Aperito Journal of Infectious Disease and Vaccines

Received: Apr 21, 2015
Accepted: May 05, 2015
Published: May 08, 2015

http://dx.doi.org/10.14437/AJIDV-1-108 Research Mahmoud M Elhaig, Aperito J Infect Dis Vaccines 2015, 1:2

Synthesis of Bioactive Glass and its Antibacterial Effect on Mupirocin Resistant, Methicillin-Resistant Staphylococcus Aureus

Samaa A Taha[1], Mahmoud M Elhaig[2*] and Azza Nady MH[3]

[1]Department of Microbiology and Medical Immunology, Faculty of Medicine, Suez Canal University, Ismailia, Egypt

[2]Department of Animal Medicine (Infectious Diseases), Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt

[3]Department of Basic Sciences, Higher Technological Institute, 10th of Ramadan City, Egypt

Abstract

Rapid resistance to mupirocin occurs among strains of Methicillin-Resistant Staphylococcus Aureus (MRSA) which the main etiological agent is causing community-acquired and hospital-acquired infections. Powdered samples of Sr-doped glass, of composition 70 SiO_2- (30–x) CaO- xSrO were prepared by sol – gel method. XRD and FTIR confirmed the calcined glass existed in the amorphous state. Sr doped glass exhibited antibacterial effect onmupirocin resistant MRSA strains by the agar-disk diffusion method. The inhibition zone increased as the concentration of Sr ions was increased. Sr release was dependent on bioactive glass composition with increasing Sr substitution resulting in higher concentrations in the medium. Sr for Ca substitution in the glass is a promising bactericidal bioactive material till 3% SrO, but SrO substitution above that ratio doesn't further improve bactericidal effect

***Corresponding Author:** Mahmoud M Elhaig, Department of Animal Medicine (Infectious Diseases), Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt; Tel: +20 643327052; E-mail: mahmoudvet1975@gmail.com

Keywords: Antibacterial Effect; MRSA; Mupirocin Resistance; Sr-doped glass; FTIR& XRD of bioactive glass

Introduction

Resistance in human pathogens is a big challenge in pharmaceutical fields and biomedicine. Antibiotic resistance profiles lead to fear about the emergence and reemergence of Multi Drug-Resistant (MDR) pathogens and parasites [1]. Infection with MDR bacteria cannot be cured easily and requires a multiple treatment of broad-spectrum antibiotics, which are less effective, more toxic and more expensive [2]. Therefore, development of or modification in antimicrobial compounds to improve bactericidal potential is a priority area of research in this modern era [3].

Methicillin-Resistant Staphylococcus Aureus (MRSA) strains are a major causal agent of community-acquired and hospital-acquired infections [4]. Mupirocin (Bactroban; SmithKline Beecham Pharmaceuticals), is one of the most effective topical antibiotics used for eradication of nasal carriage of MRSA [5, 6]. With increased use of mupirocin, staphylococcal strains with high and low levels of resistance have been reported [7]. Most commonly, low-level (MICs = 8 to 256 µg/ml) mupirocin-resistant strains are isolated, while high

level (MIC ≥ 512 µg/ml) mupirocin-resistant strains are infrequently isolated. High-level mupirocin resistance has been connected with clinical and microbiological failure [8, 9].

Nanoparticles have promising applications in diagnostics, biomarkers, cell labeling, antimicrobial agents, drug delivery systems for handling of various diseases [10, 11]. Hence, researchers are shifting towards nanoparticles to solve the problem of the emergence of multi- drug resistant bacteria [12].

A more recent field of research is the synthesis of anti-bacterial glasses as bioactive glasses are the only one, which could bond to hard and soft tissue. Biomaterials in nano-scale could stimulate the reaction between the materials and cells [13]. Continuous work is done to improve the properties of biomaterial to have antimicrobial properties. Sr–doped glasses have been shown to exhibit enhanced cellular bioactivity and to release critical concentrations of Sr ions in the range of 1–5 ppm into dissolution medium [14]. Moreover, ion release from Sr–doped silicate glasses enhances the bone cell activity and inhibited osteoclasts differentiation when directly applied in contact with cells as solid bioactive glass–discs. Bioactive glass forms a firm bond with living tissues via the establishment of a hydroxyapatite layer on their surface [15, 16].

Sol–gel technique, as a chemical method, provides an available means to synthesize bioactive glass with higher purity and homogeneity at low temperatures [17]. In recent years, strontium has been incorporated into dental and orthopaedic biomaterials to reduce microbial contamination by inhibiting bacterial growth and reproduction and impeding permeability of the cytoplasmic membrane, cell wall synthesis, replication of bacterial chromosomes and cell metabolism [18, 19]. Strontium for calcium substitution in the glass adjusted the bactericidal properties of the bone cements making strontium a promising bactericidal component of bioactive glasses and related biomaterials [20].

In the present study different compositions (x = 0, 1, 3 and 5%) of SrO substituted bioactive glasses in the (70 SiO_2 – 30 - x CaO – x SrO) system were synthesized and characterized then their antibacterial effect on mupirocin resistance MRSA was investigated.

Materials and Methods

Bacterial Isolates

Thirty nasal swabs were taken by rotating a sterile cotton swab, moistened with sterile saline, in the vestibule of both anterior nares of medical and paramedical personals working in Suez-Canal University Hospital during the period from May 2012 to October 2013. Trypticase Soy Broth (TSB) was used as a transport medium. The swabs were put directly onto Mannitol Salt Agar (MSA) (Merck, Germany) and sent to the laboratory and incubated at 35º C in a humidified incubator for 48 h. Strains that produced yellow colonies on the MSA plate, were sub cultured on a blood agar plate (Merck, Germany), for further characterization. The staphylococcal isolates were identified by conventional methods, e.g. colonial morphology, gram staining characteristics, production of catalase, coagulase tube method using rabbit plasma, DNase in tube tests and other biochemical tests [21].

MRSA Isolation

Isolates showing inhibition zones of ≤10 mm and ≤21 mm around 1 mg oxacillin (Oxoid, Cambridge, UK) and 30 µg cefoxitin (Oxoid, Cambridge, UK) disks respectively, were phenol typically characterized as MRSA.

Mupirocinsusceptibility Testing

The MICs for S. aureus isolates to mupirocin were assessed using E-test® mupirocin strips (AB-BIODISK, Solna, Sweden) according to the manufacturer's instructions. The E-test strip was applied onto each plate of Mueller-Hinton agar that was inoculated with a suspension of isolates to the optical density of a 0.5 McFarland standard with sterile forceps. Following incubation at 35° C for 24 h. E-test MIC values were read by the operator at the point where the bottom of the inhibition zone intersected with the E-test strip. Strains were considered to be susceptible if the MIC value was ≤ 4 mg/L and

levels of mupirocin resistance were defined as low-level with MIC 8–256 mg/l and high-level with MIC ≥ 512 mg/L [22].

Detection of the mupA Gene by PCR

All the strains were analyzed by Polymerase Chain Reaction (PCR) for the presence of the mupA gene. To extract the DNA, a loop full of bacterial cells from each sample was removed and resuspended in 250 µl of sterile distilled water and the suspension incubated in a 90^0C heat block for 15 min. Centrifugation followed (7500 x g, 5 min) and the supernatant containing the staphylococcal DNA extract was transferred into new test tubes and frozen for later PCR amplification. Five micro liters of the extracted DNA was transferred to 20 µl of the PCR amplification mix consisting of; 2.5 µl of 10 X buffer, 1.5 mM of $MgCl_2$, 1.5 U of Taqpolymerase, 1.25 µl of dNTPs and 1.5 µl of each primer. To detect the mupA gene, a 456-bp region was amplified by PCR, using a primer pair Mup1 (5'-TAT ATT ATG CGA TGG AAG GTT GG-3') and Mup2 (5'-AAT AAA ATC AGC TGG AAA GTG TTG-3') [23]. Cycling parameters were 94^0C for 5 min followed by 30 cycles, denaturation at 94 ^{0}C for 30 s, annealing at 52 ^{0}C for 30 s and extension at 72^0C for 30 s, and final 5 min incubation at 72^0C. Following PCR amplification, the amplified PCR products were analyzed by agarose gel electrophoresis stained with ethidium bromide and the amplicons were visualized using a UV light box.

Synthesis of Bioactive Glass

CaO was replaced by SrO on a molar base as shown in table 1, the system was produced by Sol – gel method using TetraEthylOrthoSilicates (TEOS), calcium nitrate tetra hydrate Ca $(NO_3)_2$. $4H_2O$, and strontium nitrate Sr $(NO_3)_2$ [17]. Briefly, TEOS, distilled water and 2M nitric acid were mixed in ethanol and the mixture was allowed to react for 60 min under continuous magnetic stirring for the acid hydrolysis of TEOS. Then Ca $(NO_3)_2$.$4H_2O$ was added and stirred for 30 min, the SrO was added and stirred for the same period. The mixture was left at room temperature to form gel. Each prepared gel was dried at 75^oC for 2 days in a drying oven and finally gels were stabilized by heat treatment up to 700^oC.

Table 1: Glass composition (weight %) and Sr for Ca substitutions

	Glass	SiO_2	CaO	SrO
Sample a	Sr_0	70	30	-
Sample b	Sr_1	70	29	1
Sample c	Sr_3	70	27	3
Sample d	Sr_5	70	25	5

Characterization

For characterization, the phase analysis of the samples was examined by X–ray diffractometer, model BRUKER axes, using Ni–filtered CUKa irradiation at 40 KV and 25mA.The infrared spectra of the prepared glass were obtained using Fourier Transform Infrared spectrophotometer (FT–IR) (Model 580, Perkin–Elmer). Each sample was pressed in pellet and mixed with KBr substrate. FTIR spectrum was recorded in the 4000–400 Cm^{-1} region.

Antibacterial Activity Test

The antibacterial activity of Sr–doped glasses was evaluated against mupirocin resistant MRSA strains using the agar-disk diffusion method. Freshly prepared bacterial inoculums were swabbed on sterile petri-dishes 90mm in diameter containing sterile Mueller-Hinton agaras recommended by CLSI [24]. The medium was swabbed three times by rotating the plate 60^0 after each application to ensure the spread of the bacteria over the entire surface of the plates. The discs of the prepared Sr–doped glasses were placed using sterile forceps on the seeded agar surface and incubated at 37^0c for 24hrs.The antibacterial activity was deduced from the Inhibition Zone Diameter (IZD), zone of no bacterial growth, measured under the stated experimental conditions. The antibacterial activity increases with the increase in IZD and vice versa.

Results

In this study Sol-gel method showed a fast method for preparing bioactive glasses. The XRD pattern of bioactive glass powders of Sr_0, Sr_1, Sr_3 and Sr_5 for 0 % SrO, 1% SrO, 3% SrO and 5% SrO, respectively after heating to 700 ^{0}C is presented in Figure 1. The XRD study confirmed the glass existed in an amorphous state, generally no diffraction peaks could be observed.

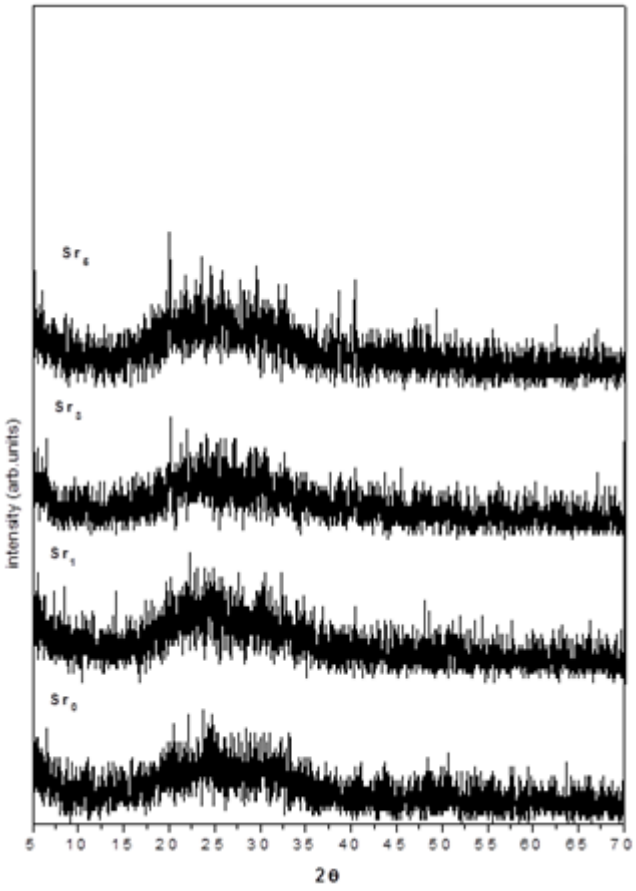

Figure 1: XRD patterns of gel glass after heating to 700 ^{0}C for Sr_0, Sr_1, Sr_3, and Sr_5.

Figure 2 showed the FTIR spectra for the bioactive glass powders after heating to 700 ^{0}C. For glass samples, the band located in the range 1000-1200 cm^{-1} corresponds to the Si–O–Si asymmetric stretching vibration, and the band located at 440 – 540 cm^{-1} corresponds to the vibrational mode of the binding of Si–O–Si. The bands located at 670-740 cm^{-1} correspond to Si–O symmetric stretch of Bridging Oxygen (BO) between tetrahedron chains, and the shoulder of spectrum at 870 – 1040 cm^{-1} is related to the Non-Bridging Oxygen (Si–O–NBO).

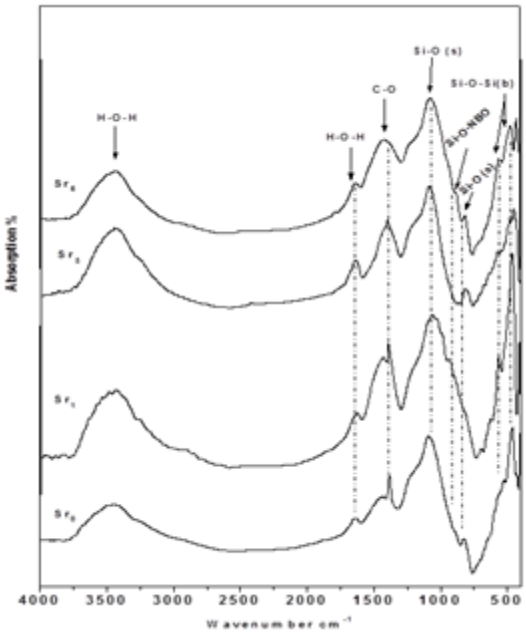

Figure 2: FTIR spectra of synthesized gel glass with different amounts of SrO (Sr_0, Sr_1, Sr_3, and Sr_5).

Out of the 30 MRSA strains tested, low-level mupirocin resistance was detected in only 2 (6.6%) MRSA strains using E test as their MICs were 12 and 16 µg / ml. PCR analysis of the mupA gene confirmed its presence in these 2 strains while the rest of the strains tested were susceptible to mupirocin (Figure 3).

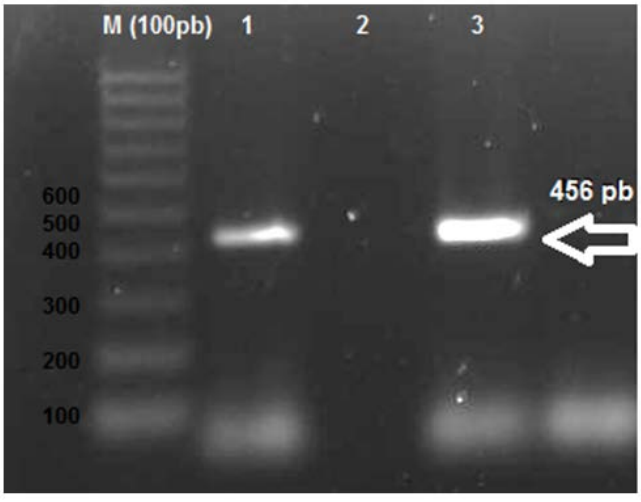

Figure 3: PCR analysis of the mupA gene using 1% agarose gel electrophoresis, M represent the DNA - size maker (100 bp

ladder); lanes 1 and 3 represent positive samples; lane 2 represents a negative sample for the mupA gene.

Figure 4 shows the antibacterial effect of the preparation Sr–doped glasses on the diagnosed mupirocin resistant MRSA strains using the agar-disk diffusion method. It was found that, Sr–doped glasses were highly effective on the tested organisms, especially sample c containing 0.619 gm of Sr–doped glasses. It showed the highest clearing inhibition zone, which was nearly 33 mm in diameter, while other samples (sample b & d) showed only 28- 30 mm inhibition zones. The sample without Sr–doped glasses (sample a) had no effect on the tested bacterial isolates.

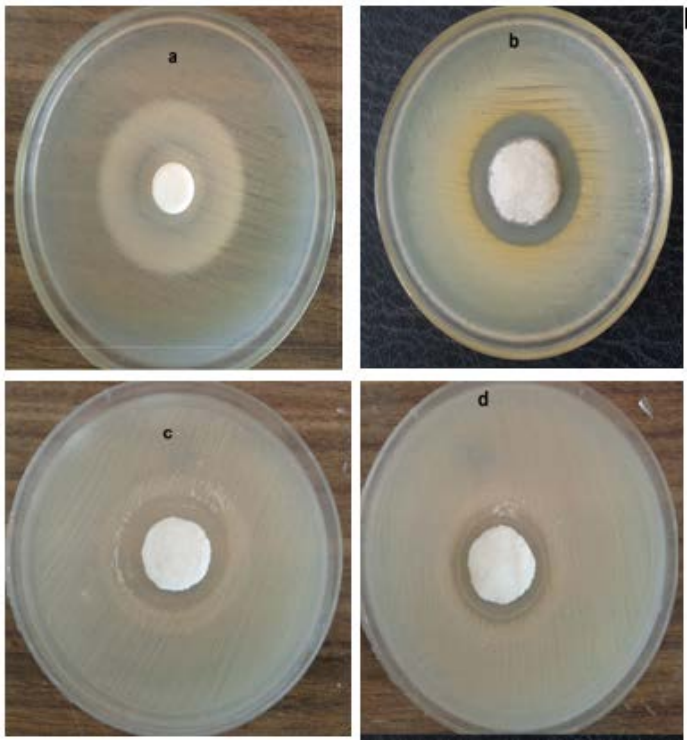

Figure 4: The antibacterial effect of the prepared Sr–doped glasses on a representative mupirocin resistant MRSA strain.

The inhibition zone increased as the concentration of Sr ions was increased as Sr release was dependent on the bioactive glass composition with increasing Sr substitution resulting in higher concentrations in the medium till 3% SrO (Figure 5).

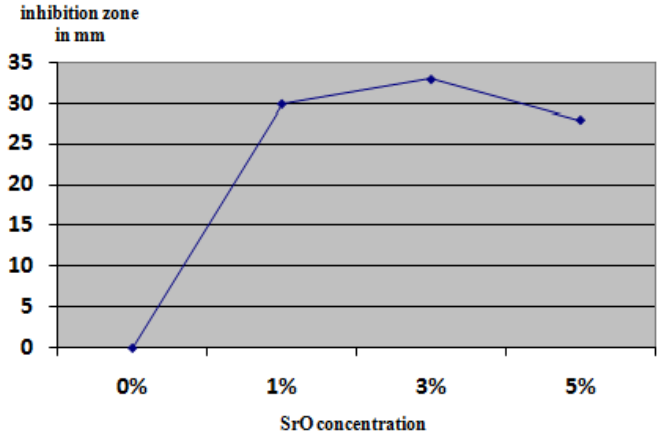

Figure 5: The relationship between SrO concentration and inhibition zone diameter in mm

Discussion

The broadening of absorption band Si – O – Si as shown in Figure 2, indicates the amorphous nature due to disorder in silicate network [17] for all samples, which was confirmed by XRD. When strontium is added to silica, the band at 823 cm^{-1} for NBO will shift to higher wave number depends on the amount of strontium. At the same time, the shift from 950 cm^{-1} to a lower wave number due to the lower charge – to – size ratio of Sr^{2+} (owing to slightly larger ionic radius) was shown to cause an expansion of the silicate network, making it less strongly ionically cross linked [20] causing breakdown of silicon network and formation of short and terminal chains.

Different frequencies of mupirocin resistance among S. aureus isolates has been clearly defined in many parts of the world, for example,Spain11.3%, USA 13.2%, Trinidad Tobago 26.1%, China 6.6%, India 6%, Turkey 45% and Korea 5%, however, it does appear to be increasing worldwide [25]. The limited number found in this study could be attributed to the limited use of that antibiotic to eliminate MRSA nasal colonization as a part of the infection control measures in our hospitals. Also, it is infrequently used for the treatment of skin infections.

The inhibition zone increased as the concentration of Sr ions was increased as Sr release was dependent on the bioactive glass composition with increasing Sr substitution resulting in higher concentrations in the medium till 3% SrO.

But above 3% SrO the inhibition zone doesn't increase with less bactericidal action which could result from decreased Sr release with strontium substitution above that level [20].

Rapid resistance to mupirocin occurs among strains of S. aureus isolated from hospitals. Therefore, monitoring for mupirocin resistance in S. aureus, especially in MRSA, is necessary to evaluate the usefulness of mupirocin in both the treatment of staphylococcal infections and infection control. Sr for Ca substitution in the glass was shown to be a promising bactericidal bioactive material till 3% SrO. But the amount of Sr above this ratio doesn't improve bactericidal action.

References

1. Tenover F, (2006). Mechanisms of antimicrobial resistance in bacteria. Am J Med 119: S3–S10.
2. Webb GF, D'Agata EM, Magal P.Ruan S, (2005). A model of 499 antibiotic-resistant bacterial epidemics in hospitals. Proc Nat AcadSci 102: 13343–13348.
3. Humberto H, Lara V, Ayala-Nunez NV, Carmen LD, Ixtepan T, Cristina RP, (2010). Bactericidal effect of silver nanoparticles against multidrug-resistant bacteria. World J MicrobiolBiotechnol 26: 615–621.
4. Askarian M, Zeinalzadeh A, Japoni A, Alborzi A, Memish ZA, (2009). Prevalence of nasal carriage of methicillin-resistant Staphylococcus aureus and its antibiotic susceptibility pattern in healthcare workers at Namazi Hospital, Shiraz, Iran.Int J Infect Dis**13** (5):e241-7.
5. PerlTM,Cullen JJ, Wenzel RP, Zimmerman MB, Pfaller MA, Sheppard D, TwombleyJ,French PP, Herwaldt LA,(2002). Intranasal mupirocin to prevent postoperative Staphylococcus aureus infections. N. Engl. J. Med. 346:1871–1877.
6. Kalmeijer MD, Coertjens H, van Nieuwland-BollenPM, Bogaers- Hofman D, de BaereGA, Stuurman A, van Belkum A, Kluytmans JI,(2002). Surgical site infections in orthopedic surgery: the effect of mupirocin nasal ointment in a double-blind, randomized, placebo-controlled study. Clin Infect Dis 35:353–358.
7. Vasquez JE, Walker ES,FranzusBW, Overbay BK, ReaganD R, SarubbiFA,(2000). The epidemiology of mupirocin resistance among methicillin-resistant Staphylococcus aureus at a Veterans' Affairs Hospital. Infect. Control HospEpidemiol 21:459–464.
8. Saderi H, Owlia P, Habibi M, (2008).Mupirocin resistance among Iranian isolates of Staphylococcus aureus. Med SciMonit 14 (10):BR210-3.
9. Lim KT, Hanifah YA, MohdYusof MY, Thong KL,(2010). Prevalence of mupirocin resistance in methicillin-resistant Staphylococcus aureus strains isolated from a Malaysian hospital. Jpn J Infect Dis 63 (4):286-9.
10. Marcato PD, Duran N, (2008). New aspects of nanopharmaceutical delivery systems. J NanosciNanotechnol 8: 2216–2229.
11. Singh R, Singh NH, (2011). Medical applications of nanoparticles in biological imaging, cell labeling, antimicrobial agents, and anticancer nanodrugs. J Biomed Nanotechnol 7: 489–503.
12. GemmellCG, Edwards DI,Frainse AP, (2006). Guidelines for the prophylaxis and treatment of methicillin-resistant Staphylococcus aureus (MRSA) infections in the UK. J AntimicrobChemother 57: 589–608.
13. Webster TJ, Ergun C, Doremus RH, Bizios R, (2002). Hydroxylapatite with substituted magnesium, zinc, cadmium, and yttrium. II. Mechanisms of osteoblast adhesion. J. Biomed. Mater. Res, 59(2), 312-317.
14. Hoppe A, Güldal NS, Boccaccini AR, (2011). A review of the biological response to ionic dissolution products from bioactive glasses and glass-ceramics. Biomaterials, 32(11), 2757-2774.
15. Gentleman E, Fredholm YC, Jell G, Lotfibakhshaieh N, O'Donnel MD, Hill RG, Stevens MM, (2010). The effects of strontium – substituted bioactive glasses on osteoplasts and osteoclasts in vitro, Biomaterials 31 (14): 3949 – 56.

16. Jebahi S, Oudadesse H, El Feki H, Rebai T, Keskes H, Pellen P, El Feki A, (2012). Antioxidative/oxidative effects of strontium-doped bioactive glass as bone graft. In vivo assays in ovariectomised rats. Journal of Applied Biomedicine, 10(4), 195-209.
17. El-Gohary MI, Tohamy KM, El-Okr MM, Ali AF, Soliman IE, (2013). Influence of composition on the in-vitro bioactivity of bioglass prepared by a quick alkali-mediated sol–gel method. Nat. Sci, 11(3): 26 – 33.
18. Alkhraisat MH, Rueda C, Cabrejos-Azama J, LucasAparicio J, Marin˜o FT, Torres Garcia-Denche J, Jerez LB, Gbureck U, Cabarcos EL,(2010). Loading and release of doxycycline hyclate from strontiumsubstituted calcium phosphate cement. ActaBiomater. 6: 1522 – 1528.
19. Dabsie F, Gregoire G, Sixou M, Sharrock P, (2009). Does strontium play a role in the cariostatic activity of glass ionomer?: Strontium diffusion and antibacterial activity. J dentistry, 37(7), 554-559.
20. Brauer DS, Karpukhina N, Kedia G, Bhat A, Law RV, Radecka I, Hill RG, (2012). Bactericidal strontium-releasing injectable bone cements based on bioactive glasses. **J. of The Royal Society Interface**, rsif20120647.
21. Halablab MA, Hijazi SM, Fawzi MA, Araj GF,(2010).Staphylococcus aureus nasal carriage rate and associated risk factors in individuals in the community. Epidemiol Infect 138 (5):702-6.
22. Saderi H, Owlia P, Habibi M. (2008).Mupirocin resistance among Iranian isolates of Staphylococcus aureus. Med Sci Moni14 (10):BR210-3.
23. Yun HJ, Lee SW, Yoon GM, Kim SY, Choi S, Lee YS, Choi EC, Kim S, (2003).Prevalence and mechanisms of low- and high-level mupirocin resistance in staphylococci isolated from a Korean hospital. J AntimicrobChemother51 (3):619-23.
24. Clinical and Laboratory Standards Institute (2009). Performance standards for antimicrobial susceptibility testing; nineteenth informational supplement M100-S19. Clinical and Laboratory Standards Institute, Wayne, PA.
25. Mohajeri P, Gholamine B, Rezaei M, Khamisabadi Y,(2012). Frequency of Mupirocin Resistant Staphylococcus aureus Strains Isolated From Nasal Carriers in Hospital Patients in Kermanshah. Jundishapur J Microbiol.5(4):560-63.