Antibacterial effects of a bioactive glass paste on oral microorganisms

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Bioactive glasses contain oxides of calcium, sodium, phosphorus, and silicon in a proportion that provides the material with surface activity and concomitantly with the property of forming a strong bond with bone. Bioactive glasses have been tested as bone substitutes in different clinical situations. In an aqueous environment, Ca^{2+}, Na^{+}, PO_4^{3-}, and Si^{4+} are released from the glass, resulting in a rise in pH and in osmotic pressure in its vicinity. Since these are factors that potentially influence the viability of oral microorganisms at the dentogingival margin, we studied the effects of bioactive glass S53P4 on the oral microorganisms *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Actinomyces naeslundii*, *Streptococcus mutans*, and *Streptococcus sanguis*. This was done by incubating each microbe in a suspension, in the presence of bioactive glass S53P4 in powder form. *A. naeslundii* was found to lose its viability within 10 min under the experimental conditions. *A. actinomycetemcomitans*, *P. gingivalis*, and *S. mutans* lost their viability within 60 min. Also for *S. sanguis* a significant loss of viability was seen within 60 min, but it was the only microbe that had any viable cells left after 60 min. Thus, in aqueous solutions the powdered bioactive glass S53P4 appears to have a broad antimicrobial effect on microorganisms of both supra- and subgingival plaque. Consequently, it could be useful as an ingredient in tooth-care products that may have beneficial effects on oral health both from a cariologic and a periodontal point of view. □ *Actinobacillus actinomycetemcomitans; Actinomyces naeslundii; Porphyromonas gingivalis; Streptococcus mutans*

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Bioactive glasses (BAG) have been used as substitutes for reconstructions of defects of the facial bones (1), rehabilitation of the dentoalveolar complex (2), regeneration of periodontal pockets (3), and recently also for treatment of hypersensitive teeth (4). The surface-reactive bioactive glass contains SiO_2, Na_2O, CaO, and P_2O_5. The chemical bond with bone in vivo is reported to result from the leaching of Na^{+}-ions and the congruent dissolution of calcium, phosphate, and silica from the glass in an aqueous environment, giving rise to an Si-rich layer on the material. The Si-rich layer acts as a templet for a calcium phosphate precipitation, which then binds to the bone (5). Bioactive glass has been successfully used for reconstructions of closed bone defects, which are not exposed to the external environment after the clinical procedure (1). However, there are a number of conditions for which bioactive glasses are used as therapeutic materials but that, at the same time, are imminently prone to microbial infections. These include clinical conditions such as infected frontal sinuses (6), periodontal pockets (3), and hypersensitive teeth as a complication of periodontal treatment or tooth wear that has resulted in the exposure of dentin and dentinal tubules (4). Obviously, the demonstration of any antibacterial activity of the BAG would add to the therapeutic value of the material in the clinical conditions described. This may be possible because of the high pH and the nonphysiologic concentration of ions caused by the presence of the glass (7).

Earlier studies have shown that the bioactive glass can act as a vehicle for Ca^{2+}, PO_4^{3}, Na^{+}, and Si^{4+}, which then mineralize type I collagen and enhance mineral formation in the dentinal tubules. Therefore, an aqueous preparation containing BAG may have potential to be used as a paste for the treatment of hypersensitive teeth with recessed gingival margins and exposed dentin. Positive effects of such treatment have been obtained after just 10–60 min, which makes this material interesting even from a clinical point of view (4).

The purpose of this study was to examine the antibacterial effects of a paste made of the bioactive glass S53P4 (8) on oral microorganisms representing periodontal pathogens, caries-associated microorganisms, and benign oral microflora. Two major pathogens were used: *Actinobacillus actinomycetemcomitans*, which has been suggested to play a role in juvenile periodontitis (9, 10), and *Porphyromonas gingivalis*, which has been associated with destructive periodontal lesions in adults (11–13). Also studied were *Actinomyces naeslundii*, which is associated with root caries; *Streptococcus mutans*, which is considered to play a major role in caries; and *Streptococcus sanguis*, as a representative of the benign oral microbiota (14).

Materials and methods

Materials

The bioactive glass powder S53P4 used in this study was produced by Abmin Technologies Ltd, Turku, Finland. The composition of the bioactive glass S53P4 by weight is SiO_2 53%, Na_2O 23%, CaO 20%, and P_2O_5 4%. The bioactive glass was prepared from reagent grade Na_2CO_3,

$CaHPO_4 \cdot 2H_2O$, $CaCO_3$ (Merck, Darmstadt, Germany), and Belgium sand. The glass batches were melted for 3 h at 1360°C. After melting the glass was cast into a plate, which was cooled from 520°C to 220°C at 1°C/min in an annealing oven. The oven was turned off and air-cooled to room temperature. The plate was crushed and dry ground in an agate mill. Powder, of particle size <45 μm (average particle size, 20 μm), was sieved out of the batch (8).

The powder was combined with a microbial suspension using a ratio of glass powder and liquid (50 mg and 30 μL, respectively), simulating the composition used for treatment of hypersensitive teeth (4).

As controls we used 1) no added glass powder and 2) an inert SiO_2 powder, containing 100% SiO_2, of particle size <45 μm (Biomaterials Project, Institute of Dentistry, University of Turku, Finland).

Microorganisms

The microorganisms used were *A. actinomycetemcomitans* (ATCC 29523), *P. gingivalis* (ATCC 33277), *A. naeslundii* (clinical isolate), *S. mutans* (NCTC 10449), and *S. sanguis* (NCTC 10904).

Preparation of the microbial suspensions

Precultivation of *A. actinomycetemcomitans*, *S. mutans*, and *S. sanguis* was performed at 37°C in BHI (Brain Heart Infusion, Unipath Ltd, Hampshire, England). After approximately 18 h of growth the cells were washed once in saline (*S. mutans* and *S. sanguis*) or in reduced transport fluid (RTF: 0.6 g/L $K_2HPO_4 \cdot 3H_2O$, 0.23 g/L NaCl, 0.23 g/L $(NH_4)_2SO_4$, 0.11 g/L KH_2PO_4, 0.1 g/L $MgSO_4 \cdot 7H_2O$, 0.37 g/L disodium ethylenedinitrilo tetraacetate; $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$, 0.4 g/L Na_2CO_3, 0.2 g/L dithiothreitol; $C_4H_{10}O_2S_2$) (15) (*A. actinomycetemcomitans*). The suspensions were adjusted with saline or RTF to an optical density of approximately 1.0 (A_{700}; corresponding to 10^5–10^7 colony-forming units (CFU)/mL). *P. gingivalis* was precultivated anaerobically (80% N_2, 10% CO_2, 10% H_2) at 37°C on Brucella agar plates (Difco Laboratories, Detroit, Mich., USA). The cells were harvested after 6 days' growth, washed once in Brewer Thioglycollate Medium (Difco; 500 g/L Beef Infusion, 5 g/L NaCl, 2 g/L K_2HPO_4, 10 g/L Proteose Peptone, 5 g/L Bacto Dextrose, 0.5 g/L Na-thioglycollate, 0.5 g/L Bacto Agar, 0.002 g/L Bacto Methylene Blue), and finally adjusted with Brewer Thioglycollate Medium to an optical density of approximately 1.0 (A_{700}; corresponding to 10^4–10^5 CFU/mL). *A. naeslundii* was precultivated in Brewer Thioglycollate Medium at 37°C for 3 days, washed once in RTF, and adjusted with RTF to a density of approximately 1.0 (A_{700}; corresponding to 10^5–10^6 CFU/mL).

Incubation experiments

The bioactive glass powder (50 mg) and 30 μL of the microbial suspension were first vortexed for thorough mixing for 10 min at room temperature in Eppendorf tubes (Sarstedt, Germany), followed by incubation without agitation for 50 min at 37°C. The controls contained 1) no added bioactive glass powder or 2) 50 mg inert SiO_2 powder (particle size, <45 μm).

For assessment of viability as CFU, the incubation was stopped by adding 470 μL RTF (*A. actinomycetemcomitans*, *P. gingivalis*, *A. naeslundii*) or saline (*S. mutans*, *S. sanguis*), followed by vortexing and gentle sonication for 2 s to detach the microorganisms from the bioactive glass powder and from the inert SiO_2 powder. The assessment of viability as CFU of the microbial suspensions was performed on solid growth media by cultivating 10 μL samples from the suspensions diluted in saline (10^1–10^7). The undiluted suspension was also cultivated by using 20 μL samples. *A. actinomycetemcomitans* and *A. naeslundii* were cultivated on blood agar anaerobically at 37°C for 3 days and approximately 8 h, respectively. *P. gingivalis* was cultivated anaerobically at 37°C for 6 days on Brucella agar plates. *S. mutans* and *S. sanguis* were cultivated on Mitis salivarius agar for approximately 18 h at 37°C aerobically in an atmosphere consisting of 74% N_2, 19% O_2, and 7% CO_2. The experiment was performed with two to three parallels and repeated once.

Ion release from the bioactive glass paste and related pH changes

The release of ions from the bioactive glass powder (50 mg) during the 60 min contact with saline (30 μL) was analyzed with a Direct Current Plasma Atomic Emission Spectroscope (DCP-AES) at the Department of Chemistry, Åbo Akademi University, Turku, Finland. The determinations were performed in triplicate. After the vortexing (10 min) and incubation (50 min), the samples were suspended in 5 mL laboratory-grade H_2O (Milli-QUF PLUS, Millipore, Molsheim, France), rapidly mixed, and immediately filtrated with a Millex-GS 0.22-mm filter (Millipore).

The time-dependent changes in the pH values of saline and Brewer Thioglycollate Medium were monitored after 10-min and 60-min incubation with the bioactive glass powder. The measurements were performed with a combination electrode, pHC4406 (Radiometer, Copenhagen, Denmark).

Results

The time-dependent release of calcium, phosphorus, silica, and sodium from the bioactive glass paste is given in Table 1. For calcium, phosphorus, and sodium, the amount of released ions did not increase during the prolonged incubation, but for silica the amount tripled during the 10–60-min incubation. The increased osmotic pressure was created mostly by the release of sodium, which corresponds to a concentration of 3.38% at 10 min and 3.50% at 60 min.

Table 1. Measurements of the release of four elements from the bioactive glass S53P4 in powdered form, showing almost instant release of calcium, phosphorus, and sodium. The amount of released silicon tripled during the time period, 10–60 min. The values shown are means ± standard deviations (*s*) in g/L

	10 min		60 min	
Element	Mean	*s*	Mean	*s*
Calcium	2.99	± 0.21	3.52	± 0.19
Phosphorus	0.17	± 0.03	0.16	± 0.00
Silicon	1.44	± 0.12	4.74	± 0.05
Sodium	33.79	± 0.54	34.98	± 0.22

During the incubation with bioactive glass powder, the mean (± standard deviation) of pH of both the saline and the Brewer Thioglycollate Medium increased in 10 min from 6.9 (± 0.3) to 10.8 (± 0.1). No further increase of the pH was seen.

A. actinomycetemcomitans totally lost its viability in contact with the bioactive glass powder within 60 min. A major decrease in the number of viable microbes was already seen within 10 min, as the number of viable microbes decreased from 9×10^5 to 9×10^2. *P. gingivalis* also lost its viability in contact with the glass powder within 60 min. After 10 min a decrease from 9×10^4 to 1×10^3 in the number of viable cells was seen. *S. mutans* lost its viability almost totally, from 6×10^6 to 0.8×10^1, after 10-min incubation with the bioactive glass powder. A total loss of viability of *A. naeslundii* was also seen already after 10 min. *S. sanguis* showed a significant loss of viability at 10 min, from 7×10^6 to 1×10^4, and at 60 min, further down to 1×10^3 (Fig. 1). However, *S. sanguis* was the only microbe that had any viable cells left after 60 min.

The incubations with the reference material, the inert SiO_2 powder, showed results similar to those of the controls with no glass powder (Fig. 2).

Discussion

In this study the bioactive glass paste showed a broad antibacterial effect on the microorganisms tested. The effect found may be due to several influences, including high pH, osmotic effects, and the Ca^{2+} concentration.

Since the bioactive glass S53P4 reacts in a surface-reactive manner in an aqueous environment, the release of ions and consequently the rise of the pH increases with an increasing surface area of the glass. In the form of a powder (<45 μm) the glass has a surface area that is large per weight unit, and thus the release of ions is high. In our experiments the surface area/volume (SA/V) ratio was very high, approximately 1920 cm^{-1}. Earlier experiments with bioactive glass S53P4 granules (297–500 μm) in which the SA/V ratio was 0.4 cm^{-1} showed almost a linear increase in the release of ions during the first 7 h (16). Owing to the high SA/V ratio in the present experiment, the release of ions was up to 300 times greater during the 60-min incubation than in the earlier experiments with granules (60 min: Ca 24 mg/L, P not detected, Si 15 mg/L, Na 12 mg/L) (16). Thus, the release of ions was faster from the bioactive powder than from the granules. Also, the pH change observed with the <45-μm glass powder within 60 min (pH 7–>11) was higher than that earlier reported with granules (pH 7–>9) (8).

Most heterotrophic bacteria grow well in media with an osmotic pressure created by 0.75% salt. Concentrations higher than 1% become inhibitory for most bacteria. However, many streptococci of the oral cavity can grow well on a 5%-sucrose medium, while the growth of most

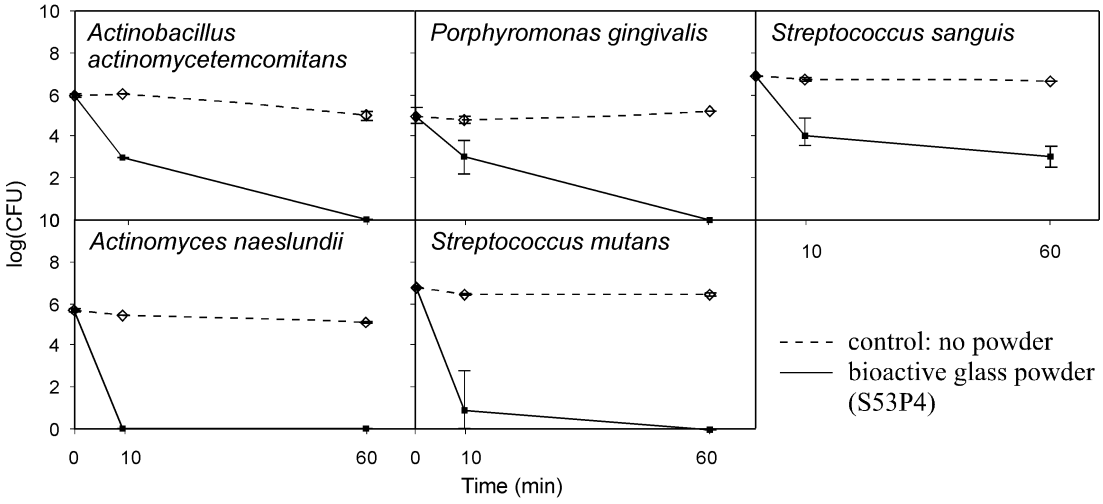

Fig. 1. The effect of the powdered bioactive glass S53P4 on viable counts of different microbes in suspension. Values shown are means; bars indicate range; $n = 3$. CFU = colony-forming units.

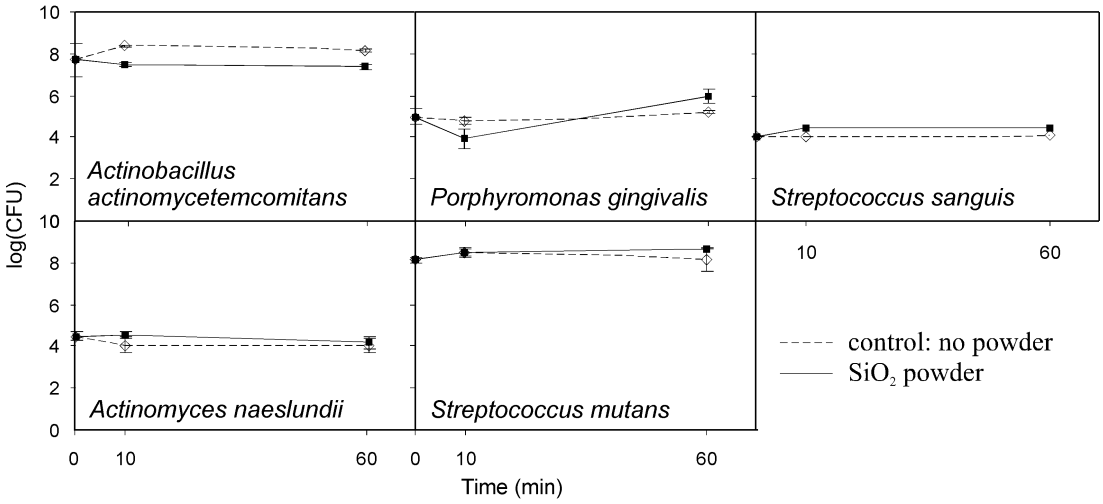

Fig. 2. The effect of SiO_2 powder on viable counts of different microbes in suspension. Values shown are means; bars indicate range; $n = 2–3$. Note that the treatment did not have any harmful effect on the anaerobic *Porphyromonas gingivalis*. CFU = colony-forming units.

other oral bacteria is inhibited under such conditions (17). As judged by the concentrations of the separate ions released from the glass powder, the total osmotic pressure was created, already after 10 min, by a concentration higher than 3% and, after 60 min, by a concentration above 4% (see Table 1). Since the outer membrane of gram-negative bacteria (*A. actinomycetemcomitans*, *P. gingivalis*) is reported to be a more efficient permeability barrier than the cell wall of the gram-positive bacteria (*A. naeslundii*, *S. mutans*, *S. sanguis*) (18), the osmotic effects due to the bioactive glass paste could partly explain the relative resistance of the gram-negative microorganisms and the more rapid loss of viability of *A. naeslundii* and *S. mutans*. Because *S. sanguis*, despite the lack of an effective permeability barrier, was the only microbe that managed to maintain some viability, other mechanisms also must be involved.

Earlier studies have shown that *P. gingivalis* is agglutinated in the presence of granules (315–500 μm) of the bioactive glass S53P4 in an aqueous environment because of Ca^{+2}-ions released from the granules (19, 5). The minimum Ca^{2+} concentration needed to induce agglutination of *P. gingivalis* was found to be 0.04 g/L (7). The Ca^{+2}-ion concentration measured in association with the glass paste was much higher (3.0–3.5 g/L). Thus, the antibacterial effects of the glass paste on *P. gingivalis* appear to be at least partly explained by a mechanism resulting in the agglutination of the bacteria.

The pH of the crevical fluid, the habitat of *A. actinomycetemcomitans* and *P. gingivalis*, ranges from 7.5 to 8.0 (20). The microorganisms of the supragingival plaque, *A. naeslundii*, *S. mutans*, and *S. sanguis*, are subjected to large pH variations in the oral cavity, for example by alkaline products used for oral hygiene and acids resulting from diet and from the fermentation of carbohydrates in plaque. However, high pH values such as those measured for the bioactive glass paste do not naturally exist in the oral cavity. In general, high pH values are considered bacteriocidic, for example when $Ca(OH)_2$ is used in the endodontic therapy of root canals (21), or as a part of the antibacterial component in dentifrices (22) and dental cements (23). Accordingly, also the antimicrobial effects of the bioactive glass paste could be partly attributed to the high pH of the paste.

In conclusion, the bioactive glass paste appears to possess a broad antimicrobial effect on microorganisms of both supra- and subgingival plaque. Consequently, the bioactive glass paste may have beneficial effects on oral health from both a cariologic and a periodontal point of view, in addition to its more direct therapeutic effect on root surface hypersensitivity. Although the results of this study do not show conclusive selectivity in the antimicrobial effect of the bioactive glass paste, further studies with different SA/V ratios may show such selective antimicrobial effects. Apparently, the clinical benefits of the bioactive glass powder when used as a paste or as a component in tooth-care products come from a combination of good influences rather than from any single property, such as its ability to reduce bacterial growth. These influences include mechanical cleaning and remineralization of both dentin and enamel.

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