Comparative antibacterial study between bioactive glasses and vancomycin hydrochloride against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*

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Received: 5 March 2019
Accepted: 22 March 2019

Egyptian Pharmaceutical Journal 2019, 18:304–310

Background
This work targets the comparison of the antibacterial activity of different bioactive glasses as particles and those coating the surface of 316 l stainless steel sheet, with that of vancomycin hydrochloride antibiotic, to determine the best efficiency of the aforementioned materials for medical and surgical purposes.
Materials and methods
Different bioactive glass composites (borate, B, S, and B5), composed of different ratios of oxides, such as SiO_2, Na_2O, CaO, B_2O_3, P_2O_5, and MgO, were prepared. The antimicrobial activity of different synthesized glasses as well as vancomycin hydrochloride antibiotic was carried out against various Gram-negative and Gram-positive pathogens. The different bioactive glasses (0.05 g) were placed each in wells (1 cm in diameter) of pathogen-seeded nutrient agar, as particles or coated on 316 l stainless steel 1.0×1.5 cm sheets for agar diffusion method. The antibacterial test of vancomycin hydrochloride in different concentrations (25, 50, 75, and 100 mg/ml in distilled H_2O) was carried out. The pathogen cell viability in presence and absence of glass composite was investigated using electron microscopy and cell count method. Nutrient broth (50 ml) was inoculated with *Staphylococcus aureus* along with 0.05 g of borate particles, incubated at 37°C and 150 rpm for 6 h. Then, the samples were examined under electron microscope, and the final pH was measured. A volume of 0.1 ml of each sample was further inoculated on solid nutrient agar, incubated at 37°C for 24 h, and then colony count was carried out.
Results and discussion
The borate bioactive glass was effective either as particles or coated on 316 l stainless steel. The other types of bioactive glasses coating the stainless steel produced a better antibacterial activity than the particles. The transmission electron microscope, showed the damaged bacterial cells of *S. aureus* after incubation with borate bioactive glass. The colony count of *S. aureus* after bioglass treatment was 18×10^2, whereas in the control sample was 25×10^6; the final pH was 10.4.

Keywords:
antibacterial and electron microscope, bioactive glass, borate, vancomycin hydrochloride

Egypt Pharmaceut J 18:304–310

1687-4315

Introduction

Hospital-acquired infections caused by nosocomial pathogens are rapidly increasing worldwide, causing great threat to the patients and public health owing to reinfection through medical devices that are not well cleaned and disinfected between patients [1]. Furthermore, there is remarkable increase of the multidrug-resistant pathogens including methicillin-resistant *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* [2,3].

Bioactive glass is mostly silica based, composed of different ratios of a number of metal oxides (mainly SiO_2, Na_2O, CaO, and P_2O_5) [1,4], giving them some mechanical support to bond to the bone tissue without malformation of the bone shape.

It was proven that when bioactive glasses were put into fluids, they promote the construction of a new layer mimicking the bone structure, playing a key role in the bone recovery process after surgeries. Moreover, the contact of bioactive glass with fluids results in the increase of osmotic pressure and pH owing to the diffusion of ions from bioactive glass granules particles into the surrounding medium, thus making the surrounding environment not suitable for the growth of a lot of microorganisms.

The bioactive glass has antimicrobial activity against a wide variety of aerobic and anaerobic bacteria, either in planktonic or as a biofilm. Furthermore, bioglass is capable of decreasing the probability of pathogens being able to form biofilm in the first place. The use of bioactive glass is a promising tool of bone defect rehabilitation, as well as for the treatment and eradication of bone infections, including bone necrosis and bone breakage that results in bone destruction [2]. The inhibitory ability of bioactive glass has been tested against a wide range of aerobic and anaerobic bacteria, both Gram positive and Gram negative [5,6]; they exhibited an inhibitory effect against pathogens without developing resistance, with a good activity against biofilm formation [7].

The use of borate-based bioactive glasses has proven to be more effective than silicate-based bioactive glasses and is now considered as a fairly new trend in the field of bioremediation materials [8]. The reaction rate of bioactive glass can easily be modified by modifying the boron content of the glass composite [9]. In addition, borate glasses have many other benefits, for instance, borate-based bioactive glasses can promote cell division and proliferation [10] and induce healing of the tissues after surgeries [11]. Borate alone was used several decades before for wound healing and for its antimicrobial properties [12]. Borate chemistry in a solution has been studied to try to understand its mode of action; several suggestions propose energy depletion by binding to the energy reservoirs of the cells (NAD and NADH) [13,14] and binding to ribose groups causing the destruction of the cell DNA [13,15]. Moreover, binding the bioactive glass to a polysaccharide polymer like in case of chitosan has the advantage of being used in different biomedical purposes, such as antibacterial properties [1]. The combination of polymers with glass gives composite materials that mimic bone structure. Furthermore, polymers offer the advantage of low-temperature processing of composite materials. Bioactive glass composite coatings for metal implants have been successfully used owing to their superior properties [16–19].

Many silicate, borate, and phosphate glass compositions have been proposed for a wide range of biomedical applications in contact to both hard and soft tissues, as reviewed elsewhere. However, owing to their poor mechanical properties (especially tensile strength and fracture toughness), bioactive glasses alone cannot be used for structural purposes where metallic alloys are still the materials of choice. Two valuable options to solve this problem involve either the combination of the glass with a fracture-tough phase, such as a metal or a polymer, to produce a composite, or the application of the glass as a coating on a mechanically stronger and tougher substrate as in the case of stainless steel. In the biomedical field, coatings have been used in a variety of applications to modify the surface of implants and, in some cases, to create an entirely new surface that gives the implant additional properties which are quite different from those of the uncoated device. The aim of this study was the comparison of the antibacterial activity of different bioactive glasses as particles and those coating the surface of 316 l stainless steel sheet, to that of vancomycin hydrochloride antibiotic, to determine the best efficiency of the aforementioned materials for medical and surgical purposes.

Materials and methods

Preparation of the materials

Synthesis of the glass composites

A variety of glass particles were prepared, characterized as described previously and will be referred to as the glass composite [18]. The composition of the different types of glass composites is shown in Table 1. All types of the synthesized glass composite particles were transparent and colorless without any crystalline inclusions.

Preparation of glass composite-coated stainless steel sheet

Stainless steel 316 l sheets used in this study were cut into small pieces with the desired dimensions (1.0×1.5 cm). The synthesized glass composite particles (Borate, S, B, and B5 composites) described previously were deposited on the stainless-steel sheets by electrophoretic deposition technique. In vitro degradation tests of glass-coated substrate were followed in simulated body fluids (solution ISO 23317), as well as Dulbecco's modified Eagle medium solution by measuring the ionic concentrations of the released species from glass coatings and stainless steel substrates [18].

Table 1 Composition of different synthesized glass particles used in this study

Oxides	Percentage (w/w) of different oxides in the glass composite			
	Borate	S	B	B5
SiO_2	–	45	40	40
Na_2O	20	24.5	25	24.5
CaO	10	24.5	20	24.5
B_2O_3	60	–	5	5
P_2O_5	–	6	10	6
MgO	10	–	–	–

According to the electrochemical measurements, coatings proved noticeable improvement for corrosion protection that satisfies the medical requirements of the selected materials [18].

Antibacterial activity

Antibacterial activity of the prepared glass composites

The antibacterial activity of the prepared glass composites was determined using Agar diffusion assay. In brief, nutrient agar medium was prepared and inoculated with 1-ml cell suspension of each bacterial pathogen separately, including Gram-negative bacteria such as *E. coli* ATCC25922 and *P. aeruginosa* ATCC27953 and gram-positive bacteria such as *S. aureus* ATCC29213. Thereafter, 1-cm diameter wells were made in the nutrient agar medium using sterile cork borer, and 50 mg of each glass composite was placed into the wells under aseptic conditions either as free glass particles or coated on the stainless steel sheets. Then 0.1 ml of sterile distilled H_2O was added to the composite in each well. The plates were incubated at 37°C for 48 h, and the inhibition zones were measured. However, stainless steel sheets without the composites were used as a control. The experiments were carried out in triplicate, and the mean values were recorded and/or plotted.

Antibacterial activity of vancomycin hydrochloride

Overall, 0.5 g of vancomycin hydrochloride was dissolved in 10 ml sterile distilled H_2O and then was diluted in sterile distilled H_2O to give a final concentrations of 25, 50, 75, and 100 mg/ml. Wells of 1 cm diameter of each of the bacterial pathogen-seeded solid nutrient agar medium were inoculated with 0.1 ml of each antibiotic dilution. The agar plates were incubated at 37°C for 48 h, and clear zones were measured. The experiment was performed in triplicate, and the mean values were recorded and/or plotted.

Bacterial viability testing of *Staphylococcus aureus*

The *S. aureus* cells' viability in the presence and absence of the glass composite was investigated using transmission electron microscopy and bacterial cell count.

Transmission electron microscope method

A volume of 50 ml of sterile nutrient broth (pH 7) in 250 ml conical flasks, inoculated with *S. aureus*, was prepared, and 50 mg of borate glass particles was added to the inoculated medium. The control sample was the bacteria-inoculated medium without any glass particles. The flasks were incubated in a shaker incubator adjusted to 37°C and 150 rpm for 6 h. After the incubation period, the tested sample and the control were examined under transmission electron microscope (TEM) available in the National Research Centre (Dokki, Giza, Egypt) facilities (JEOL JEM-2100 Electron Microscope; Jenway, Jenway LTD., Feasted, Dunmow, Essex, UK).

Bacterial cell count

A volume of 0.1 ml of the aforementioned prepared *S. aureus* cultures in the presence or absence of the glass composite was withdrawn, and further inoculated on fresh agar medium and incubated for 24 h at 37°C. After the incubation period, a colony count was carried out. The experiment was performed in triplicate, and the mean values were recorded.

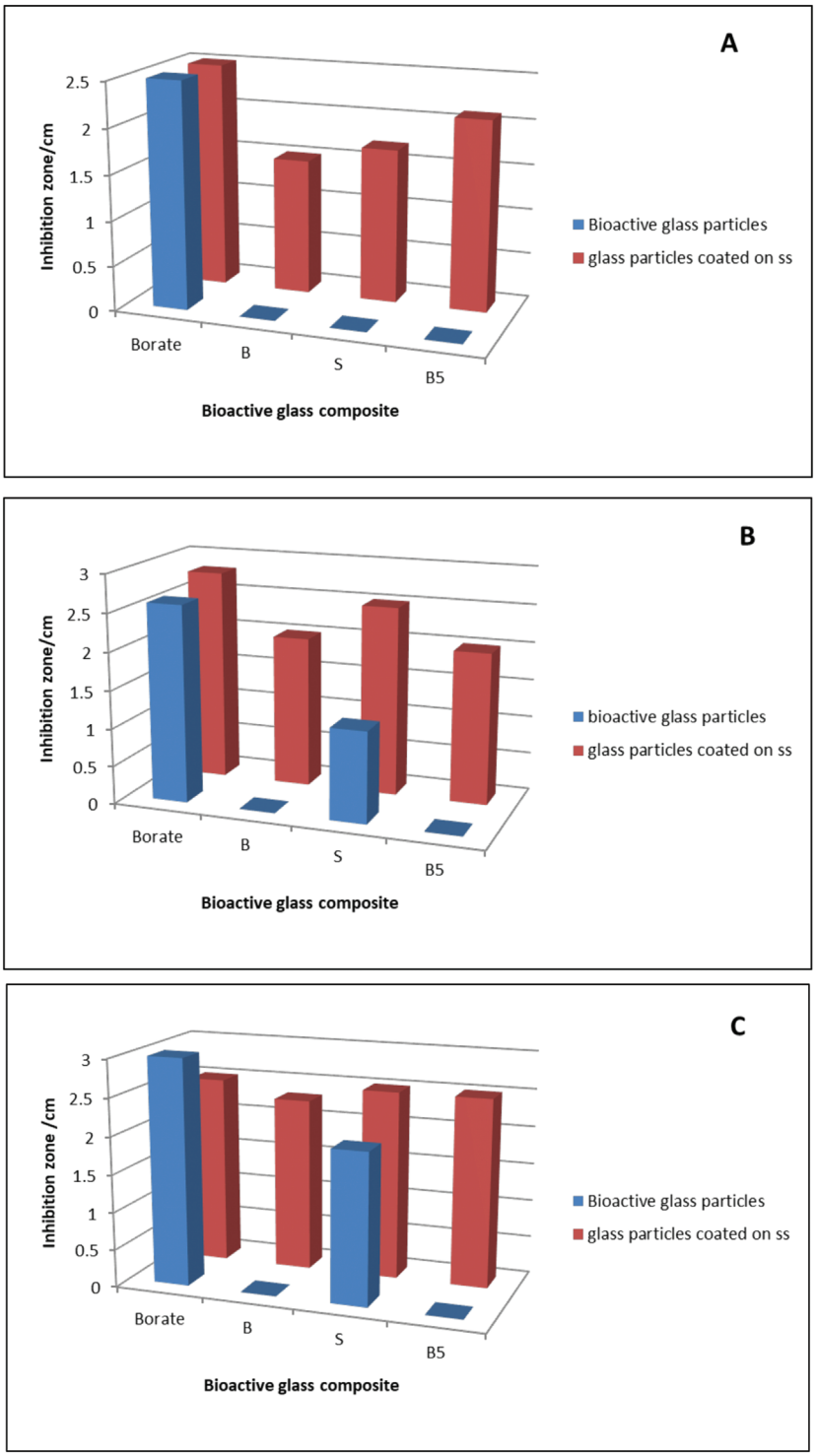
Results and discussion

The use of bioactive glass as an alternative to graft materials used to date has been strongly proposed [2]. It has been reported that the antimicrobial capabilities of borate-based biomaterials were used at the infection site [20].

In this research, it was found that the inhibitory effect of the glass composite was maximized when the glass composite particles were coated on stainless steel sheets. In case of *S. aureus* (Fig. 1a), only the borate particles in the free form caused inhibition of the cells' growth, producing an inhibition zone of 2.5 cm, whereas the other free composite (B, S, and B5) particles showed no antimicrobial activity in the free form (B, S, and B5). However, upon coating on stainless steel sheet, all of the tested composites exhibited antibacterial activity. The borate composite coated on stainless steel sheets showed the highest activity with inhibition zone of 2.5 cm, followed by the B5 (2.1 cm), S (1.7 cm), and B stainless steel-coated composites (1.5 cm).

The results shown in Fig. 1b indicated also that, in case of *E. coli*, only the borate and S composite particles inhibited the bacterial growth showing inhibition zones of 2.6 and 1.2 cm, respectively. On the contrary, application of the composites as stainless steel sheets coated by various glass composites resulted in an increase of the antibacterial activity of borate and S composite by ~2.1- and 1.7-fold, respectively. Furthermore, although the B and B5 composite in the free granules form showed no antibacterial activity, the composites coated on the stainless steel sheet exhibited activity against *E. coli* with inhibition zones of 2 cm for both composites. Similar pattern was shown in case of *P. aeruginosa* that

Figure 1



Antibacterial activity of different bioactive glass composites as free particles or coated on stainless steel sheets against (a) *Staphylococcus aureus* (b) *Escherichia coli*, and (c) *Pseudomonas aeruginosa*.

only free granules borate and S composite caused inhibition of the bacterial growth, whereas the four type of the composites coated on stainless steel exhibited cell growth inhibition (Fig. 1c). The bacterial strains under test were mostly inhibited by borate glass and silicon-based glass composites, respectively. This can be owing to the effect produced as a result of the presence of boron and silicon ions.

As shown in Fig. 2, different tested glass composites coated on stainless steel were able to inhibit both *E. coli* and *P. aeruginosa* bacterial cells with variable efficiency.

Glass composite effect versus vancomycin hydrochloride antibiotic

Vancomycin hydrochloride, a water-soluble antibiotic drug, is commonly used for preventing osseous staphylococcal infections after surgery [21]. It is widely used in orthopedic surgical-site infections to treat prosthetic infections (hip, knee and shoulder) [22,23]. In addition, vancomycin is used widely because it is a wide-spectrum antibiotic used against bacteria and it is low in cost.

To evaluate the glass composite prepared and tested in this study, its antibacterial activity was compared with the activity of vancomycin as a potent antibiotic, as shown in Fig. 3.

The results shown in Table 2 revealed the antimicrobial activity expressed as inhibition zones of 50 mg of each glass composite coated on stainless steel sheet was equivalent to the activity of 50 mg of the vancomycin. These results indicated the potency of the prepared composite coated on stainless steel sheet and its potential applications.

Figure 2

(A) **(B)**

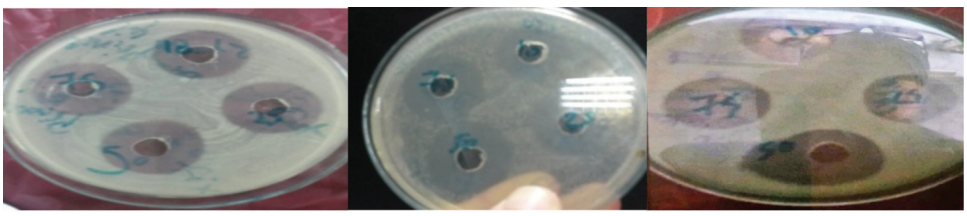
Antibacterial activity of the synthesized composites coated on the stainless steel sheet against (a) *Escherichia coli and* (b) *Pseudomonas aeruginosa*.

Cell viability testing

The pathogen cell viability in the presence and absence of the glass composite was investigated using electron microscopy and bacterial cell count. Figure 4 shows TEM images of the viable *S. aureus* cells grown in the absence and presence of stainless steel sheets coated with borate composite. In absence of any composite, the *S. aureus* cells showed intact cell membrane with no cell damage (Fig. 4a), whereas after 1 h incubation with borate composite, the disintegration of the *S. aureus* cells started (Fig. 4b), and complete cell damage was seen after about 6 h of incubation (Fig. 4c).

Furthermore, the cell viability of the *S. aureus* was investigated by colony count of culture grown in presence and absence of borate glass-coated stainless steel sheet. The results shown in Table 3 demonstrated clearly the severe reduction of viable *S. aureus* cells in the sample after the incubation with the borate composite coated on stainless steel sheet compared with the control. In addition, the pH value of the medium was shifted from pH 7.0–10.4. Interestingly, the ability of one type of bioglass to reduce biofilm produced by *S. aureus* was also shown by Coraça-Huber and colleagues. They emphasized a marked reduction of biofilm mass after being in contact with bioactive glass, as well as a significant decrease in the staphylococci cell count when treated with this type of bioglass.

Figure 3



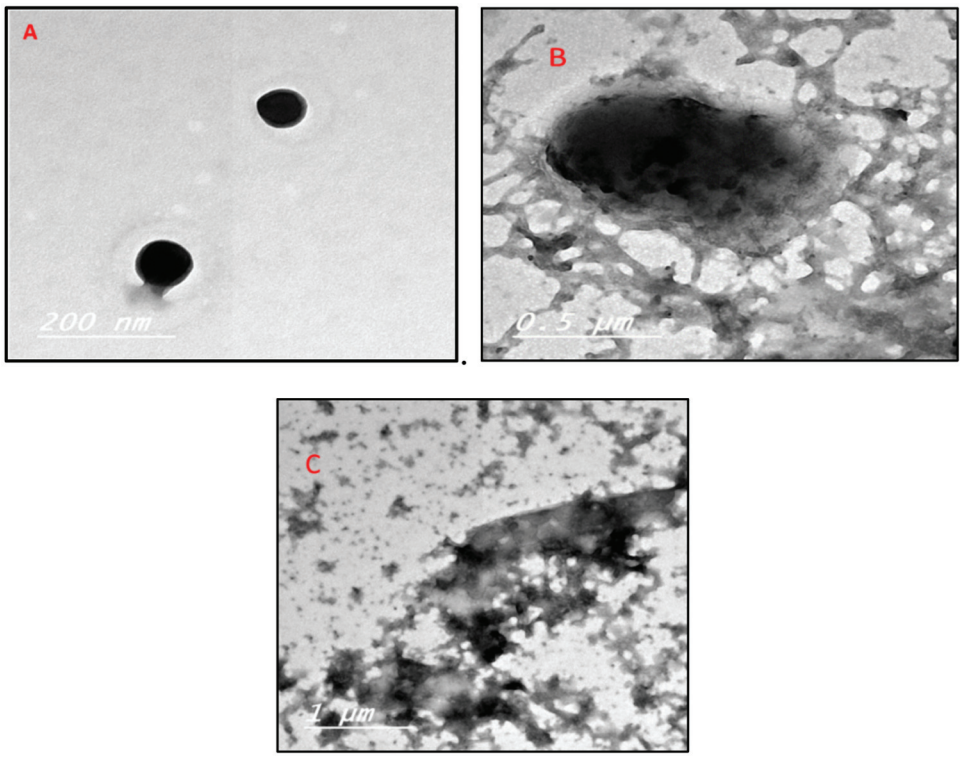
(A) **(B)** **(C)**

Inhibition zones of vancomycin hydrochloride for (a) *Escherichia coli*, (b) *Pseudomonas aeruginosa*, and (c) *Staphylococcus aureus.*

Table 2 Inhibition zones of vancomycin hydrochloride and glass composites against different bacterial pathogens

	Inhibition zone (cm)							
	Vancomycin (mg/ml)				Composite (50 mg)			
Pathogen	100	75	50	25	Borate	B	S	B5
Staphylococcus aureus	3	2.7	2.5	2	2.5	1.5	1.7	2.1
Escherichia coli	2.9	2.9	2.2	1.5	2.8	2	2.5	2
Pseudomonas aeruginosa	4.2	3.9	3.1	2.5	2.5	2.3	2.5	2.5

Figure 4



Transmission electron microscope images showing the effect of borate glass on the *Staphylococcus aureus* cell integrity. (a) *S. aureus* cells in absence of any glass granules, (b) *S. aureus* cell after incubation with borate granules coated on stainless steel sheet for 1 h and (c) after 6 h incubation.

Table 3 The bacterial cell count and final pH of *Staphylococcus aureus* before and after incubation with borate glass composite

	CFU/ml	Final pH
Control (in the absence of borate glass)	25×10^6	7.0
Sample (in presence of borate composite)	18×10^2	10.4

In general, the results revealed that, in case of composite particles, the most effective bioglass composite was the borate glass containing 60% B_2O_3 (wt/wt) followed by the phosphate glass containing 6% P_2O_5 (wt/wt). On the contrary, the inhibitory effect of all composite types under test was enhanced upon coating on the stainless steel sheet. This antibacterial effect was suggested to be referred to several factors, including the release of ions in the medium causing an alteration in the pH value of the surrounding medium [17,18]. Similar to the findings in this study, found that the pH of the medium containing bioglass raised to 10 times of its original value in 1 h [18]. The researchers found that the level of antibacterial effect of bioglass in a broth medium can be similar to that of the same effect in a broth medium adjusted with NaOH, which suggests that high pH alone could be responsible for antibacterial effects. The results presented here confirm that the raised pH value is a potential cause for the reduction of bacterial count [18,19]. Other investigators have suggested that high concentrations of some ions may cause destruction of bacterial cell membranes [6,11]. The effects of changing ion concentrations on bacterial viability should be further investigated.Several methods have been reported in the literature to try to explain the antibiofilm activity of the bioactive glass [2]. In a similar research, borate (B_2O_3) alone inhibited the growth of *E. coli*, *Shigella sonnei*, *Vibrio natriegens*, *Staphylococcus epidermidis*, *Serratia marcescens*, and methicillin-resistant *S. aureus* [8].

Conclusion

Bioactive glasses show great promise as antimicrobial biomaterials that can be used in medical implants and can reduce the risk of bone and joint infections resulting in improving public health. Bioactive glass shows strong antibacterial effects for a wide range of aerobic and anaerobic bacteria, owing to the increase of pH and changing the osmotic pressure of the surrounding environment. Borate-based glass can be used for coatings of medical devices to prevent infection by inhibition of bacterial growth.

In addition to stimulating osteogenesis, the ability of bioactive glasses to inhibit the growth of or kill bacteria

commonly found in a clinical situation is an important application of devices that incorporate this material.

Conflicts of interest

There are no conflicts of interest.

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