



Full Length Research Paper

Bioactive glass with antimicrobial agents: *In vitro* evaluation

Monize F. F. De Carvalho^{*a}; Rosana Z. D. Fernandes^b; Ângela L. Andrade^c;
Vagner R. Santos^d; Karine A. T. Tavano^e; Walison A. Vasconcelos^f

^a DDS, MS Student Post-graduate Program in Dentistry Faculty of Sciences of Health, Department of Dentistry, Federal University of Vales do Jequitinhonha e Mucuri, Diamantina, Minas Gerais, Brazil.

^b Professor Department of Chemistry - ICEX, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

^c Professor Department of Chemistry - ICEB, Federal University of Ouro Preto, Ouro Preto, Minas Gerais, Brazil

^d Professor Department of Microbiology and Biomaterials, School of Dentistry, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

^e Professor Department of Dentistry, Federal University of Vales do Jequitinhonha e Mucuri, Diamantina, Minas Gerais, Brazil

^f Professor Department of Restorative Dentistry, School of Dentistry, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

*Corresponding authors E-mail: monize_c@hotmail.com; Cel: +55-31-9383-2692

ABSTRACT

Bioactive glass is biocompatible and has the ability to stimulate bone formation. The aim of the present study was to evaluate the efficiency of bioactive glass in releasing tetracycline and/or hydrocortisone through an *in vitro* growth inhibition study involving microorganisms commonly found in the oral cavity: *Staphylococcus aureus* (ATCC 27664), *Enterococcus faecalis* (ATCC 12399) and *Streptococcus mutans* (ATCC 70069). The bioactive glass was prepared using the sol-gel method and had a molar composition of 80% SiO₂, 4% P₂O₅ and 16% CaO. Four groups were formed: a drug-free control group (CG); a hydrocortisone group (HG) with 2% (in weight) of the drug incorporated into the bioactive glass; a tetracycline group (TG) containing 2% (in weight) of the drug; and a hydrocortisone-tetracycline group (HTG) containing 2% of each drug. Test specimens were distributed in Petri dishes containing agar previously inoculated with the microorganisms and incubated for 24 hours. Statistical analysis involved the Kruskal-Wallis and Mann-Whitney tests. The CG and HG proved ineffective in inhibiting the growth of the microorganisms. The TG exhibited inhibition zones on all microorganisms tested. The HTG had the largest inhibition zones for *Staphylococcus aureus* (ATCC 27664) and *Enterococcus faecalis* (ATCC 12399), with statistically significant differences in comparison to the other groups ($p < 0.001$). The present study demonstrates that bioactive glass is able to release tetracycline for effective antibiotic action.

Keywords: Bioactive glass, tetracycline, hydrocortisone

INTRODUCTION

Bioactive glass is a biocompatible synthetic material capable of inducing the formation of a layer of hydroxyapatite on its surface and generally composed of SiO₂, Na₂O, CaO and P₂O₅ (Dorozhkin and Eppler 2002; Laurence and Hillier 2003; Cholewa-Kowalska et al. 2009; Son et al. 2011; Jiang et al. 2012). Bioactive glass

can be used to release medications (Piattelli et al. 2000; Amaral and Costa 2003) and induce the migration, replication and differentiation of osteogenic cells from bone marrow (Forsback et al. 2004; Bakry et al. 2013). In the field of dentistry, this product is a viable alternative for repairing bone defects (Laurence and Hillier 2003) and

the remineralization of dentinal tubules (Hu et al. 2009; Malachowa and DeLeo 2010).

The oral cavity is colonized by Gram-positive microorganisms, which, despite being commensal, also constitute opportunistic pathogenic agents (Andrade et al. 2009). In dentistry, antibiotics are used to control infections caused by these microorganisms (Bellantone et al. 2002). Thus, the incorporation of an anti-inflammatory drug, such as hydrocortisone, and an antibiotic, such as tetracycline, into bioactive glass may be a viable option for controlling infection and inflammatory processes while also promoting bone formation.

The aim of the present study was to evaluate the efficiency of bioactive glass in releasing tetracycline and/or hydrocortisone through an *in vitro* growth inhibition study involving microorganisms commonly found in the oral cavity: *Staphylococcus aureus* (ATCC 27664), *Enterococcus faecalis* (ATCC 12399) and *Streptococcus mutans* (ATCC 70069).

MATERIALS AND METHODS

Preparation of Samples

Bioactive glass samples were prepared using the sol-gel method at room temperature. Sol was composed of $(\text{SiO}_2)_{0.80}(\text{P}_2\text{O}_5)_{0.04}(\text{CaO})_{0.16}$ and prepared using tetraethyl orthosilicate (TEOS), triethyl phosphate (TEP) and calcium chloride (CaCl_2) as precursors of silicon, phosphorus and calcium, respectively. All chemical reagents were obtained from Fluka Production GmbH (Germany).

Briefly, a mixture of ethanol, hydrochloric acid (pH 1.7) and TEOS (molar ratio: 1:4:1) was stirred for 30 min. In samples containing tetracycline (Fluka Production GmbH – Germany), an amount of the drug corresponding to 2% in weight was added to the solution, which was stirred for another 30 min. TEP was then added and the solution was stirred for another 30 min, following which CaCl_2 was added. The solution was stirred until solidifying. For the samples with hydrocortisone, 0.04 g of hydrocortisone succinate (União Química Farmacêutica, Brazil) was incorporated after the inclusion of all other reagents. Following gelification, all samples were maintained in an amber vial in an atmosphere with 100% relative humidity for 12 hours and then dehydrated in increasing concentrations of acetone in 15-second baths. The samples were then maintained in the dark to avoid photodecomposition (Bellantone et al., 2002).

The following groups were formed: a drug-free control group (CG); a hydrocortisone group (HG) with 2% (in weight) of the drug incorporated into the bioactive glass; a tetracycline group (TG) containing 2% (in weight) of the drug; and a hydrocortisone-tetracycline group (HTG)

containing 2% of each drug. Moreover, a tetracycline disc group (TDG) consisting of standardized (35 mg) tetracycline discs (CECON, Sao Paulo, Brazil) was used as a positive control of growth inhibition for subsequent comparison with the antibiotic incorporated into the bioactive glass. The antimicrobial susceptibility test was performed through diffusion on agar, following the norms recommended by the Clinical and Laboratory Standards Institute (2006).

Microorganisms

The freeze-dried microorganisms were cultivated in brain-heart infusion broth (Difco, USA) and added to a blood-agar culture medium. Following overnight incubation, aliquots of 500 μL containing 1.0×10^8 colony forming units/mL of the microorganism cultures (*Staphylococcus aureus* ATCC 27664, *Enterococcus faecalis* ATCC 12399 and *Streptococcus mutans* ATCC 70069) were inoculated on Mueller-Hinton agar (Difco, USA). Test specimens (6.0 mm indiameter) containing the samples from the different groups were placed on the surface of the agar and incubated at 37 °C in a CO_2 atmosphere. After 24 hours of incubation, the inhibition zones were measured.

Statistical analysis

Mean and standard deviation values were determined from triplicate analyses. The Shapiro-Wilk test demonstrated non-normal data distribution ($p < 0.05$). Therefore, the nonparametric Kruskal-Wallis and Mann-Whitney tests were employed for the comparisons of the groups. Statistical analysis was performed with the aid of the SPSS program, version 20.0 (SPSS Inc., Chicago, USA).

RESULTS

The CG and HG were ineffective in inhibiting the growth of *Staphylococcus aureus*. The TDG (positive control) had the largest inhibition zone for this microorganism, with a statistically significant difference in comparison with the other groups (Table 1).

Statistically significant differences were found between the TG and all other groups for the growth inhibition of *Enterococcus faecalis*. No statistically significant difference was found between the HTG and TDG regarding this microorganism (Table 2).

The TG was the most effective at inhibiting the growth of *Streptococcus mutans*, whereas the CG and HG exhibited no inhibition zones. Statistically significant differences among the groups were found for this microorganism (Table 3).

Table 1. Susceptibility of *S. aureus* to different groups

<i>S. aureus</i>	n	Minimum	Maximum	Mean	SD	p*
CG	15	0.00	0.00	0.00	0.00	a
TG	15	16.00	16.00	16.00	0.00	b
HG	15	0.00	0.00	0.00	0.00	a
HTG	15	19.80	20.20	20.00	0.76	c
TDG	15	20.60	21.20	21.00	0.17	d

SD – standard deviation; *Different letters denote statistically significant differences ($p < 0.001$)

Table 2. Susceptibility of *E. faecalis* to different groups

<i>E. faecalis</i>	n	Minimum	Maximum	Mean	SD	p*
CG	15	0.00	0.00	0.00	0.00	a
TG	15	19.00	19.00	19.00	0.00	b
HG	15	0.00	0.00	0.00	0.00	a
HTG	15	19.00	22.20	21.00	0.74	c
TDG	15	21.00	21.00	21.00	0.00	c,d

SD – standard deviation; *Different letters denote statistically significant differences ($p < 0.001$)

Table 3. Susceptibility of *S. mutans* to different groups

<i>S. mutans</i>	n	Minimum	Maximum	Mean	SD	p*
CG	15	0.00	0.00	0.00	0.00	a
TG	15	23.20	25.00	24.00	0.50	b
HG	15	0.00	0.00	0.00	0.00	a
HTG	15	17.60	18.20	18.00	0.15	c
TDG	15	18.80	19.40	19.00	0.15	d

SD – standard deviation; *Different letters denote statistically significant differences ($p < 0.001$)

DISCUSSION

As demonstrated elsewhere (Amaral and Costa 2003), it is possible to incorporate tetracycline and hydrocortisone in bioactive glass samples prepared using the sol-gel process at room temperature. In the present study, the HTG and TG demonstrated antimicrobial activity against *Staphylococcus aureus* (ATCC 27664), *Enterococcus faecalis* (ATCC 12399) and *Streptococcus mutans* (ATCC 70069).

The antibacterial action against microorganisms of the oral cavity occurred through the interaction of the bioactive glass and the drugs in the HTG and TG. This finding is in agreement with data reported in a previous study, in which silver ions were added to bioactive glass in sol-gel form and the interaction produced a significant increase in antibacterial action (Andrade et al. 2006; Balamurugan et al. 2008). A study involving 45S5 bioglass also found antibacterial action against pathogenic strains, but the authors attributed this action

to the increase in pH caused by the material (Bellantone et al. 2002).

The incorporation of hydrocortisone and/or tetracycline into bioactive glass produced an antibacterial effect in the present study, but statistically significant differences were found regarding the inhibition zones. The largest inhibition zones for *S. aureus* and *E. faecalis* occurred in the HTG, whereas the largest inhibition zone for *S. mutans* occurred in the TG. These findings suggest a different diffusion pattern of the drugs following their interaction. However, a previous study found that concentrations of hydrocortisone and/or tetracycline used in the preparation of bioactive glass triggered no chemical reaction or interaction between the drugs (Amaral and Costa 2003).

Despite the need for further experimental and clinical investigation, the present findings suggest that the addition of drugs to bioactive glass is a viable alternative for the health field, implying a reduction in inflammatory responses during surgical procedures (Barie 2002;

Cheadle 2006) as well as a reduced risk of contamination or recontamination by microorganisms of the oral cavity (Rocas et al. 2004; Schirrmeyer et al. 2009). Moreover, this method favors bone formation (Son et al. 2011) and offers greater patient comfort.

CONCLUSION

Bioactive glass with tetracycline in its composition demonstrated effective antibiotic action, whereas no antibiotic action was found with the incorporation of hydrocortisone.

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REFERENCES

- Amaral M, Costa MA (2003). Si₃N₄-bioglass composites stimulate the proliferation of MG63 osteoblast-like cells and support the osteogenic differentiation of human bone marrow cells. *Biomaterials*. 23: 4897-906.
- Andrade AL, Souza DM, Vasconcelos WA, Ferreira RV, Domingues RZ (2009). Tetracycline and/or hydrocortisone incorporation and release by bioactive glasses compounds. *J Non-Cryst Solids*. 355: 811–16.
- Bakry AS, Takahashi H, Otsuki M, Tagami J (2013). The durability of phosphoric acid promoted bioglass–dentin interaction layer. *Dent Mater*. 29: 357-64.
- Balamurugan A, Balossier G, Laurent-Maquin D, Pina S, Rebelo AH, Faure J, Ferreira JM (2008). An in vitro biological and anti-bacterial study on a sol-gel derived silver-incorporated bioglass system. *Dent Mater*. 24: 1343 - 51.
- Barie PS (2002). Surgical site infections: Epidemiology and prevention. *Surg Infect (Larchmt)*. 3 Suppl 1: S9 - 21.
- Bellantone M, Williams HD, Hench LL (2002). Broad-spectrum bactericidal activity of Ag(2)O-doped bioactive glass. *Antimicrob Agents Chemother*. 46: 1940- 5.
- Cheadle WG (2006). Risk factors for surgical site infection. *Surg Infect (Larchmt)*. 7 Suppl 1: S7 - 11.
- Cholewa-Kowalska K, Kokoszka J, Łaczka M, Niedź LW, Madej W, Osyczka AM (2009). Gel-derived bioglass as a compound of hydroxyapatite composites. *Biomed Mater*. Sep 25; 4: 055007. doi:10.1088/1748-6041/4/5/055007. PubMed PMID: 19779249.
- Dorozhkin SV, Epple M (2002). Biological and medical significance of calcium phosphates. *Angew Chem Int Edit*. 41: 3130 – 46.
- Forsback AP, Areva S, Salonen JI (2004). Mineralization of dentin induced by treatment with bioactive glass S53P4 In vitro. *Acta Odontol Scand*. 62:14 – 20.
- Hu S, Chang J, Liu M (2009). Study on antibacterial effect of 45S5 Bioglass. *J Mater Sci- Mater M*. 20: 281-6.
- Jiang P, Qu F, Lin H, Wu X, Xing R, Zhang J (2012). Macroporous/mesoporous bioglasses doped with Ag/TiO₂ for dual drug action property and bone repair. *IET Nanobiotechnol*. 6: 93 - 101.
- Laurence P, Hillier IH (2003). Towards modeling bioactive glasses: quantum chemistry studies of the hydrolysis of some silicate structures. *Comp Mater Sci*. 28: 68 - 75.
- Malachowa N, DeLeo FR (2010). Mobile genetic elements of *Staphylococcus aureus*. *Cell Mol Life Sci*; 67: 3057–71.
- Piattelli A, Scarano A, Piattelli M (2000). Bone regeneration using bioglass: an experimental study in rabbit tibia. *J Oral Implant*. 26: 257 - 61.
- Rocas IN, Siqueira JF, Santos KR (2004). Association of *Enterococcus faecalis* with different forms of periradicular diseases. *J Endodont*. 30: 315 - 20.
- Schirrmeyer JF, Liebenow AL, Pelz K, Wittmer A, Serr A, Hellwig E, Al-Ahmad A (2009). New bacterial compositions in root-filled teeth with periradicular lesions. *J Endodont*.; 35: 169 - 74.
- Son JS, Kim SG, Oh JS, Appleford M, Oh S, Ong JL, Lee KB (2011). Hydroxyapatite/poly(lactide) biphase combinations scaffold loaded with dexamethasone for bone regeneration. *J Biomed Mater Res Part A*. 15; 99: 638 - 47.

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