Scholars LITERATURE

La Prensa Medica Argentina

Research Article

DOI: https://doi.org/10.47275/0032-745X-299
Volume 105 Issue 6

Optimization of Facial Silicone Elastomers Antimicrobial Activity: *In-Vitro* Study

Hamed Alzoubi[1]*, Muhanad Moh'd Hatamleh[2,3]

[1]Associate Professor, Department of Microbiology and Immunology, Faculty of Medicine, Mu'tah University, Jordan
[2]Assistant Professor, Vice Dean, Luminus Technical University College, Amman, Jordan
[3]Senior Clinical Scientist, Dental Hospital, King's College Hospital, London, United Kingdom

Abstract

Objective: Maxillofacial prostheses when worn can be contaminated by oral and skin microflora, which risk the patient's tissue. Also, skin around craniofacial implants retaining maxillofacial prosthesis shows a microflora of potential pathogens causing peri-abutment infection. This study aimed to investigate the effect of Zinc Oxide nano particles (ZnO-NP) and Chlorohexidine Diacetate Salt (CHX) inserts on the antimicrobial activity of maxillofacial silicone elastomer at three difference concentrations (1%, 3%, and 5%).

Materials and Methods: a commonly used maxillofacial silicone elastomer (M511, Technovent, UK) was mixed with ZnO-NP and CHX at 1%, 3%, and 5% concentrations (by weight). Then it was packed inside disc-shaped steel moulds (40 mm diameter and 0.5 mm height) and cured for 1 hour at 100°C then smaller silicone discs (10 mm diameter) were produced. Sixty specimen per insert were made for the three concentrations. Each concentration included 20 sample which were divided equally between two inoculants; *Staphylococcus aureus* (*S. aureus*) and *Candida albicans* (*C. albicans*). Eighty samples of silicone without any insert were made and divided into 4 groups acting as negative control (NC) and 4 groups acting as double negative control (DNC). The upper surface of each specimen was inoculated with 200µl of inoculant (106 cfu). NC specimens were inoculated with 200 µl of inoculant (*S. aureus* or *C. albicans*) without CHX or ZnO-NP. DNC specimens were inoculated with 200 µl sterile distilled water and it did not have CHX or ZnO-NP nor *S. aureus* nor *C. albicans*.

Results: There was an observed decrease in colony forming units of *S. aureus* and *C. albicans* when tested against 1%, 3%, and 5% concentrations of CHX and ZnO-NP. However, a complete growth inhibition of both organisms was only obtained at 5% concentration of CHX where there was zero number of colonies (100% growth inhibition). No colonies were detected at the DNC disc as expected. The NC specimens showed numerous uncountable colonies as expected.

Conclusion: CHX exhibited antibacterial activity against some oral pathogenic strains better than ZnO-NP. It has the potential to completely eliminate bacteria when mixed at 5% (by weight), thus, contributing to the disinfection of silicone-based materials and the preventing infections caused by *Candida albicans* or *Staphylococcus aureus*.

Keywords: Maxillofacial prostheses; Silicone; Zinc oxide; Nanoparticles; Chlorhexidine; Antimicrobial

Correspondence to: Hamed Alzoubi, Department of Microbiology and Immunology, Faculty of Medicine, Mu'tah University, Jordan; E-mail:- dr_alzoubi@mutah.edu.jo

Citation: Alzoubi H, Hatamleh MM (2019) Optimization of Facial Silicone Elastomers Antimicrobial Activity: *In-Vitro* Study. Prensa Med Argent, Volume 105:6. 299. DOI: https://doi.org/10.47275/0032-745X-299.

Received: October 25, 2019; **Accepted:** November 11, 2019; **Published:** November 16, 2019

Introduction

Maxillofacial prostheses are an efficient treatment option when maxillofacial defects resulting from ablative surgery, trauma, or congenital anomalies cannot be surgically reconstructed. Silicone elastomers, mainly polydimethylsilioxane (PDMS) have been used for over 50 years in constructing maxillofacial prostheses for individuals with facial defects [1]. Such prostheses play integral role in improving patient's quality of life. However, they usually last for 18 to 24 months as they deteriorate in structure, colour fade and become stained requiring their remake [2].

The silicones are porous in nature [3], making those favourable sites for bacterial colonization causing prosthesis staining and infections [4]. A black discoloration is usually evident on the prosthesis's inside surfaces after they have been worn for a period of time (Figure 1).

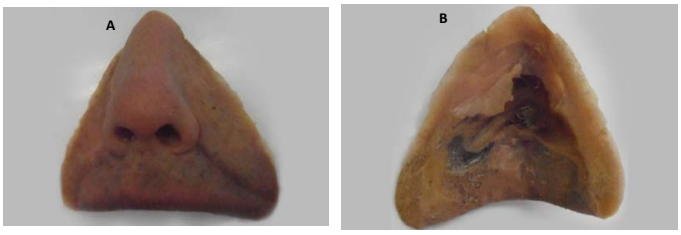

Figure 1: (a) Example of large midfacial prosthesis of intra-oral communication and (b) showing black discolouration on the prosthesis fitting surface.

Microbiologic techniques and SEM studies revealed that a fungus belonging to the genus Penicillium is associated with such discolored areas. Furthermore, disk diffusion tests determined that the antifungal agent clotrimazole, when incorporated into silicone samples, was effective in inhibiting it's in vitro growth [5,6]. Titanium oxide (TiO_2)

has been advocated as an antibacterial agent based on its photocatalytic properties proposing its use for preventing bacterial contamination and disinfection [7]. Other oxides of similar anti-fungal activities were ZnO, SiO_2 and Al_2O_3 [8]. But there is contradictory results of their effects requiring further evidence.

For example, ZnO which is a bio-safe material that possesses photo-oxidizing and photocatalysis impacts on chemical and biological species. It exhibits attractive antibacterial properties due to increased specific surface area as the reduced particle size leading to enhanced particle surface reactivity but research still inconclusive about its antibacterial effect [9]. CHX is well known for its antibacterial and antifungal activities and have been commonly used in different fileds of dentistry [10]. It affects the colonization rate of oral bacteria on the enamel surface by coating the surface of the bacteria which results in prolonged and persistent antimicrobial action.

The use of the above mentioned inserts in maxillofacial silicone prostheses is limited. Thus, this study aimed to investigate the effect of Zinc Oxide nano particles (ZnO-NP) and Chlorohexidine Diacetate Salt (CHX) inserts on the antimicrobial activity of maxillofacial silicone elastomer at three difference concentrations (1%, 3%, and 5%).

Materials and Methods

Preparing Silicone Samples

Commonly used maxillofacial silicone elastomer (M511, Technovent, Newport, Wales) was mixed according to manufacturer instructions with ZnO nano particles (ZnO-NP) (BET= 67 m^2/g) and Chlorohexidine Diacetate Salt (CHX) at different concentrations: 1%, 3% and 5% (by weight). The mixture was manually mixed for 5 sec and placed in a Speed Mixer at 2000 rpm (revolution per minute) for 60 sec (Speedmixer, Dac 150, Hauchild Engineering, Germany). Then it was packed inside disc-shaped steel moulds (40 mm diameter and 0.5 mm height) and cured for 1 hour at 100 °C. Once curd, smaller silicone discs (10 mm diameter) were produced. Sixty specimen per insert were made available for the three concentrations. Each concentration included 20 specimen which were divided equally between two inoculants; *Staphylococcus aureus* (*S. aureus*) and *Candida albicans* (*C. albicans*). Eighty specimens of silicone without insert were made and divided into 4 groups acting as negative control (NC) and 4 groups acting as double negative control (DNC). All groups are present in table 1.

Preparation of the Microbial Test Inoculum

S. aureus ATCC 25923 and *C. albicans* ATCC 10231 were obtained from the microbiology laboratory, Faculty of Medicine, Mutah University. Microorganisms were retrieved from the -70°C and streaked using sterile inoculating loop onto nutrient and hypertonic Dextrose Agar (SDA) (BBL Microbiology Systems, Becton Dickinson, Company, Sparks, MD, U.S.A.) to obtain single colonies. Plates were incubated at 36 °C ± 1 overnight. Growing *S. aureus* colonies were confirmed by their typical colony morphology, Gram's staining, catalase production and tube coagulase test. *C. albicans* was confirmed by colony appearance, gram stain and germ tube test. Ten colonies of each growing organism were suspended separately in 5 ml brain heart infusion and incubated at 36 °C ± 1 overnight. The culture suspension of *S. aureus* and *C. albicans* was utilized to calculate the neat colony forming units for each organism using serial dilution method.

Testing Silicone Discs

Testing the Sterility of the Silicone Discs: Sterility of silicone discs was carried out by washing them using either 70% ethanol, distilled water or nothing. The discs were then incubated at 36 °C ± 1 overnight. After that, the uppermost face of each disc was then cultured using sterile forceps by placing onto nutrient agar for 15s then removed. Agars were then incubated at 36 °C ± 1 overnight before being examined for any growth of microorganisms.

Antimicrobial Inhibitory Effect of Silicone Discs: Antimicrobial activity of the silicone discs were assessed as previously described [11]. S. *aureus* and *C. albicans* suspensions were adjusted to $5x10^6$ colony forming units/ml (cfu/ml). For *S. aureus*, the first test group was silicone discs that contains 1%, 3% or 5% chlorhexidine in triplicates. The second test group was silicone discs with 1%, 3% or 5% ZnO-NP in triplicates. The silicone samples were aseptically placed in a sterile petri dish using sterile forceps with their release liner facing up.

The upper surface of each disc was inoculated with 200 µl of *S. aureus* suspension (10^6 cfu) using a sterile pipette. One of the silicone discs with 0% concentration was inoculated with 200 µl sterile distilled water and considered as double negative control (DNC), since it has no CHX or ZnO-NP and no bacteria was added. Another 0% disc was inoculated with 200 µl *S. aureus* suspension and considered as a negative control (NC), since it has no CHX or ZnO-NP but with bacteria added. To prevent inoculum leakage, drying and to ensure proper contact to the discs, the inoculated silicone samples were covered with a sterile cover film.

The discs were incubated at 36 °C ± 1 overnight. Thereafter, excess inoculum was removed using filter paper and the bacterial-exposed face of each disc was cultured by placing onto nutrient agar for 15s then removed. Plates were then incubated at 36 °C ± 1 for 18-24 hours

Table 1: Test groups presented.

Group (n=10)	Group description	Group (n=10)	Group description
1	Silicone plus CHX (1%) inoculated with S.aureus	**7**	Silicone plus ZnO (1%) inoculated with S.aureus
2	Silicone plus CHX (3%) inoculated with S.aureus	**8**	Silicone plus ZnO (3%) inoculated with S.aureus
3	Silicone plus CHX (5%) inoculated with S.aureus	**9**	Silicone plus ZnO (5%) inoculated with S.aureus
DNC	Silicone plus 0% CHX	**DNC**	Silicone plus 0% ZnO
NC	Silicone plus 0% CHX	**NC**	Silicone plus 0% ZnO
4	Silicone plus CHX (1%) inoculated with C. albicans	**10**	Silicone plus ZnO (1%) inoculated with C. albicans
5	Silicone plus CHX (3%) inoculated with C. albicans	**11**	Silicone plus ZnO (3%) inoculated with C. albicans
6	Silicone plus CHX (5%) inoculated with C. albicans	**12**	Silicone plus ZnO (5%) inoculated with C. albicans
DNC	Silicone plus 0% CHX	**DNC**	Silicone plus 0% ZnO
NC	Silicone plus 0% CHX	**NC**	Silicone plus 0% ZnO

Abbreviations: DNC: Double negative control where silicone discs contained no inoculum and the discs were inoculated with distilled water only; NC: Negative control where silicone discs contained no inoculum and the discs were inoculated with 200 µl of suspension of either microorganisms. *S. aureus*: Stapylococcus aureus; *C. albicans*: Candida albicans.

and observed for bacterial growth. Experimental procedure for testing discs against *C. albicans* using SDA was the same as described above for *S. aureus*.

Results

The demonstration of inhibitory effect of CHX and ZnO-NP variable concentrations is shown in Figure 2. There was a significant decrease in colony forming units of *S. aureus* and *C. albicans* when tested against 1%, 3%, and 5% concentrations of CHX and ZnO-NP.

Table 2 shows that both inserts reduced bacterial and fungal number of colony forming units compared to the negative control with CHX showing an overall better antimicrobial activity than ZnO-NP regardless of their concentration. However, the complete inhibition of *S. aureus* and *C. albicans* growth was zero number of colonies (100% growth inhibition) and only obtained using the 5% concentrations of CHX compared to other CHX and ZnO-NP concentrations.

No colonies were detected at the DNC disc as expected since this control was inoculated with sterile distilled water only. For the NC disc, numerous uncountable colonies were detected as expected. Images of silicone samples without any insert were captured using

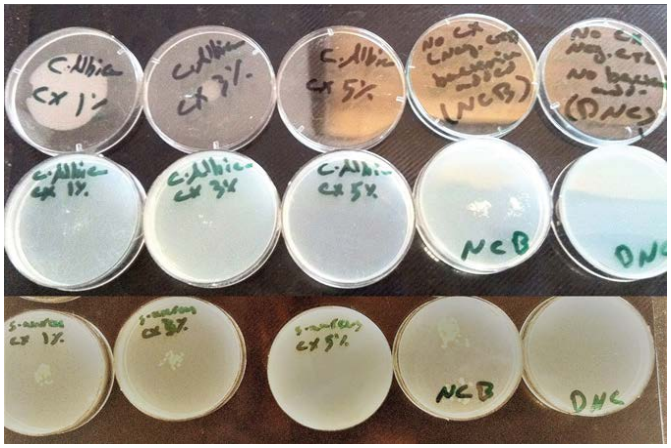

Figure 2: Antimicrobial effect of CHX and ZnO-NP concentrations (1%, 3%, and 5%) on *C. albicans*.

Table 2. Silicone discs impregnated with different concentrations of CHX and ZnO, and tested against *S .aureus* and *C. albicans*

Organism	***S. aureus***	***C. albicans***
Presence of growth	(CFUs)	(CFUs)
Silicone and 1% CHX	60±7	7±2
Silicone and 3% CHX	40±9	4±1
Silicone and 5% CHX	0(complete inhibition)	0(complete inhibition)
Silicone and 0% CHX, distilled water added (CHX DNC)	0	0
Silicone and 0% CHX Organism added (CHX NC)	heavy growth (uncountable)	heavy growth (uncountable)
Silicone and 1% ZnO	130 ±15	70 ±12
Silicone and 3% ZnO	100 ±15	30 ±8
Silicone and 5% ZnO	70±9	40±11
Silicone and 0% ZnO, distilled water added (ZnO DNC)	0	0
Silicone and 0% ZnO Organism added (ZnO NC)	heavy growth (uncountable)	heavy growth (uncountable)

Abbreviations: CFUs: Colony Forming Units; CHX: Chlorhexidine; DNC: Double Negative Control; NC: Negative Control; ZnO: Zinc Oxide.

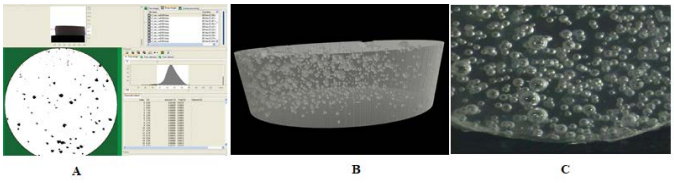

Figure 3. Manually mixed silicone sample with no inserts scanned using micro-Computer Tomography (u-CT) at high resolution (slice thickness 14.21um). (A) Air bubbles pores are clearly present under three views: black dots 2D cross-sectional slice, (B) 3D reconstruction of the 2D slices and (C) 2D image using optical microscope.

Micro Computer Topography (u-CT) and optical microscope (X40 magnification). The images show pores trapped as a result of the manual mixing of the silicone elastomer as shown in Figure 3.

Discussion

Facial silicone prostheses communicating with the oral cavity may be contaminated with oral microflora. These microorganisms can grow over a wide temperature range (40 to 45ºC). Human skin especially around craniofacial percutaneous implants shows a microflora consisting of *Staphylococcus aureus Streptococcus species*, *bacilli* and *Candida parapsilosis*, that cause peri-abutment infection [5,6]. They are associated with reported infections of medical devices [12], especially maxillofacial silicone prostheses [4,13]. This study aimed to investigate the effect of two different inserts (ZnO-NP and CHX) at three different concentrations (1%, 3%, and 5%) on the antibacterial and antifungal activities of maxillofacial silicone elastomer. There was significant effect of insert and concentration on bacterial and fungal growth. However, the complete growth inhibition was only observed at 5% CHX concentration, this inhibition was maintained at 18 and 24 hours of incubation which indicates strong bactericidal effect of 5% CHX. The CHX and ZnO-NP were suggested to have such antibacterial activity [14,15,16]. Different concentrations were tested against *C. albicans* and *S. aureus* as they are common organisms that may lead to prosthesis infection [11,12,14]. Both inserts decreased bacterial and fungal growth with CHX showing an overall better antimicrobial activity than ZnO-NP regardless of their concentration.

CHX impregnated in medical devices kills organisms and protects against microbial colonization and subsequently biofilm development by disrupting the pathogen's cell membrane [10]. It markedly decreased the growth of both organisms at 1% and 3% concentrations, and completely inhibited the growth at 5% concentration. Its antimicrobial effect is novel and attributed to its broad-spectrum biocide effect against fungi and bacteria. It has both bacteriostatic (inhibits bacterial growth) and bactericidal (kills bacteria) mechanisms of action, depending on its concentration [17].

Taking into consideration that CHX has been used safely in human [14]; incorporating it in silicone would have potential clinical applications in medical devices such facial prosthetics, indwelling catheters, needleless connectors and antimicrobial dressings [18]. A study found that CHX antimicrobial activity can last for 48 hours on the skin when used topically and is not affected by the presence of blood or other body fluids [18]. Hence, it is anticipated that facial prosthetics impregnated with CHX can be worn for long hours without any CHX inferior effect on the skin.

ZnO-NP were previously found to have antimicrobial effect which is enhanced by the surface area to volume ratio of the particles [15]. However, in our study, the antimicrobial effect of ZnO-NP against *S. aureus* and *C. albican* was not as effective as CHX. That was evidenced

by the remaining microbial growth the silicone discs with ZnO-NP of the three different concentrations. Therefore, the current study limited *in vitro* antimicrobial benefits of using zinc oxide in silicone discs.

ZnO-NP antibacterial activity is directly correlated with their concentration and size. Larger surface area and higher concentration are accountable for ZnO-NP antibacterial activity [19]. This was evident in current study as samples impregnated with 5% ZnO had the least growth of microbes. However, size effect was found to be inconclusive as some studies indicated no effect [20], while others reported increased antibacterial activity with decreased size [21]. Other factors such as material aging and presence of surface defects may affect the antibacterial activity of ZnO-NP [9,22]. For example, some studies suggested that freshly mixed zinc has shown better antibacterial effect than aging mixtures, probably because increased zinc is released from fresh material and such effect becomes less over time [22,23]. Also, the insolubility nature of the ZnO-NPs was suggested to hinder the diffusion of enough antibacterial amount of Zn to the disc surface environment leading to a decreased visible antibacterial effect [24]. Toxicity mechanism of ZnO-NP differs among studies due to variances in test conditions and mechanisms being investigated [9].

Facial silicone prostheses are porous in nature. Clinically, pigments and colourants are added to the external surfaces of the prostheses for better camouflage effect and skin-tone matching of surrounding tissues. Patients are advised not to use any corrosive solvents, household soaps, or industrial detergents but tap water with mild soap in cleaning their prosthesis [2,25, and 26]. While such protocol can maintain the colour and properties of the silicone prosthesis, however, it is insufficient to kill all bacterial growth as patients notice black staining present inside the fitting surfaces of the prostheses over time. This is likely fungal and bacterial growth inside the pores. Manually mixed silicones have significantly high percentage of pores (0.97-2.15%) and pore volume (0.64-1.43 mm^3) when compared to mechanical mixing under vacuum [3]. Such pores, especially on prosthesis fitting surface are favourable sites for microbial colonization and cannot be reached by the brush during normal prosthesis cleaning [4]. Hence it is advocated to use anti-microbial silicone-cleaning solution, however it can inferiorly affect silicone's tear strength [25]. A side from this, maxillofacial silicone composition is polydimethylsiloxane chains with no antimicrobial ingredients [1]. Thus, incorporating anti-microbial agent can be the ideal solution. But more studies need to be carried out to determine its influence on silicone's prosthetic materials properties and color.

This study complemented the ongoing in vitro work on silicone disinfection, however its results is restricted to baseline, which is considered a limitation. It needs to be widened to other types of bacteria that exist on silicone prostheses surface.

Conclusion

Within the limitations of this study Chlorohexidine diacetate salt exhibited antibacterial activity against some oral pathogenic strains better than ZnO nano particles. It has the potential to completely eliminate bacteria that adhere to maxillofacial elastomer when mixed at 5% (by weight), thus, contributing to the disinfection of silicone-based materials and the destruction of the source of infections caused by *Candida albicans* or *Staphylococcus aureus.*

References

1. Hatamleh MM, Polyzois GL, Nuseir A, Hatamleh K, Alnazzawi A (2016) Mechanical properties and simulated aging of silicone maxillofacial elastomers: advancements in the past 45 years. J Prosthodont 25: 418-426.
2. Hatamleh MM, Haylock C, Watson J, Watts DC (2010) Maxillofacial prosthetic rehabilitation in the UK: a survey of maxillofacial prosthetists' and technologists' attitudes and opinions. Int J Oral Maxillofac Surg 39: 1186-1192.
3. Hatamleh MM, Watts DC (2011) Porosity and color of maxillofacial silicone elastomer. J Prosthodont 20: 60-66.
4. Taylor RL, Liauw CM, Maryan C (2003) The effect of resin/crosslinker ratio on the mechanical properties and fungal deterioration of a maxillofacial silicone elastomer. J Mater Sci Mater Med 14: 497-502.
5. Abu-Serriah MA, Bagg J, McGowan DA, Moos KF, MacKenzie D (2000) The microflora associated with extra-oral endosseous craniofacial implants: a cross-sectional study. Int J Oral Maxillofac Surg 29: 344-350.
6. Pigno MA, Goldschmidt MC, Lemon JC (1994) The efficacy of antifungal agents incorporated into a facial prosthetic silicone elastomer. J Prosthet Dent 71: 295-300.
7. Cho M, Chung H, Choi W, Yoon J (2004) Linear correlation between inactivation of E. coli and OH radical concentration in TiO2 photocatalytic disinfection. Water Res 38: 1069-1077.
8. Choi JY, Kim KH, Choy KC, Oh KT, Kim KN (2007) Photocatalytic antibacterial effect of TiO2 film formed on Ti and TiAg exposed to lactobacillus acidophilus. J Biomed Mater Res Part B: Appl Biomater 80: 353-359.
9. Sirelkhatim A, Mahmud S, Seeni A, Kaus NH, Ann LC, et al. (2015) Review on zinc oxide nanoparticles: antibacterial activity and toxicity mechanism. Nanomicro Lett 7: 219-242.
10. Ilango P, Arulpari M, Medona M, Abirami T (2013) Chlorhexidine - a miracle chemical. Int J Cur Res Rev 5: 26-34.
11. Shi Y, Song W, Feng ZH, Zhao YT, Li F, et al. (2009) Disinfection of maxillofacial silicone elastomer using a novel antimicrobial agent: recombinant human beta-defensin-3. Euro J Clin Microbiol Infect Dis 28: 415-420.
12. Kojic EM, Darouiche RO (2004) Candida infections of medical devices. Clin Microbiol Rev 17: 255-267.
13. Nikawa H, Jin C, Hamada T, Makihira S, Polyzois G (2001) Candida albicans growth on thermal cycled materials for maxillofacial prostheses in vitro. J Oral Rehabil 28: 755-765.
14. Darouiche RO, Mansouri MD, Kojic EM (2006) Antifungal activity of antimicrobial-impregnated devices. Clin Microbiol Infect 12: 397-399.
15. Padmavathy N, Vijayaraghavan R (2008) Enhanced bioactivity of ZnO nanoparticles-an antimicrobial study. Sci Technol Adv Mater 9: 035004.
16. Sánchez-López E, Guerra M, Dias-Ferreira J, Lopez-Machado A, Ettcheto M, et al. (2019) Current applications of nanoemulsions in cancer therapeutics. Nanomater 9: 821.
17. McDonnell G, Russell AD (1999) Antiseptics and disinfectants: activity, action, and resistance. Clin Microbiol Rev 12: 147-179.
18. Hibbard JS (2005) Analyses comparing the antimicrobial activity and safety of current antiseptic agents: a review. J Infus Nurs 28: 194-207.
19. Peng X, Palma S, Fisher NS, Wong SS (2011) Effect of morphology of ZnO nanostructures on their toxicity to marine algae. Aquat Toxicol 102: 186-196.
20. Franklin NM, Rogers NJ, Apte SC, Batley GE, Gadd GE, et al. (2007) Comparative toxicity of nanoparticulate ZnO, bulk ZnO, and ZnCl2 to a freshwater microalga (Pseudokirchneriella subcapitata): the importance of particle solubility. Environ Sci Technol 41: 8484-8490.
21. Yamamoto O (2001) Influence of particle size on the antibacterial activity of zinc oxide. Int J Inorg Mater 3: 643-646.
22. 22. Hojati ST, Alaghemand H, Hamze F, Babaki FA, Rajab-Nia R, et al. (2013) Antibacterial, physical and mechanical properties of flowable resin composites containing zinc oxide nanoparticles. Dent Mater 29: 495-505.
23. Jatania A, Shivalinga BM (2014) An in vitro study to evaluate the effects of addition of zinc oxide to an orthodontic bonding agent. Eur J Dent 8: 112-117.
24. Aydin Sevinc B, Hanley L (2010) Antibacterial activity of dental composites containing zinc oxide nanoparticles. J Biomed Mater Res B Appl Biomater 94: 22-31.
25. Hatamleh MM, Polyzois GL, Silikas N, Watts DC (2011) Effect of extraoral aging conditions on mechanical properties of maxillofacial silicone elastomer. J Prosthodont 20: 439-446.
26. Hatamleh MM, Watts DC (2010) Effect of extraoral aging conditions on color stability of maxillofacial silicone elastomer. J Prosthodont 19: 536-543.