Synthesis and Determination of Antimicrobial Activity of Visible Light Activated TiO$_2$ Nanoparticles with Polymethyl Methacrylate Denture Base Resin Against Staphylococcus Aureus

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Abstract

Objectives: The aim of this study was to synthesize visible light activated titanium dioxide (TiO$_2$) nanoparticles using titanium nitride (TiN) by solution route to determine its antimicrobial activity against Staphylococcus aureus when combined with heat cure polymethyl methacrylate denture base resins (PMMA) in three different combinations.

Methods: Visible light activated as-prepared TiO$_2$ photocatalyst was synthesized by peroxide based solution route technique using TiN. Part of the as-prepared TiO$_2$ was annealed and anatase form was obtained. Physical properties of the material were evaluated by X-ray diffraction (XRD), scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDAX) and Fourier transform infrared spectroscopy (FTIR). TiO$_2$ (3 w%, 5 w%)-PMMA composite samples (incorporated, precuring or post-curing coating) and unblended acrylic samples were prepared. They were placed in saline and exposed to light for 1 day, 1 week, 1 month, 3 months and 6 months in a halogen light system specially fabricated for this study. Then the samples were subjected to microbiological tests to determine the inhibitory activity against $S$. aureus. Based on visible turbidity changes in the broth containing samples the antibacterial activity was reported. Statistical analysis of the data was done to interpret the results.

Results: XRD, SEM, EDAX and FTIR showed better patterns with anatase form. This material showed consistent and reliable inhibitory activity in all forms when combined with PMMA in three different combinations. The best results were achieved at 3 w% concentration of TiO$_2$.

Conclusion: 3 w% of anatase TiO$_2$ upon incorporation and postcuring coating with PMMA shows promising inhibitory activity against $S$. aureus.

Clinical significance: To decrease the load of oral microflora on dentures by utilising visible light activated TiO$_2$ photocatalyst especially in elderly and handicapped denture wearers.

Keywords: Polymethyl methacrylate denture base resin; Acrylic resin; Titanium dioxide nanoparticles; Visible light activated photocatalysis; $S$. aureus; Oral colonizers

Introduction

Dentures are generally used as alternatives for natural teeth for rehabilitating lost oral functions. Acrylic resin is the material widely used in the fabrication of dentures [1]. Plaque is a mass of bacteria protected by a biofilm and it adheres to dentures with a wider adhesion area than the natural teeth [2-4]. The composition of oral microflora varies at different surfaces within the mouth because of the physical and biological properties of each site. These properties include the number of receptors for the microbial adhesion, the redox potential of the site and the provision of essential nutrients. The composition of the oral flora of particular site changes with the age of the host. These changes with denture wearing increase the prevalence of $S$. aureus, $L$. acidophilus, yeasts and $A$. viscosus [5-7].

This increased the quest for a material which is active in inhibiting these oral microflora. It has been shown that most denture cleansers are not effective in reducing plaque accumulation [8]. In addition, the routine use of denture cleansers may be unaffordably prohibitive in cost especially in elderly and handicapped denture wearers. Hence, there is a need to develop a single, economical and effective method to achieve denture hygiene.

Photocatalysis is a technique wherein UV rays activate the photocatalyst. TiO$_2$ is one of the prevailing materials in the field of photocatalysis. TiO$_2$ is used as an inorganic additive as it is chemically stable, biocompatible and non-toxic [9]. Studies have been reported where TiO$_2$ nanoparticles were added in dental restorative materials like glass ionomer [10] and epoxy resins [11,12]. However, there were no identified reports on the antimicrobial activity of visible light activated photocatalytic TiO$_2$ nanoparticles upon incorporation in PMMA denture base resins. It was found that the commercially available TiO$_2$ had negligible visible light photocatalytic activity since it contains high concentrations of defects which cause rapid recombination of photo generated electron-hole pairs [13]. Therefore to establish a clean and safe photocatalytic reaction system using the solar beam and/or visible light, it was vital to develop titanium dioxide photocatalyst that can absorb visible light and operate with high efficiency under solar beam and/or visible light. TiO$_2$ prepared by nitrogen doping with TiN as a precursor was known to be a visible light sensitive photo catalyst [13]. Therefore, an active intermediate form of visible light activated yellow

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The dough stage was heat cured thoroughly mixed and on reaching the gel of polymer (DPI Heat Cure, India) was added to the same. It was mixed with 1.7 ml of methyl methacrylate monomer and then 8.55 g by weight of polymer i.e. 0.3 g and 0.5 g of nanoparticles were weighed and mixed. Preparing of samples with TiO\(_2\) nanoparticles against S. aureus, Staphylococcus aureus, is a common oral microflora. The null hypothesis was that there was no inhibitory activity of visible light activated TiO\(_2\) nanoparticles against S. aureus when incorporated or coated on PMMA resins.

**Materials and Methods**

**Synthesis of visible light activated TiO\(_2\)**

The visible light activated TiO\(_2\) nanoparticles was synthesized using the peridioxide based technique using TiN (Sigma-Aldrich) as precursor and yellow TiO\(_2\) nanoparticles were obtained. A part of the as prepared nanoparticles (yellow TiO\(_2\)) was annealed to a temperature of 800°C in a furnace by raising the temperature uniformly i.e. 5°C/minute. At 800°C temperature, a holding period for two hours was maintained and was cooled after the holding period was over. This gave an anatase form of TiO\(_2\).

**XRD, EDAX and FTIR analysis of TiO\(_2\)**

XRD, EDAX and FTIR of as-prepared and anatase forms of TiO\(_2\) photocatalyst were obtained. XRD (Sietronics XRD scan, Australia) analysis was done using Cu k- alpha 1 radiation (\(\lambda\) = 1.5418 Å) with a scan step of 0.02 degree/second and 20 degree value of 20 -70 degrees. The nature and phase of the as-prepared and anatase nanoparticles was studied by XRD analysis. The peak patterns and values obtained were compared with the standard patterns and values. It was observed that the patterns and values of as-prepared material did not correspond to the standard anatase visible light activated phase, while XRD of annealed matched (Figure 1). FTIR showed band patterns corresponding to organic impurities (Figure 2). EDAX measurements showed presence of organic impurities in the as-prepared nanoparticles which significantly reduced on annealing (Figure 3,4). Annealing helped eliminate all the above impurities and imperfections. Therefore, the as-prepared material was discarded. The anatase form showed required visible light activated photocatalytic phase and was used to assess the antimicrobial activity of TiO\(_2\) required to have inhibitory activity against S. aureus.

**Pilot study**

A pilot study was performed to assess the antimicrobial activity of 3 w% and 5 w% of TiO\(_2\) in PMMA in three forms, using methodology described below. Since comparable antimicrobial activity against S. aureus was seen in both concentrations, 3 w% of nanoparticles was considered, for economic reasons to the end user and less effect on the mechanical properties of denture base resins.

**Preparation of samples with TiO\(_2\)**

Sample preparation of TiO\(_2\)-PMMA composite was done by 3 methods.

Mould spaces for specimens were prepared using wax pieces (5 mm\(^2\) 5 mm\(^2\) 2 mm thickness) in a dental flask. 3 w% and 5 w% TiO\(_2\) by weight of polymer i.e. 0.3 g and 0.5 g of nanoparticles were weighed and mixed with 1.7 ml of methyl methacrylate monomer and then 8.55 g of polymer (DPI Heat Cure, India) was added to the same. It was thoroughly mixed and on reaching the dough stage was heat cured as per manufacturers’ instructions. After curing, the samples were trimmed, sandpapered and polished [14]. They were referred to as “Group A”. Surface coating of acrylic with nanoparticles was done at two stages, viz, pre-curing coating and post-curing coating to increase the percentage of nanoparticles on the surface of the specimens. In both cases chloroform was used as a binder. TiO\(_2\) nanoparticles were dispersed in chloroform and was then coated on to the acrylic material at pre-curing (Group B) and post-curing stages (Group C). Control specimens used were plain acrylic specimens unblended with nanoparticles were referred to as ‘Group D’.

**FTIR, SEM and EDAX of TiO\(_2\)-PMMA composite**

A FTIR (BRUKER-FTIR Alpha-T, Germany) was used to analyze the bonding between TiO\(_2\) and PMMA in the test specimens. The characterization of bonds present in TiO\(_2\) and unblended acrylic samples were separately studied and compared with the study specimens. EDAX was done to study the presence of TiO\(_2\) in the specimens. SEM (JEOL JSM-6308 LA, Japan) was used to study the surface morphology of all the combinations of TiO\(_2\) and PMMA powder (Figures 5,6,7). All the materials were sputtered with a thin layer of gold and imaged. (JEOL JFC-1100E, Japan)

**Halogen light system**

A halogen bulb was enclosed in a wooden box for purposes of easy handling and safety. Reflecting surfaces were created by using mirrors on the inner surfaces of the box so as to allow all the surfaces of all the specimens to be exposed to visible light. After polymerization, test specimens were placed into glass beakers filled with normal saline. Desiccation of samples was avoided as saline was replaced periodically. The beakers were made of borosilicate glass (Rivera laboratory glassware, Mumbai, India) which could transmit visible light, and its transmission rate was over 80 %. They were exposed to light in the form of visible light over a period of 24 hours. Control specimens were immersed in normal saline and stored in desiccators. After 24 hours, Staphylococcus aureus colonies were spread onto the inner surfaces of the boxes and incubated for 24 hours at 37°C to determine the growth of bacteria.
specially designed and fabricated halogen light chamber at a distance of 30 cm for a period of 4 hours to ensure maximum activation of photo catalytic sites. This was continued for a period of 6 months. The samples were removed and tested for microbiological activity at 1 day, 1 week, 1 month, 3 months and 6 months durations. At each time interval 5 samples were subjected to microbiological tests.

**Microbiological tests**

On completion of the defined time periods, the specimens were removed from saline and were tested for their inhibitory effect on *S. Aureus* which was isolated from salivary sample of a healthy volunteer and identified by standard tests. All the procedures were performed in a biosafety cabinet (Level 2 A II). Any possible contamination of specimens while handling was eliminated by dipping the specimens in 1 % sodium hypochlorite for one hour followed by repeated washing with sterile distilled water to remove traces of sodium hypochlorite which would otherwise affect the growth of organism on inoculation in the broth. The sterile specimens were then individually placed in sterile brain heart infusion (BHI) broth (5 ml) in penicillin bulbs. The broth was infected by inoculating 100 µl of overnight culture of
S. aureus and was incubated for 24 hours. Uninoculated controls were run with each batch. After 24 hours, the specimens were removed from the infected broth with sterile forceps and dried in a biosafety cabinet. They were transferred to sterile 5 ml beakers containing sterile saline, sealed carefully with sterile plastic film and exposed to halogen light for 4 hours. After exposure, specimens were transferred to sterile BHI broth in penicillin bulbs and incubated. Subcultures were made from the turbid broth to reidentify the growth of S. aureus that helped to rule out accidental contamination.

**Statistical Analysis**

A chi square test done (using SPSS 16.0 version) for the difference between proportions as shown below

Chi square \((\chi^2)\) = 1.58  
Degree of Freedom (DF) = 2  
Significance level \((p)\) = 0.45

With a Degree of Freedom (DF) of 2, a significance level \((p)\) of 0.45 indicated that there was no statistically significant difference in the antimicrobial activity observed across the groups (Table 1).

**Results**

**XRD studies**

XRD patterns of the nanoparticles in as-prepared and anatase form are shown in Figure 1. It is clear that as-prepared material is crystalline in nature. After annealing it to a temperature of 800ºC there was improvement in the crystallinity. The obtained XRD pattern of the annealed form matches with the visible light activated anatase form of TiO\(_2\). Further the particle size was estimated using Debye Scherer's formula.

**FTIR**

The recorded FTIR spectrum of as-prepared material was in the range of 400 to 4000. It could be observed that the spectrum of as-prepared samples showed pronounced bonds of “OH” stretching and bending at 3132 and 1393 were numbers. In addition to these bands the spectrum also showed other bands at different wave numbers that could be assigned to various modes of organic contamination in TiO\(_2\) nanoparticles. A broad and intense band around 495 wave-numbers could be assigned to Ti-O-Ti stretching mode (Figure 2).

After annealing, (spectrum b) there was significant reduction in (organic contamination) bonds. But the Ti-O-Ti band appeared quite significant. The results clearly indicated that annealing at 800ºC removes organic contamination of TiO\(_2\) nanoparticles that occur during solution route synthesis.

**SEM**

The SEM photomicrograph of anatase material showed improvement in the growth of nanoparticles i.e. the presence of spherical particles. SEM images of denture composites prepared by incorporating and surface coating of nanoparticles were recorded (Figures 5,6,7). It was observed that there was more number of active nanoparticles on the surface coated specimens.

**Antibacterial activity**

Presence of turbidity in the broth containing specimens indicated growth when compared with a clear uninoculated media control.
Absence of turbidity in broth suspension indicated inhibitory activity. Inhibitory activity was seen with all groups of specimens, while the controls always showed growth (Figures 8, 9). Comparable antimicrobial activity was seen in all three groups of test samples containing visible light activated TiO$_2$ photocatalyst at a concentration of 3 w%.

The total number of PMMA-TiO$_2$ samples was 75, which were divided equally for three different combinations viz, incorporated, pre-curing and post-curing. For every timed category 2 PMMA controls, and 1 media control was used, resulting totally in 10 and 5 samples respectively. The samples were studied for durations of 1 day, 1 week, 1 month, 3 months and 6 months. Each time 5 samples of each group were studied. The growth of bacteria in each timed category was noted. The observations were made for growth in the media control as well as the controls. No growth was seen in the media controls whereas growth was observed in all the controls.

Thus the confounding variable of media being contaminated was nil. Whereas growth in controls was always present. So any suppression of growth could be attributed to the antimicrobial activity of the test material only.

**Sample size:** The samples used were 75 in number. The optimum sample size for a proportion of 72% at a proportion of error level of...
5% and a precision of 20% worked out to 76.2. Hence, we have studied an adequate sample size of 75 samples. The observation of nil growth was in 54 out of 75 samples, whereas growth was found in 21 samples. It was observed that 72% of antimicrobial activity was seen across all categories while growth was seen in 28% of the specimens across the categories. This showed that there was no significant difference in the antimicrobial activity across the categories.

Discussion

The null hypothesis which stated "there was no inhibitory activity of visible light activated TiO₂ nanoparticles against S. aureus when incorporated or coated on PMMA resins" was rejected based on the results of this study.

On TiO₂ nanoparticles

Literature reveals many investigations wherein antifungal & antiseptic agents were incorporated/coated on denture base resins or soft liners. However their effect was not persistent, as these agents were rapidly released [15,16]. Hence, the main objective of this study was to develop and evaluate antibacterial activity of a denture base resin with minimum percentage of visible light photocatalytic additive. Various techniques of preparing visible light activated TiO₂ have been proposed - including sol-gel technique, N doped, C doped, Co ion doped – TiO₂ photocatalyst. [17-19]. The disadvantages of doped materials were low surface energy because of using high temperature, requirement of expensive chemicals and synthesis methods and an increase in carrier recombination [13]. Randron et al., [13] recommended the use of TiN as an alternate precursor in peridioxide based route because of its air stability, organic and chloride ion free route, simplicity and low cost. In concurrence with the above study, authors would like to state here that TiO₂ nanoparticles were synthesised by a simple and organic free precipitate technique using TiN as a precursor. XRD patterns of the as-prepared & anatase phase confirmed the respective phases of TiO₂. The particle size of TiO₂ as calculated by Debye Scherer equation was 31 nm. The obtained phase transformation from yellow amorphous to anatase at 800°C and particle size were consistent with the previous work [13,20]. Since the anatase form of TiO₂ showed photocatalytic phase which was the required form in our work, we continued the use of anatase form in our work.

Sample preparation techniques

Investigators have suggested incorporation of TiO₂ photocatalyst nanoparticles in denture base acrylic and also coating on soft liners [21,22]. The benefit of incorporation of TiO₂ within the material itself allows constant presence of photocatalyst even if the denture base material wears off. At the same time, the number of active sites available drastically reduces as evident in SEM. Therefore grafting of nanoparticles with a suitable binder (chloroform) was done. Therefore, coating of nanoparticles on heat cure denture base acrylic was done at two different stages viz., pre curing and post curing. This was to determine the technique that would give better and more functionally active photocatalytic sites. The SEM of the above two coated samples revealed a halo of TiO₂ nanoparticles around the polymer particles in post curing coated samples. The photocatalytic particles were far greater in number than incorporated or precurring. Post-curing application of nanoparticles had increased the scope of extending the use TiO₂ nanoparticles to old denture wearers.

The use of chloroform in our study as a binder appeared to involve a less time consuming technique and economical as compared to precoating the surface of PMMA denture base with acrylicoxyproul trimethoxysilane based agents followed by surface coating with TiO₂ as it is a two step procedure [23]. At the same time, the presence of photocatalytic nanoparticles in our study has been positive as demonstrated by 6 month microbiological results. Nonetheless, it is very important at this stage to evaluate the presence of the material after clinical use without undermining the photocatalytic activity of TiO₂ nanoparticles when evaluated invitro over a period of 6 months.

Exposure of specimens to light

The specimens were tested for inhibitory effect at time intervals of 1 day, 1 week, 1 month, 3 months and 6 months. Exposure of the specimens to visible light was done during these time intervals for 4 hours. The conventional and ideal source of visible light was sunlight. However, the uneven occurrence and distribution of sunlight (natural source of visible light) and seasonal variations caused a setback. There were identified reports which suggested that wavelength of light emitted by halogen light bulb is comparable to sunlight (3900Å to 7500Å). Therefore halogen light was used as a source of visible light in this study. All these ensured that all the photocatalyst containing sites would be activated. Desai and Kaushik synthesized TiO₂ by sol-gel technique and studied its inhibitory photocatalytic activity against some common pathogenic micro organisms such as E. coli, P. aeruginosa, K. pneumoniae, S. aureus under visible illumination [24]. The antimicrobial activity of TiO₂-PDMA combination is in accordance with the study conducted by Desai and Koske et al. which evaluated the photocatalytic bactericidal effects of TiO₂ on S. aureus when irradiated. They concluded that treating the surgical devices with TiO₂ particles mixture could create a nearly sterile environment when illuminated, even at lower intensities.

Saline was used to maintain a wet field and prevent dessication of organisms. Dessication is known to result in microbial killing. Normal saline is known to be isotonic and reverse osmosis would be avoided.
Microbiological tests

Photocatalytic activity was assessed against *S. aureus* as it is a common oral colonizer in denture wearing elderly patients [6]. Repeated tests and observations showed consistent inhibitory activity of TiO$_2$ nanoparticles. These observations indicated that TiO$_2$ nanoparticles had inhibitory activity against *S. aureus* when blended with PMMA by all techniques of sample preparation as described earlier. But more efficacious inhibitory activity against *S. aureus* observed at 3 w% concentration of nanoparticles proved anastase form of TiO$_2$ to be a better material [15]. Both 3 w% and 5 w% concentration of nanoparticles yielded similar results, 3 w% concentration of TiO$_2$ was preferred for reasons explained above. Primarily, it is important to note that the photocatalytic ability of TiO$_2$ nanoparticles was present till 6 months regardless of the duration of the experiment. The average antimicrobial activity as analyzed statistically was 72 % in all categories and 28% growth was seen in all categories. Statistically there was no significant difference in the antimicrobial activity across the groups in the study conducted over the time periods. Availability of photocatalytic sites was higher in post curing coated material compared to precurring material as revealed by SEM. However, microbiological tests indicated no significant difference in the performance of all the three groups tested. This disagreement in the SEM and microbiological results need further confirmation by repetitive studies. This will certainly be useful in the development of novel self cleansing denture base material.

The materials were exposed to visible light for periods from 1 day to 6 months to assess the requirement of induction or preactivation of photocatalytic activity. The results obtained after 1 day pre-exposure and 6 months pre-exposure to visible light did not provide any comparable difference in the activity. We therefore conclude that preactivation of the photocatalytic sites in the denture base material with light is not necessary. Patients can start using the dentures immediately after delivery.

Conclusions

Based on the results presented and within the limitations of this study, following conclusions could be drawn:

1. Visible light activated TiO$_2$ nanoparticles can be synthesized using TiN by solution based technique.
2. Organic impurities present in as-prepared TiO$_2$ were eliminated by annealing, as demonstrated by XRD, EDAX and SEM.
3. FTIR studies showed significant and adequate bonding between nanoparticles and PMMA.
4. 3 w% of TiO$_2$ shows antimicrobial activity against *S. aureus* almost uniformly in all three different combinations (viz. incorporation, pre-curing and post curing coating) with PMMA.
5. The inhibitory effect indicates reduction in microbial number and an inadequate number of bacteria prevents quorum sensing . These mechanisms ultimately prevent plaque formation on denture surfaces. TiO$_2$ when used with PMMA would contribute to have better denture hygiene by using cheaply available solar energy/light.
6. The results obtained in the study encourage further extension of the definitive scope of study by invivo methods and also to check the inhibitory activity of TiO$_2$ photocatalyst against other oral colonizers.
7. Further studies to assess the mechanical properties, consistent presence of TiO$_2$ nanoparticles over a period of time when used by patients are essential.

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References

1. Rawl HR (2009) Dental Polymers. Philips Science of Dental Materials (11th edition): Elsevier (India)
2. Fure S, Zickert I (1990) Salivary conditions and cariogenic micro-organisms in55, 65, and 75 year old Swedish individuals. Scand J Dent Res 98: 197-210.
3. Segal E, Kremer I, Dayan D (1992) Inhibition of adherence of Candida albicans to acrylic by a chitin derivative. Eur J Epidemiol 8: 350-355.
4. Costerton JW, Stewart PS, Greenberg EP (1994) Bacterial biofilms: a common cause of persistent infections. Science 284: 1318-1322.
5. Percival RS, Challacombe SJ, Marsh PD (1991) Age-related microbiological changes in the saliva and plaque microflora of healthy adults. J Med Microbiol 35: 5-11.
6. Glass RT, Conrad RS, Bullard JW, Goodson LB, Mehta N, et al. (2010) Evaluation of microbial flora found in previously worn prostheses from the Northeast and Southwest regions of the United States. J Prosthent Dent 103: 384-389.
7. Marsh PD, Percival RS, Challacombe SJ (1992) The influence of denture-wearing and age on the oral microflora. J Dent Res 71: 1374-1381.
8. Walker DM, Stafford GD, Huggett R, Newcombe RG (1981) The treatment of denture-induced stomatitis. Br Dent J 151: 416-419.
9. Akira Fujishima (May 12 2005) Discovery and applications of photocatalysis-Creating a comfortable future by making use of light energy. Japan Nanonet Bulletin 44.
10. Elsaka SE, Hamouda IM, Swain MV (2011) Titanium dioxide nanoparticles addition to a conventional glass-ionomer restorative: Influence on physical and antibacterial properties. J Dent 39: 589-598.
11. Wetzel B, Rosso P, Haupert F, Friedrich K (2006) Epoxy nanocomposites-fracture and toughening mechanisms. Eng Fract Mech 73: 2375-2398.
12. Xia Y, Zhang F, Xie H, Gu N (2008) Nanoparticle-reinforced resin-based dental composites. J Dent 36:450-455.
13. Random C, Irvine JTS, Robertson P (2008) Synthesis of Visible-Light-Activated Yellow Amorphous TiO$_2$ Photocatalyst. Int J Photoenergy.
14. Winkler S, Woelfel JB (2000) Processing dentures; in Essentials of complete denture prosthodontics (2nd edition) AITBS (India).
15. Akiba N, Hayakawa I, Keh ES, Watanabe A (2005) Antifungal effects of a tissue conditioner coating agent with TiO$_2$ photocatalyst. J Med Dent Sci 52: 223-227.
16. Desai VS, Koveshk M (2009) Antimicrobial activity of titanium dioxide nanoparticles synthesized by sol-gel technique. Res J Microbiol 4: 97-103.
17. Asahi R, Morikawa T, Ohwaki T, Aoki K, Taga Y (2001) Visible-Light Photocatalysis in Nitrogen-Doped Titanium Oxides. Science 293: 269-271.
18. Iwasaki M, Hara M, Kawada H, Tada H, Ito S (2000) Cobalt ion doped TiO$_2$ photocatalyst response to visible light. J Colloid Interface Sci 224: 202-204.
19. Wong MS, Chu WC, Sun DS, Huang HS, Chen JH, et al. (2006) Visible-Light-Induced Bactericidal Activity of a Nitrogen-Doped Titanium Photocatalyst against Human Pathogens. Appl Environ Microbiol 72: 6111-6116.
20. Gao Y, Masuda Y, Seo WS, Ohta H, Koumoto K (2004) TiO$_2$ nanoparticles
prepared using an aqueous peroxotitanate solution. Ceramics International 30: 1365-1368.

21. Cheng Y, Sakai T, Moroi R, Nakagawa M, Sakai H, et al. (2008) Self-cleaning Ability of a Photocatalyst-containing Denture Base Material. Dent Mater J 27: 179-186.

22. Shibata T, Hamada N, Kimoto K, Sawada T, Sawada T, et al. (2007) Antifungal effect of Acrylic Resin Containing Apatite-coated TiO₂ Photocatalyst. Dent Mater J 26: 437-444.

23. Amano D, Ueda T, Sugiyama T, Takemoto S, Oda Y, et al. (2010) Improved brushing durability of titanium dioxide coating on polymethyl methacrylate substrate by prior treatment with acryloxypropyl trimethoxysilane-based agent for denture application. Dent Mater J 29: 97-103.

24. Koseki H, Shirai K, Asahara T, Tsurumoto T, Shindo H, et al. (2009) Photocatalytic bactericidal action of fluorescent light in a titanium dioxide particle mixture: an in vitro study. Biomed Res 30: 189-192.