTorre GP. Effect of surface area to volume ratio on in vitro surface reactions of bioactive glass particulates. In: Anderson OH, Yli-Urpo A, editors. Bioceramics, vol. 7. Oxford: Butterworth-Heinemann Ltd, 1994. p. 55–60.