Asian Journal of Chemistry; Vol. 27, No. 9 (2015), 3152-3156

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2015.18299

Effect of Immersion in Cu(II)-Natural Zeolite on Copper Sorption and Colour Stability of Acrylic Resins

D. Irnawati[1,*], W. Widjijono[1], K. Wijaya[2] and W. Asmara[3]

[1]Dental Biomaterials Department, Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta, Indonesia
[2]Physical Chemistry Laboratory, Chemistry Department, Faculty of Math and Natural Sciences, Universitas Gadjah Mada, Yogyakarta, Indonesia
[3]Microbiology Department, Faculty of Veterinary Science, Universitas Gadjah Mada, Yogyakarta, Indonesia

*Corresponding author: Fax: +62 274 515307; Tel: +62 274 562965; E-mail: ninnad38@gmail.com

Received: 12 June 2014; *Accepted*: 29 August 2014; *Published online*: 26 May 2015; AJC-17214

Denture cleansers ideally are bactericidal, fungicidal and harmless to denture materials. Copper(II)-zeolites showed antimicrobial activities, but its effect on denture materials is unknown. The purpose of this research was to investigate the effect of immersion in copper(II)-zeolites on copper sorption and colour stability of acrylic resins. The copper(II)-zeolites (CuZA) were made by mixing natural zeolite (ZA) with 0.1 M, 0.2 M, 0.4 M, 0.6 M and 0.8 M $CuCl_2$ solutions at 60 °C for 1 h. Acrylic resins specimens (20 mm × 20 mm × 2 mm) were immersed in natural zeolite and copper(II)-zeolites suspension in distilled water (70 mg/mL) for 60 days. The results showed that copper amount in acrylic resins varied from not detected (ZA group) to 2.82 ± 1.03 ppm (CuZA-0.1 group) and the ΔE^*_{ab} (L*a*b) from 1.207 ± 0.155 (ZA group) to 1.584 ± 0.313 (distilled water group). Variation of immersing suspensions did not influence the copper sorption and colour stability of acrylic resins ($p > 0.05$). Sixty days immersion in Cu(II)-natural zeolites did not afffect copper sorption and colour stability of acrylic resins.

Keywords: Copper, Natural zeolite, Sorption, Colour stability, Acrylic resins.

INTRODUCTION

The majority of denture bases are fabricated using polymethyl methacrylate resins (acrylic resins)[1]. Denture surfaces could be adhered by plaque and microorganisms such as *Candida albicans*, *Lactobacillus* and *Streptococcus mutans*[2,3]. Denture plaque may promote denture stomatitis, caries and periodontal diseases, therefore the denture cleanliness needs to be considered[2].

Denture cleanliness can be achieved by immersing in denture cleansers for several minutes till overnight[3,4]. An ideal denture cleanser should be non-toxic, leaves no irritant material, harmless to the constituent material of denture (polymers, alloys, porcelain) and preferably bactericidal and fungicidal[4]. The types of commercial denture cleansers are abrasive cream, alkaline hypochlorite, alkaline perborate, dilute acids and enzyme[3,4]. Those denture cleansers have disadvantages. Abrasive cream can abrade acrylic resins, chlorine solutions tend to darken Co-Cr or Ni-Cr alloys and alkaline hypochlorite may cause bleaching and leave an odor on denture[1,4].

Recently, inorganic antibacterial materials of heavy metals such as silver, copper, zinc loaded in zeolite have been developed. Copper has better antifungal properties, chemical stability and lower cytotoxicity than silver. Unfortunately, copper can cause an allergic reaction[5,6]. Zeolites are crystalline, hydrated alumino-silicates with three dimensional framework structures. Zeolites consists of the non-framework exchangeable cations such as Na^+, K^+, Ca^{2+} and Mg^{2+}. Natural zeolites are generally a group of minerals consists of several types including clinoptilolite and mordernite[7]. Zeolites have good properties such as, porous, cation exchange, non-toxic and have chemical and biological long-term stability[8].

Copper ions can be impregnated in zeolite through batch technique by mixing zeolite powder with 0.1-0.85 M/L copper salt solutions[9,10]. Copper ions can be adsorbed by the zeolite because its non-framework cations are not tightly bound and are exchangeable[7]. Copper ions can be released from zeolites by ion exchange, because zeolites have been known to be the carrier and slow releaser of heavy metals with oligodynamic properties[8]. Natural zeolites are widely available in Indonesia such as in Wonosari Regency of Yogyakarta Province. The Wonosari natural zeolite were mixed with 0.05 to 0.25 M copper chloride ($CuCl_2$) solutions and produced Cu(II)-natural zeolites with copper content of 0.441 to 0.737 %. This materials showed antifungal activity towards *C. albicans*[11,12].

The application of Cu(II)-natural zeolite as antimicrobial material for denture cleansers by immersion method will cause long term interaction with acrylic resins of denture base materials. Acrylic resins can absorb liquids and soluble materials from its environment. On the other hand, denture base materials should have colour stability and biocompatibility to be clinically accepted[3,13]. The purpose of this study was to investigate the effect of acrylic resins immersion in Cu(II)-natural zeolites towards Cu sorption and acrylic resins colour stability.

EXPERIMENTAL

Natural zeolite from Wonosari Regency of Yogyakarta Province, $CuCl_2 \cdot 2H_2O$ powder (Merck, Germany) and polymethyl methacrylate resins (Stellon, England) were used in this research. The Cu(II)-natural zeolites were prepared based on the previous research at Physical Chemistry Laboratory, Department of Chemistry, Faculty of Math and Natural Sciences Universitas Gadjah Mada, Indonesia[13]. The natural zeolite powder was washed using distilled water, filtered and dried at 200 °C for 2 h. It was then crushed and sieved to obtain 100 mesh size powder. Copper chloride solutions of 0.1, 0.2, 0.4, 0.6 and 0.8 M concentrations were prepared. Activated natural zeolite and 0.1 M solution of $CuCl_2$ in 50 g/250 mL ratio was mixed for 1 h at 60 °C. Subsequently, the suspension was filtered, the precipitate was washed using distilled water and dried at 100 °C for 24 h. Then it was re-sieved to obtain Cu(II)-natural zeolite powder in 100 mesh size (CuZA-0.1 group). The steps were repeated using different concentrations of $CuCl_2$ solutions to obtain of Cu(II)-natural zeolite powder for CuZA-0.2; CuZA-0.4; CuZA-0.4 and CuZA-0.8 groups.

Heat cured acrylic resins samples (20 mm × 20 mm × 2 mm) were made for Cu adsorption test (30 samples), colour stability test (40 samples) and scanning electron microscopy (SEM) test (2 samples). The powder and liquid of acrylic resins (23 g/10 mL) were mixed into dough phase, put in a plaster mold in the metal denture flask and heated in a waterbath at 73 °C for 1.5 h and 100 °C for 0.5 h. Acrylic resin excess in each samples were removed using arkansas stone burs, finished with coarse and fine sandpaper, washed and then soaked in distilled water for 24 h at room temperature.

Fifty mL suspensions of natural zeolite and copper(II)-zeolites in distilled water (70 mg/mL) were prepared in glass bottles. Acrylic resin samples for Cu adsorption test (5 samples for each group) and SEM test (1 sample for distilled water group and 1 sample for CuZA-0.8 group) were immersed in natural zeolite and copper(II)-zeolites suspensions for 60 days at room temperature (29 ± 2 °C) as a simulation of overnight immersion (8 h) for 180 days. The bottles were shaken for 15 s everyday and the suspensions were replaced every 20 days. The acrylic resin samples were washed using distilled water, dried with tissue paper and pulverized into powder using Arkansas bur stone.

Acrylic resin samples for colour stability tests were divided into 8 groups of 5 samples. Initial colour of acrylic resin samples were measured before treatments. Seven groups were immersed in natural zeolite suspension, copper(II)-zeolites suspension and distilled water in the same way as Cu absorption samples. One group was not given the soaking treatment. Acrylic resin samples colour were measured again after treatments.

Copper content in acrylic resin samples were measured using Atomic Absorption spectrophotometer equipment (Perkin-Elmer 3110, USA) with 324.8 nm wavelength (λ) at Analytical Chemistry Laboratory, Department of Chemistry, Faculty of Math and Natural Sciences UGM. The copper concentrations were calculated with the accuracy of 0.01 ppm. The surface characterization of SEM samples were examined using SEM (JEOL JSM-6510LA, Japan) at Laboratory of Integrated Research and Testing Unit I UGM. The colour of acrylic resin samples for colour stability test were measured using the Commission Internationale de l'Eclairage (CIE)L*a*b instrumental system technique using Chromameter (Konica Minolta Chromameter CR 400, Japan) at Medical Technology Laboratory, Faculty of Medicine, Universitas Gadjah Mada, Indonesia. The colour changes were calculated using the formula[3]: $\Delta E^*_{ab}(L^*a^*b) = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$. The Cu amount in acrylic resin and acrylic resin colour changes data were statistically analyzed using one way ANOVA with 0.05 significant level.

RESULTS AND DISCUSSION

The Cu(II)-natural zeolites powder obtained in this research has yellow creamy colour (Fig. 1). The results of Cu absorption in acrylic resins test are presented in Table-1. The Cu content was not detected in ZA group and the highest mean of Cu content showed in CuZA-0.1 group. The one way ANOVA analysis showed that immersion material variations did not influence the Cu concentration in acrylic resins ($p > 0.05$) (Table-2). The SEM micrographs are presented in Fig. 2. The results showed that copper(II)-zeolites powder were retained in the irregularities of acrylic resin surface. The colour differences of acrylic resins before and after immersion treatments are presented in Fig. 3. The acrylic resins specimens of distilled water group and CuZA-0.8 group showed slight colour brightness after immersion treatments. The results of colour stability test are presented in Table-3. The lowest mean of acrylic resins colour change was from the non-immersion group and the highest was from the distilled water group (Table-3). Acrylic resins that were subjected to immersion showed the value of $\Delta E^*_{ab}(L^*a^*b^*)$ 4 to 5 times greater than acrylic resins that were not soaked. One way ANOVA test results showed that there was no effect of immersion materials variations towards discolouration of acrylic resins ($p > 0.05$) (Table-4).

Fig. 1. Copper(II)-zeolites powder

TABLE-1
MEANS AND STANDARD DEVIATIONS OF COPPER AMOUNT IN ACRYLIC RESINS

Groups	Number of samples	Mean and standard deviation (ppm)
Natural zeolite	3	Not detected
CuZA-0.1	5	2.82 ± 1.03
CuZA-0.2	4	1.41 ± 0.96
CuZA-0.4	5	2.57 ± 0.83
CuZA-0.6	5	2.41 ± 1.40
CuZA-0.8	5	2.57 ± 0.99

TABLE-2
SUMMARY OF ONE WAY ANOVA OF COPPER AMOUNT IN ACRYLIC RESINS

	Sum of squares	Df	Mean square	F	Sig.
Between groups	5.118	4	1.279	1.127	0.374
Whiten groups	21.578	19	1.136		
Total	26.698	23			

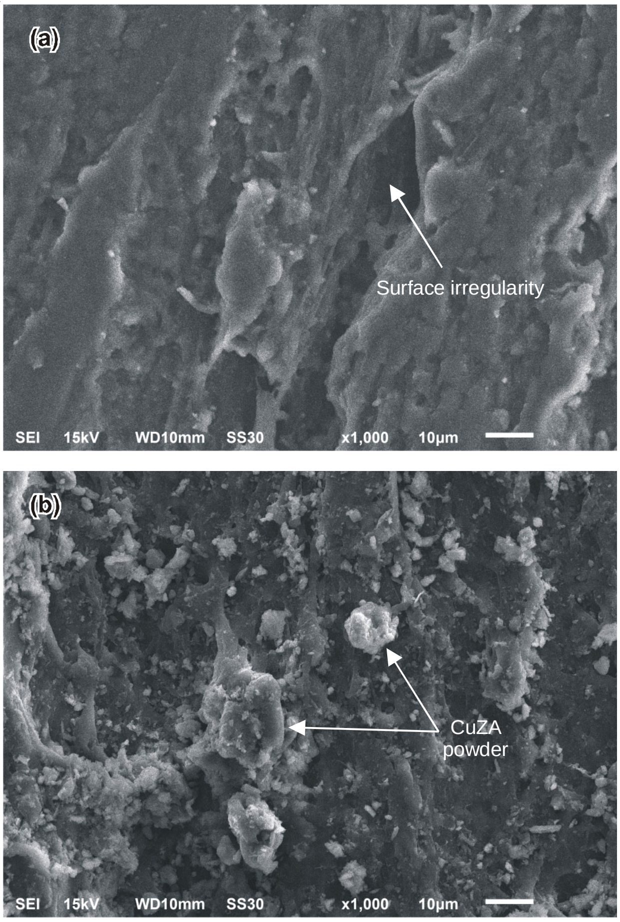

Fig. 2. SEM micrographs of acrylic resins surfaces after immersion; (a) distilled water; (b) CuZA-0.8

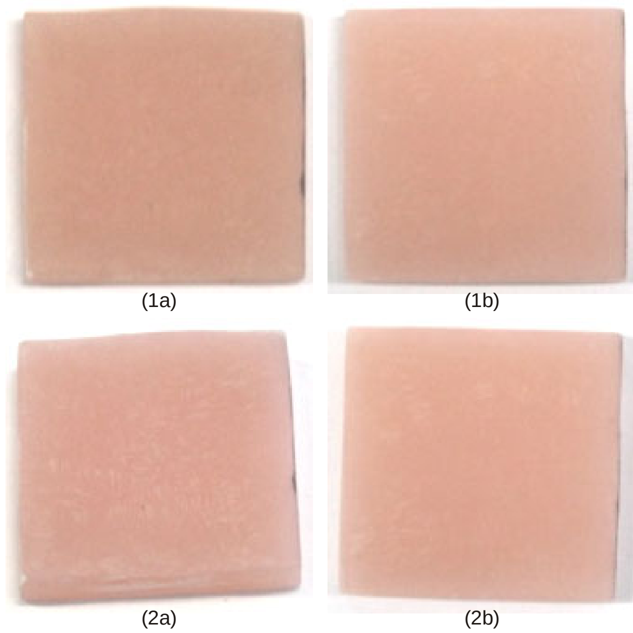

Fig. 3. Acrylic resins samples before and after immersion treatments; (1a) distilled water before treatment; (1b) after treatment; (2a) CuZA-0.8 before treatment, (2b) after treatment

TABLE-3
MEANS AND STANDARD DEVIATIONS OF $\Delta E^*_{ab}(L^*a^*b^*)$ ACRYLIC RESINS

Group	Number of samples	Mean and standard deviation
Non-immersion	5	0.334 ± 0.162
Distilled water	5	1.584 ± 0.313
Natural zeolite	5	1.207 ± 0.155
CuZA-0.1	5	1.445 ± 0.294
CuZA-0.2	5	1.347 ± 0.213
CuZA-0.4	5	1.244 ± 0.266
CuZA-0.6	5	1.517 ± 0.699
CuZA-0.8	5	1.361 ± 0.317

TABLE-4
SUMMARY OF ONE WAY ANOVA OF ACRYLIC RESINS DISCOLOURATION (ΔE)

	Sum of squares	Df	Mean square	F	Sig.
Between groups	0.570	6	0.095	0.728	0.531
Whiten groups	3.657	28	0.131		
Total	4.227	34			

The reaction of natural zeolite powder with different concentration of $CuCl_2$ solutions increased Cu amount in zeolites. Copper ions were impregnated into zeolites by adsorption process[7]. Metal ions move through the pores of zeolite mass and the channels of the lattice to replace exchangeable cations[14]. The forces involved in adsorption are weak and physical van der Waals force, ion exchange and chemical interaction[15]. Metal cations interact with zeolites by two mechanisms, ion exchange on Al exchange sites and electrostatic adsorption at silanol groups[16]. Ion exchange reactions in zeolites required an excess of solution and the cations amount adsorbed was depended on the solution concentration[7]. The 0.05 to 0.25 M $CuCl_2$ solution concen-trations affected the increase of Cu amount in natural zeolites[12]. The copper(II)-zeolites powder in this study were expected to have varying Cu content with the lower concentration of copper chloride solution resulting in the Cu(II)-natural zeolite with less Cu amount compare to the higher concentration of copper chloride solution.

The result showed that less than 3 ppm Cu was detected in acrylic resins after immersion in copper(II)-zeolites suspensions for 60 days. The immersion of acrylic resin into natural zeolite and copper(II)-zeolites suspensions in water caused an interaction among those materials. Copper ions could be leached out from zeolite structure and acrylic resins would absorbed water and this leaching components. Copper ions can be released from zeolites by ion exchange, but theoretically these ions can not be released in water because the neutral condition has low solution ionic strength[17]. Copper ions might be released from zeolites through the leach out of copper ions that interact with zeolites by electrostatic adsorption at silanol groups or that have weak and physical van der Waals force[15,16]. Metal ionic species also could be absorbed on the surface of zeolite. These ions are not stably attached to the surface and could released from zeolite[18]. Previous study showed that less than 2 mg/L copper leached-out in running distilled water[14].

Copper ions that leached-out into distilled water would be absorbed by acrylic resins because polymethyl methacrylate tends to absorb water through adsorption and absorption processes[1,3]. Polymethyl methacrylate slowly absorbs water and acrylic resin denture base required 17 days to become fully saturated with water. Water was absorbed through diffusion mechanism and facilitated by the polarity of polymethyl methacrylate molecules that are caused by unsaturated molecules or unbalanced intermolecular forces[1,19]. Water molecules penetrate through the porosity and intermolecular space of acrylic resin mass and occupies a position between polymer chains[1,13].

The results showed that the variation of immersion materials didnot influence the Cu concentration in acrylic resins. Copper was detected in acrylic resins that were soaked in all CuZA groups. It was assumed that acrylic resins retained copper not only by absorption of leaching copper ions in water, but also by retaining copper(II)-zeolites powder on its surfaces. The natural zeolite and copper(II)-zeolites powder were contact with acrylic resins surfaces in immersion treatments. When liquid was absorbed by acrylic resins, natural zeolite and copper(II)-zeolites with 100 mesh (150 μm) size powder could be attracted to acrylic resins. Those powder were attached to the surface of acrylic resin by mechanical adhesion through interlocking or penetration on acrylic resin surfaces[3]. The copper(II)-zeolites powder were retained in the irregularities surfaces of acrylic resins (Fig. 2).

Acrylic resins samples retained less than 3 ppm Cu and those concentrations were higher than Word Health Organization (WHO) recomendation of copper content in drinking water (2 ppm)[20]. In this research, the acrylic resins were immersed continually for 60 days as simulations of overnight immersion (8 h) for 180 days and the zeolite powder were continually contact to the acrylic surface. On daily application, denture is washed after soaked in denture cleanser. It was predicted that in intermitten immersion the acrylic resins were retained less Cu than in continue immersion.

Water and Cu ions sorption as well as the attachment of natural zeolite and copper(II)-zeolites caused discolouration of acrylic resins. The results showed no significant effect of immersion variations (distilled water, natural zeolite and copper(II)-zeolites) to acrylic resins colour. This suggested that the natural zeolite and copper(II)-zeolites were not a dominant factor in the discolouration of acrylic resins. Colour change was expected to occur due to intrinsic factors of acrylic resin which involved chemical changes through amine accelerator oxidation and long term immersion in distilled water[21]. When the material has not been exposed to the dye, physicochemical changes that affect the colour was a result of internal reactions. Those reactions might be caused by oxidation, water absorption, acrylic resin component releasing (plasticizers, monomers and pigments) and chemical degradation[22-24].

Oxidation of amine accelerators cause polymer degradation due to free radicals play a role as an initiator that resulting in discolouration of the acrylic resins[22,24]. Water sorption could affect acrylic resins colour because water molecules in acrylic would influence the reflection or transmission of light beam thus affecting translucency of acrylic resins[3,22]. Interaction of acrylic resin and water could lead to hydrolysis process. Water molecules break the vulnerable polymer chains into shorter chains and lead to polymer degradation. Hydrolysis process caused reduction of polymer molecular weight and light molecular weight component to dissolve in water[23].

Structural changes in acrylic resin influenced the dominant wavelength (*hue*) and colour intensity (*chroma*) that is indicated by the change of a* and b* values. Initial hue and chroma values were inherent material properties due to the presence of pigments and fillers with varying translucency and opacity. Acrylic resin discolouration may occur due to alteration or molecular reaction on material components and the loss of plastisizers, monomers and colour pigments[1,24].

In this study, the acrylic resins showed colour change value of $\Delta E^*_{ab}(L^*a^*b^*)$ between 1.2 and 1.5, lower than the results of the previous studies. Immersion of acrylic resins in distilled water for 60 days showed that the $\Delta E^*_{ab}(L^*a^*b^*)$ greater than 2[21]. The acrylic resins showed $\Delta E^*_{ab}(L^*a^*b^*)$ value of 2.31 for distilled water and 2.22 for denture cleanser after immersion at 12 h/day for 180 days[22]. Acrylic resin discolouration in this study was clinically acceptable as the material discolouration was clinically appropriate with the value of ΔE^* ($L^*a^*b^*$) less than 3.3[3].

Conclusions

Within the limitation of this study, the following conclusions could be made:

• The acrylic resin showed insignificant Cu absortion and discolouration after immersion in Cu(II)-natural zeolite suspension for 60 days.

• The Cu(II)-natural zeolite variations did not affect the Cu sorption and colour stability of acrylic resins.

• The acrylic resins colour was still clinically acceptable after immersion in Cu(II)-natural zeolite suspension for 60 days.

Further research needs to be conducted to investigate the biocompatibility aspects such as cytotoxicity and hypersensitity of Cu(II)-natural zeolite as antimicrobial material in denture cleanser.

REFERENCES

1. K.J. Anusavice, Phillips' Science of Dental Materials, Saunders, St. Louis, p. 34-5, 474-95 (2013).
2. G.A. Zarb and C.L. Bolender, Prosthodontic Treatment for Edentulous Patients Complete Denture and Implant-Supported Prostheses, Mosby Inc., St. Louis, pp. 34-50 (2004).
3. J.M. Powers and R.L. Sakaguchi, Craig's Restorative Dental Materials, Mosby Elsevier, St. Louis, pp. 18-30/513-553 (2006).

4. R.G. Craig, J.M. Powers and J.C. Wataha, Dental Materials Properties and Manipulation, Elsevier, St. Louis, pp. 270-295 (2004).
5. B. Li, S. Yu, J.Y. Hwang, and S. Shi, *J. Miner. Mater. Character. Eng*., **1**, 61 (2002).
6. G. Schmalz and D. Arenholt-Bindslev, Biocompatibility of Dental Materials, Springer, Berlin, p. 234 (2009).
7. H. Hamdan, Introduction to Zeolites Synthesis Characterization and Modification, Universiti Teknologi Malaysia, Johor Baru, pp. 1-49 (1992).
8. C. Orha, M. Likarec, R. Pode and G. Burtica, *Chem. Bull. Politechnica Univ. (Timisoara)*, **64**,152 (2005).
9. R. Niira, Y. Niira, K. Niira, H. Niira, T. Yamamoto and M. Uchida, US Patent 4938958 (1990).
10. Z. Hagiwara, S. Hoshino, H. Ishino, S. Nohara, K. Tagawa and K. Yamanaka, US Patent 4911899 (1990).
11. D. Irnawati and W. Widjijono, K. Wijaya and W. Asmara, In Proceeding Book of the International Symposium on Oral and Dental Sciences, The IJDR, Indonesia, pp. 39 (2013).
12. D. Irnawati, P. Agustiono and E.H. Wardhani, In Program Book International Joint Symposium: The University of Tokushima, Universitas Gadjah Mada, Niigata University, Indonesia, p. 31 (2010).
13. J.L. Ferracane, *Dent. Mater.*, **22**, 211 (2006).
14. watersensitivescities.org.au/resource-library/removal-and-inactivation-of-e-coli-from-water-using-copper-modified-natural-zeolite.
15. M.K. Doula and A. Ioannou, *Micropor. Mesopor. Mater.*, **58**, 115 (2003).
16. M. Schreier, S. Teren, L. Belcher, J.R. Regalbuto and J.T. Miller, *Nanotechnology*, **16**, S582 (2005).
17. Y. Petrakakis, E. Mylona, D. Georgantas and H. Grigoropoulou, *Global Nest J.*, **9**, 207 (2007).
18. S. Demirci, Z. Ustaoglu, G.A. Yilmazer, F. Sahin and N. Bac, *Appl. Biochem. Biotechnol.*, **172**, 1652 (2014).
19. V.M. Miettinen and P.K. Valittu, *J. Prosthet. Dent.*, **77**, 531 (1997).
20. P.G. Georgopoulos, A. Roy, M.J. Yonone-Lioy, R.E. Opiekun and P.J. Lioy, The International Copper Association, New York, USA (2001).
21. S. Buyukyilmaz and I.E. Ruyter, *Int. J. Prosthodont.*, **7**, 372 (1994).
22. G. Hong, H. Murata, Y.A. Li, S. Sadamori and T. Hamada, *J. Prosthet. Dent.*, **101**, 205 (2009).
23. S. Lyu and D. Untereker, *Int. J. Mol. Sci.*, **10**, 4033 (2009).
24. I. Kostoulas, G. Polyzois, A. Mitsoudis, V. Kavoura and M. Frangou, *Gerodontology*, **29**, e239 (2012).