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Original Research Article

Effect of Zinc on Antibacterial Action of Bioactive Glass Coating for Dental Implant

Ahmed Salah Hameed* Ahmed Muhammed Abass Luma Jasim Hamood
Israa Abass Obaid
College of Dentistry, University of Babylon , Hilla , IRAQ

*E-mail: aalnoaman7@gmail.com

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Abstract

Peri-implantitis is one of the causes of implant failure. Attempts have been done to coat dental implant with bioactive glasses to enhance osseo-integration and reduce microbial attachment. However, the anti-bacterial action of these coatings was limited to certain bacterial strains . Metals such as zinc and silver have been added to these glasses to improve their anti-microbial action.

In this study zinc (3 mole%)-containing and zinc-free bioactive glasses were synthesized using melt-derived route. The glass coatings on titanium discs were prepared using enameling technique. The antibacterial action of zinc-containing and zinc-free glass coatings was studied against *Streptococcusmutants*, *Staphylococcus aureus* and *Porphyromonasgingivalis* by disc diffusion method. These bacterial strains were obtained by swab samples. The results showed that zinc at 3 mole% had no effect on antibacterial action of bioactive glasses and both glass coatings exhibited similar effect on bacterial strains used in this study. Since these glass coatings have similar effect on aerobic and anaerobic bacteria, these glass coatings can be used in various medical applications.

Key words: Bioactive glasses, Anti-bacterial, Dental implant, Titanium.

الخلاصة

التهاب الانسجة حول زرعة الاسنان هي واحدة من اسباب فشل زراعة الاسنان. محاولات عديدة قد جرت لاحاطة زرعة الاسنان بمادة الزجاج الحيوي لتحسين عملية الالتحام مع العظم وتقليل الالتصاق البكتيري عليها. رغم ذلك لقد وجد ان الفعل المضاد لهذه الزجاجات الحيوية محدد لانواع معينة من البكتريا وغير واسع. لذا فان بعض المعادن مثل الزنك والفضة قد اضيفت الى هذه الزجاجات الحيوية لغرض تحسين الفعل المضاد للبكتريا.

في هذه الدراسة الزجاجات الحيوية التي تحتوي على مادة الزنك بنسبة ٣ مول قد تم تحضيرها بطريقة الذوبان المستخلصة. هذه الزجاجات الحيوية قد صنعت كغطاء لاقراص التيتانيوم بطريقة التلميع. الفعل المضاد للبكتريا لهذه الزجاجات الحيوية والحاوية لمادة الزنك والخالية من الزنك قد درست بطريقة القرص الانتشاري على انواع *Streptococcusmutants,Pyrophyromonasgingivalis, Staphylococcusaureus,* هذه البكتريا تم الحصول عليها بطريقة المسحة من الفم. النتائج اثبتت ان مادة الزنك بنسبة ٣ مول ليس لها اي تاثير على الفعل المضاد للبكتريا لهذه الزجاجات الحيوية وان الزجاجات الحاوية على الزنك والخالية من الزنك لها نفس التاثير على انواع البكتريا المستخدمة في هذه الدراسة. هذه الزجاجات الحيوية يمكن استخدامها في مجالات طبية عديدة مادام لها نفس التأثير على البكتريا الهوائية واللاهوائية.

Introduction

Peri-implantitisis one of the causative factor of dental implant failure. Thisbacterial infectioncauses destruction of bone surrounding dental implant in a mannersimilar to that of periodontitis, an inflammatory condition affecting natural dentition. Titanium has an excellent biocompatible properties, but does not exhibit antimicrobial action. One approach to achieve better disinfection and biocompatibility is to modify titanium implant surfaces[1]. Surface modification of titanium by coating or adding antibacterial properties of metals or alloys to reduce microbial attachment seems an efficient way to achieve success outcome[2]. Bioactive glasses have the ability to dissolve in biological fluids and release ions such as silica, sodium and calcium. This ionic dissolution facilitates hydroxyapatite formation and direct bonding to bone and soft tissues[3]. In addition, these ionic dissolution and the rapid change in the pH of the surrounding medium enable these glasses to exhibit anti-bacterial action[4,5]. Hence, these glasses can be used as coatings for prosthetic metallic implants [6].

The antibacterial action of silica based melt-derived bioglass® was investigated against certain types of microorganisms and the results were promising[7]. Though, the antimicrobial activity of this glass was limited to certain types of microorganisms. Therefore, elements such as zinc, silver and copper have been incorporated into glass composition to broaden the antibacterial action of these glasses. Many studies stated that zinc has antibacterial action [8,9,10,11]. It was thought that the antibacterial action of zinc is attributed to the release of hydrogen peroxide from its surfaces [12]. However, other reportsstated that the inhibition of bacterial growth is related to the change in protein structure and this causes inhibition of specific metabolic enzymes, thus leading to inhibition of bacterial growth [13, 14 ,15].

In this study the effect of Zn-containing bioactive glass coatingson*Porphyromonasgingivalis*, *Strpt.mutants* and *S.aureus*were studied. These bacteria were chosen due to their relevancy in the development and establishment of prei-implantitis.Characterization, bioactivity and cytotoxicity of the glass coatings were investigated previously and the coatings were able to exhibit surface apatite after 1 month immersion in simulated body fluid[16].The prepared glasses have been synthesized by melt-derived route and coated on pure titanium discs by enameling technique. The composition of the prepared glasses is more intricate compared to bioglass®, as they are multi-component system and contains additional elements such as Zn and Mg.

Materials and Methods

Glass synthesis

The zinc-containing glass and zinc-free glass were prepared using reagent grade chemicals (SiO_2, MgO,$CaCO_3$, Na_2CO3, K_2CO_3,MgF_2, ZnO and P_2O_5) in the appropriateproportions. $MgF_2$was substituted partially for ZnO in zinc-free glass. The composition of theseglassesare listed in table 1. The glass batch was melted in a 300 mL platinum–rhodiumalloy crucible using an electric furnace (Hope Valley, LentonThermalDesigns, UK) at temperatures between 1450 and 1460 °C for one anda half hours. The melts were then rapidly quenched in deionized water to prevent crystallization and phase separation. The glass fritproduced was collected in a sieve and dried overnight at 120 °C. Thedried frit was then ground in a Gyro Mill(Glen Creston, UK) for 14 min. and sieved for 60min in a sieve shaker (Retsch,VS1000, Germany) andseparated into more and less than 45 μmparticle size groups.

Table1: Chemical composition (mole%) of QM_5MgO (Zn-containing glass) and QM_5MgF_2 (Zn-free glass)

Glass	SiO_2	CaO	MgO	Na_2O	K_2O	P_2O_5	ZNO	MgF_2
QM5MgO	41.7	36.3	7.8	5.2	1.0	4.7	3.0	………
$QM5MgF_2$	41.7	36.3	9.93	5.2	1.0	4.7	…….	1.0

Glass Coating

A glass coating on a disc of commercially pure titanium (Advent researchmaterials Ltd. U.K. purity≥99.6%) was synthesized by vitreousenamelling technique, using a porcelain furnace (Centurion QuartzNEY). The precipitation method was used in order to make bioactiveglass to settle on a disk of commercially pure titanium. The suspensionwas prepared by dispersing 2.5 g of selected glass particles (<45 µm)in 50 ml ethanol by stirring thoroughly. A disc of commercially pure titaniumof 10 mm, which had been previously polished with 1 mm diamondpaper and cleaned in acetone and ethanol in ultrasonic path, wasdropped in glass powder suspension. This was left in a vacuum oven at80 °C for 1 h so that the glass powder was settled on metal substrate,producing a uniform surface. Confirming that the glass powder completelydeposited, the specimen was introduced in a pre-heated dentalfurnace to 300 °C. Afterwards, it was heated at a rate of 60 °C/min to740 °C followed by 30 min hold at that desired temperature. Duringheating process, the furnace was evacuated to 100% vacuum pressure(0.1 atm).

Samples Collection

Isolation and all biochemical tests for diagnosis of (*S.aureus*,*Strept.mutants*, and *Pyrophyromonasgingivalis*) were carried out according to standard methods [17, 18].

1- Incubation under aerobic condition: By swabs from caries activated in brain heart infusion broth (B.H.I) for one hour. The samples then cultured in blood agar and incubated for 24 hours. The growth appeared as mixing of microorganisms (*Streptococcus*, *Staphylococcus*, Lactobacilli and fungi). Laboratory diagnosis was carried out by gram stain and biochemical test (catalase, coagulase tests) and antibiotic sensitivity test on Muller Hinton agar with 5% blood.

2- Incubation under anaerobic condition: The subgingival plaque samples were inoculated into 2 ml of Brucella broth supplemented with 0.4-µl/ml vitamin K and 5µg/ml hemin. After that, they were diluted and plated onto trypticase soy agar supplemented with 10% defibrinated horse blood,5mg/ml hemin and 0.4 µl/ml vitamin K. The plates were incubated and duplicated in anaerobic atmosphere for 7-10 days or in air plus 10% CO_2 for 2-4 days and staining. The anaerobic bacteria were identified by API20 and rapid ID32A, biochemical tests. Black pigmented,anaerobic ,gram negative rods is considered *Porphyromonasgingivalis*.

Antimicrobial activity

Antibacterial action of zinc-containing and zinc-free glass coatings was investigated*in vitro* using disc diffusion method. Bacterial strains (*S.aureus*, *Strept.mutants*, and *P.gingivalis*) were obtained by swab samples and used to analyze the antimicrobial action. The antimicrobial tests were carried out on the previously prepared solidified and sterilized Mueller-Hinton agar plates. The coated samples were sterilized by dipping in 100% ethanol for 1 minutes.After dryness, they were placed in the agar plates and incubated for 48 hours at 30 ^{o}C . After the incubation period, the inhibitory zone was measured in millimeter (mm) using a transparent ruler. Uncoated titanium disc was used as a control.

Results and Discussion

The antimicrobial efficacy of Zn-containing and Zn-free glass coatings were investigated against bacterial cultures of *S. aureus*, *Strept. mutants*and *P.gingivalis* by disc diffusion method. The two bioactive glasses produced almost equal zones of inhibition ranged between 35-45mm as shown in table 2 and figure 1. However, Zn-containing coating showed slightly wider inhibition zone (50 mm) against *P.gingivalis* compared to Zn-free glass coating (45 mm). These results indicated that zinc at 3 mole% has little, if any, effect on antibacterial action of bioactive glasses. Zinc at 3 mole% was used in order to prevent cytotoxicity and not to interfere with glass solubility and bioactivity.Ainaand her-coworkers[19] studied the effect of incorporating zinc at 5-20 wt% into bioglass® composition and concluded that high zinc content in bioglass retards glass dissolution and enhances cytotoxicity.

The results of present study are consistent with the ionic dissolution studies of the glass coating containing zinc, as the concentration of the released zinc in the biological fluid was not significant; because this ion has the ability toenter the glass network and form Si-O-Zn bonds and is therefore not released in the medium[16].Hence, zinc was unable to potentiate the antibacterial action of the Zn-containing glass.

In general, the antibacterial action of Zn-containing and Zn-freeglass coatings could be ascribed to the high concentration of certain ions such as calcium and silica. It is thought that calcium stimulates auto-agglutination of bacteria on the glass surface [4]; whereas silica inhibits bacterial growth by promoting calcium-phosphate layer formation [20],which could interfere with the integrity of bacterial cell wall. Furthermore, the antibacterial action of these glasses could be assigned to the ionic dissolution of these glasses which increases the alkalinity of the physiological medium[5,21].

Table2: Sensitivity of bacterial strains to QM5MgO and QM5MgF_2 glasses on Mueller-Hinton agar plates

Glass coatings	Bacterial strains	Inhibition zone (mm)
QM5MgO QM5MgF_2	*Staphylococcus aureus*	35 45
QM5MgO QM5MgF_2	*Streptococcusmutans*	45 45
QM5MgO QM5MgF_2	*Porphyromonasgingivalis*	50 45

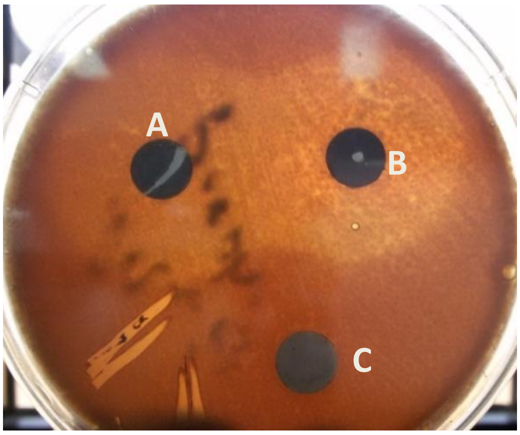

Figure 1: Effect of (A) Zn-containing (B) Zn-free bioactive glass coatings against *S. aureus* (C) is uncoated (control) titanium disc. The coated surfaces facing bacterial growth.

This study showed that the investigated glasses (with and without Zn) have antibacterial action against aerobic and anaerobic bacterial strains found in the oral cavity.For this reason, these glass coatings can be used to coat the body and the neck of dental implant to prevent the establishment of peri-implantitis.In addition, this feature renders these glasses are useful for other clinical applications such as coating of surgical sutures and pin fixation used in oral and maxillofacial surgery.

The uncoated titanium discs did not show zone of inhibition. This indicates that titanium has no antimicrobial effect on the bacterial strains used in the present study.

Conclusion

Zinc at 3 mole% has no effect on antibacterial action of bioactive glass. The glass containing zinc and zinc free glasses exhibit similar inhibitory action on growth and multiplication of *S. ureus*, *Strept.mutants* and*P.gingivalis*.These glasses can be used in a variety of medical and dental applications.

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