<table>
<thead>
<tr>
<th><strong>Title</strong></th>
<th>Biocompatibility of a titanium dioxide coating method for denture base acrylic resin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Author(s)</strong></td>
<td>辻 将</td>
</tr>
<tr>
<td><strong>Journal</strong></td>
<td>() -</td>
</tr>
<tr>
<td><strong>URL</strong></td>
<td><a href="http://hdl.handle.net/10130/3422">http://hdl.handle.net/10130/3422</a></td>
</tr>
<tr>
<td><strong>Rights</strong></td>
<td></td>
</tr>
</tbody>
</table>
Biocompatibility of a titanium dioxide coating method for denture base acrylic resin

Masashi Tsuji

Department of Removable Prosthodontics & Gerodontology, Tokyo Dental College, Tokyo, Japan
Abstract

Objectives: Ease-of-denture-cleaning is of paramount importance in geriatric patients and those with limited dexterity. We have previously investigated methods of coating dentures with titanium dioxide (TiO_2) and reported the effects of such treatments in *in vitro* studies. This study was to verify the biocompatibility of a TiO_2-coated acrylic resin produced by the new coating method with spray-coating technique.

Methods: Specimens were prepared from denture base acrylic resin and polished up to grit #1000. The TiO_2 coating agent was sprayed onto the specimens using an air-brush gun. Specimens were then divided into ‘PMMA’, ‘primer-coated PMMA’ and ‘TiO_2-coated PMMA’ groups to be evaluated for biological safety using a hamster oral mucosa irritation test, a guinea pig skin sensitization test and a rabbit intracutaneous test. The biological reaction was scored.

Results: Reaction scores were considerably less than 1.0, the acceptable limit set by the ISO, in all three tests. Indeed, in most samples there was no deleterious effect at all.

Conclusion: These results demonstrate that denture base resin coated with TiO_2 by this method does not cause irritation or sensitization of the oral mucosa, skin or intracutaneous tissue, and is therefore good biocompatibility for use in close proximity to oral mucosa and skin.

Keywords: Dental Materials; Dentures; Titanium dioxide; Coated Materials, Biocompatibility; Animal Models; Geriatric Dentistry
Introduction

Polymethyl methacrylate (PMMA) resin has long been used as a denture base material\(^1\), because of its hardness and rigidity under masticatory pressure, ease of handling, good esthetics and low price. However, despite its obvious suitability as a denture base material\(^2,3,4\), PMMA is also susceptible to deterioration\(^5,6\), surface roughness following fatigue, microbial adherence\(^7\) and colonization by bacteria due to water absorption\(^1\).

Microbe adhesion to the denture surface befouls the oral cavity and can cause systemic infections (e.g. aspiration pneumonitis)\(^8,9\). Geriatric patients and those with limited dexterity (e.g. maniphalanx dysfunction or rheumatoid arthritis) find oral hygiene (OH) troublesome and require assistance from family or nursing staff. However, although various mechanical, ultrasonic and chemical cleaning methods have been proven efficacious\(^10\), these helpers often lack the OH knowledge to apply them. Hygienic denture materials would be invaluable in improving OH in denture wearers. We have previously reported on the photocatalytic effect of titanium dioxide (TiO\(_2\)) application (i.e., oxidation decomposition and super-hydrophilicity), and the viability of making dentures that can be cleaned simply by rinsing in water\(^11,12,13\). TiO\(_2\) can be incorporated into the resin\(^10,14,15\) to achieve the photocatalytic effect but requires ~5wt% TiO\(_2\), which can weaken the material and cause internal decomposition through its photocatalytic effect. A preferable option is to use TiO\(_2\) coating method, which does not alter the resin itself. Coating can be by brushing, dipping or spraying, which will influence the coating thickness and resilience to detachment. We used a spray-coating technique using an air-brush gun, which produces a thin coating\(^11\) and reportedly changes the surface characteristics of the acrylic resin, making it less likely to accumulate bolus residue and subsequently easier to clean\(^11\). This TiO\(_2\) coating of acrylic resin also inhibits the adhesion of microbes\(^12\), and is sufficiently durable to withstand normal oral function\(^13\). Unlike the mixing method, the spray-coating technique is not material-dependent and can be applied to all denture components, including the base, artificial teeth and major and minor connectors. Finally,
because the coat is concentrated at the surface, where it is most useful, the photocatalytic effect can be achieved with much lower amounts of TiO$_2$.

The TiO$_2$ coating material contains TiO$_2$ and organic material (primer) $^{13}$. This organic material, and the solvent in which it is solubilized, are the most likely candidates for inducing an inflammatory response in tissues. The application protocol minimizes the risk of deleterious effects from these components, but although the material has been validated \textit{in vitro}, the biological safety of the TiO$_2$ coat remains to be verified. The purpose of this study was to investigate the biocompatibility of this TiO$_2$ spray-coating method for acrylic resin dentures.
Materials and methods

Plate specimens

PMMA specimens were prepared from wet-heat-curing acrylic resin (Acron, GC Corporation, Tokyo, Japan). The volume ratio of powder : liquid was 1 : 0.43. The resulting mixture was packed into a plaster mold and placed in 60 °C water for 60 min (for primary polymerization) and then boiling water for 60 min (for secondary polymerization). Specimens were polished up to the equivalent of grit #1000 by wet abrasive paper.

TiO₂ coating

Specimens were pre-treated with a primer agent (Paltitan PTI5603S; Nihon Parkerizing, Kanagawa, Japan), whose main component is acryloxypropyltrimethoxysilane in ethanol, sprayed for 2 s from an air-brush gun (Super Airbrush Advance; WAVE, Tokyo, Japan) then dried for 10 min at 70 °C in air. TiO₂ coating agent (Paltitan PTI5603S, Nihon Parkerizing, Kanagawa, Japan), containing 2.0% anatase-type TiO₂ in water and ethanol, was then sprayed onto the substrate for 2 s and dried in an oven for 10 min at 70 °C.

Animals

All animals used in these experiments were obtained from Japan SLC, Inc. (Hamamatsu, Japan), housed in an air-conditioned room (temperature: 21±2 °C; humidity: 55±10%) under a 12-h light/dark cycle (lights on: 6:00 am – 6:00 pm) and maintained on commercial laboratory chow and water for at least one week before being used. Animals were treated according to the Guidelines for the Treatment of Experimental Animals approved by the Tokyo Dental College (No.242607, No.232606, No.242606).

Oral mucosa irritation test
The oral mucosa irritation test was performed using 5-week-old male Syrian hamsters. Animals were divided into 4 groups as follows: ‘positive control’, ‘PMMA’, ‘primer-coated PMMA’ and ‘TiO₂-coated PMMA’. Cheek pouches were everted and rinsed with physiological saline before suturing a specimen (a 5 mm diameter × 0.8 mm height disc or cotton wool with liquid of resin as positive control) into the right-hand cheek pouch with non-resorbable nylon thread. The left cheek pouch was sutured without a specimen insertion as negative control. After 24 hours, animals were euthanized with an excess of pentobarbital and the cheek pouches were excised, fixed with formalin and embedded in paraffin. Serial sections, stained with hematoxylin and eosin, were examined for microscopic evidence of epithelial and connective tissular reaction. Every challenge site was scored (0–4 points) for erythema by 2 judges independently.

**Sensitization test**

The skin sensitization test was performed using 6-week-old male Hartley guinea pigs divided into 5 groups as follows: ‘negative control’, ‘positive control’, ‘PMMA’, ‘primer-coated PMMA’ and ‘TiO₂-coated PMMA’. Hair was clipped from the back region and 0.1 ml of emulsified Freund's complete adjuvant was applied intradermally at the four corners of a 2×4 cm area before a criss-cross lattice of abrasions was made at the injection site. Specimens (as for the mucosal irritation test, above) or 0.1 ml of 0.5% 2,4-dinitrochlorobenzene (as positive control) in acetone were applied to the abraded area of skin for 24 hours. Abrasion and patch application was repeated on each of the following two days. Sodium lauryl sulphate (10%) was applied to the injection site after six days, and again on the seventh day in combination with specimen or 0.2 ml 0.5% 2,4-dinitrochlorobenzene. This was then left for 48 hours. Negative control animals were similarly treated, but without the test materials. At fourteen days after the last induction, all animals were again challenged with the specimen or 0.01 ml of 0.1% 2,4-dinitrochlorobenzene in acetone together. Every challenge site was scored (0–4 points) for
erythema and swelling respectively at 24 hours and 48 hours after application by 2 judges independently.

**Intracutaneous test**

The intracutaneous test was performed using male rabbits, weighing 2.5–2.8 kg, divided into 4 groups as follows: ‘positive control’, ‘PMMA’, ‘primer-coated PMMA’ and ‘TiO2-coated PMMA’. Extraction liquid from each group of specimens was acquired by soaking a disc of each material (18 mm diameter, 1.0 mm height) in polar solvent (physiological saline) or non-polar solvent (sesame oil) for 3 days at 37 °C with stirring. Positive control was kappa-carrageenan in saline and liquid of resin in oil. Back fur was closely clipped, allowing sufficient space either side of the spine for injection of 0.2 ml of test liquid (injected intracutaneously at five sites on one side) and solvent only (injected at five sites on the contralateral side as negative control). Each injection site was evaluated for signs of skin reaction immediately and at 24, 48 and 72 hours after injection by 2 judges independently, with reaction scored on a scale of 0–4 points for erythema and swelling respectively. A net score was obtained by subtracting the negative control score from the test score. A score of 1.0 or less was deemed to be non-irritant.
Results

Oral mucosa irritation test

Table 1 shows the results of the oral mucosa irritation test. Marked erythema was found in all animals in the positive control group, with a mean score of 2. In contrast, no erythema was apparent in the other groups (mean score: 0). An example of histological evaluation of hamster cheek pouch mucosa exposed to liquid of resin as positive control (A) and TiO\textsubscript{2}-coated PMMA (B) are shown in Figure 1. Abnormal finding on the (B) was not observed. However, the coagulation necrosis both epithelium and connective tissue layers, and pressure bulla were observed on the (A).

Sensitization test

Table 2 shows skin sensitization rates. In the negative control group, the score in each animal was zero. In the positive control (2,4-dinitrochlorobenzene), marked erythema and swelling were apparent in all animals, with mean scores at 24 hours and 48 hours after application being 5.8 and 6.2, respectively. Overall, there was no evidence of significant skin reaction in the ‘PMMA’, ‘primer-coated PMMA’ or ‘TiO\textsubscript{2}-coated PMMA’ groups (mean scores: 0.2, 0 and 0.2, respectively), although minor erythema (1 point) was observed in one animal in each of the ‘PMMA’ and ‘TiO\textsubscript{2}-coated PMMA’ groups at 24 hours after application. However, this had disappeared at 48 hours.

Intracutaneous test

Table 3 shows the intracutaneous reactivity data. Marked erythema and swelling were found in all positive control animals (kappa-carrageenan in saline or liquid of resin in oil; net scores: 5.93 and 5.18, respectively). No significant reaction was observed in any of the test groups (net scores: <<1.0, the acceptable limit of intracutaneous reaction), although minor erythema (1 point) was observed.
**Discussion**

Despite evidence of functional efficacy, the biocompatibility of this coating material remained unknown. The denture base resin contacts not only the wearer’s oral mucosa, but also his or her skin and that of the dentist and dental technician\(^1\) so a guarantee of high safety had to be established prior to clinical use. Many studies have evaluated the safety of denture base resins\(^{16,17,18}\), mostly by evaluating *in vitro* cellular reactions to the resin monomer\(^19\). However, reactions at the cellular level are not representative of the systemic biological inflammatory reaction, so we evaluated our TiO\(_2\)-coated denture base material in three whole-animal models to assess its biocompatibility.

The safety evaluation was planned in accordance with ISO10993-10, which requires an “oral mucosa irritation test”, a “sensitization test”, and an “intracutaneous test”\(^20\). The oral mucosa irritation test determines the reaction against the material in direct contact with the oral mucosa\(^21\), while the sensitization test evaluates irritation reaction and sensitization effects in contact with skin\(^22\). However, these tests do not take account the risk of elution of unpolymerized organic components and residual organic solvent into the saliva, hence the inclusion of the intracutaneous test to assess component penetration into tissues.

There are differences among the surface texture and character of PMMA, primer-coated PMMA and TiO\(_2\)-coated PMMA. We have previously reported that the surface roughness (R\(_{\text{max}}\)) of the polished resin plate, which was 1.82 ± 0.83 µm, was increased up to 2.67 ± 0.26 µm by this TiO\(_2\) coating and the water contact angle of the resin plate was dropped from 70° to 10° after TiO\(_2\) coating, i.e. the wettability of the surface was increased\(^{11}\). These differences might have any effect on biological reaction. Therefore, in the oral mucosa irritation test, the specimens should not over-expand the cheek pouches, so were kept relatively small (5 mm
diameter, 1 mm thickness). Also, to eliminate irritation related to friction between the specimen and oral mucosa, the test period was kept relatively short (24 hours), allowing chemical-induced reaction to be studied selectively.

In the sensitization test, guinea pigs were tested using Freund’s Complete Adjuvant (to amplify antibody production) and focal stimulation with sodium lauryl sulfate to induce sensitization\textsuperscript{22}. Positive skin reactions were not found in the ‘primer-coated PMMA’ group, and there was only one case of minor erythema in each of the ‘PMMA’ and ‘TiO\textsubscript{2}-coated PMMA’ groups, which disappeared after 24 hours. With a mean score of 0.2 (“negligible (0–0.4)”, according to the ISO skin irritation index), these low-grade reactions do not alter the conclusion that the PMMA, primer-coated PMMA and TiO\textsubscript{2}-coated PMMA do not cause significant skin irritation.

The intracutaneous test employed liquids infused with any leached unpolymerized monomer from the specimens\textsuperscript{23}, and tested this on rabbit back skin, which is known to be highly sensitive. Extraction of monomer from the specimens was performed at 37 °C for 72 hours, more severe than the clinical situation. In the oral cavity, the solvent is saliva, which is polarized due to its electrolyte content. Moreover, lipophilic components elute into organic salivary elements (e.g., proteins and amino acids), hence our study considered both polar and non-polar solvents. To eliminate the irritation of the intracutaneous injection and by the solvent itself, the negative control received an injection of solvent with no extract. In the specimen groups, the test scores were never higher than 1.0, and the mean scores were all less than 1.0, meeting the ‘non-irritant’ requirements of the ISO test (mean net score: <1.0). The results indicate that neither the primer nor the TiO\textsubscript{2} components of the coating material (residual reactive component and organic solvent) produce any reaction above that caused by PMMA itself, which is recognized as a highly biocompatible denture base material. This suggests that the TiO\textsubscript{2}-coated PMMA material should also be highly biocompatible.
This TiO\textsubscript{2} coating agent contains nano size TiO\textsubscript{2} particles (5-10 nm in diameter). If the nano particles were released from the coating, it might cause cytotoxicity. However, TiO\textsubscript{2} particles were bound to each other and to primer agent chemically. The most likely deleterious components in this coating method were the organic material in the primer agent and the organic solvent required to adapt the TiO\textsubscript{2} particles for spray application. Acryloxypropyltrimethoxysilane, which is main component in the primer agent, binds TiO\textsubscript{2} on acrylic resin, but has irritant property. However, the specimens were warm-dried for 10 min after both the primer application and the TiO\textsubscript{2} coating, and the absence of obvious reaction in our three safety tests suggests that this protocol leaves negligible amounts of residual reactive components. Indeed, the coating can be as thin as 2 \( \mu \text{m} \), so most organic solvent will be volatilized during the drying stage, minimizing the amount of potentially injurious solvent in the coating layer.

**Conclusion**

This study has demonstrated that the TiO\textsubscript{2}-coated denture base resin has no irritation to the oral mucosa, nor does it cause skin sensitization. Any elution of components from the coating has no deleterious effects for tissues.

**Acknowledgements**

The author would like to thank Professor Dr. Kaoru Sakurai, Professor Dr. Mitsuru Kawaguchi, Professor Dr. Kohei Sawaki and Associate Professor Dr. Takayuki Ueda for their kind advice.
References

1) Pfeiffer P, Rosenbauer EU.
Residual methyl methacrylate monomer, water sorption, and water solubility of hypoallergenic denture base materials.

2) Cimpan MR, Cressey LI, Skaug N, Halstensen A, Lie SA, Gjertsen BT, Matre R.
Patterns of cell death induced by eluates from denture base acrylic resins in U-937 human monoblastoid cells.

3) Silva Cde S, Machado AL, Chaves Cde A, Pavarina AC, Vergani CE.
Effect of thermal cycling on denture base and autopolymerizing relining resins.

4) Marra J, Paleari AG, Rodriguez LS, Leite AR, Pero AC, Compagnoni MA.
Effect of an acrylic resin combined with an antimicrobial polymer on biofilm formation.

5) Sato M, Ohshima T, Maeda N, Ohkubo C.
Inhibitory effect of coated mannan against the adhesion of Candida biofilms to denture base resin.

6) Nam KY, Lee CH, Lee CJ.
Antifungal and physical characteristics of modified denture base acrylic incorporated with silver nanoparticles.
7) Sahin C, Ergin A, Ayyildiz S, Cosgun E, Uzun G.
Effect of biofilm formation, and biocorrosion on denture base fractures.

8) Sumi Y, Kagami H, Ohtsuka Y, Kakinoki Y, Haruguchi Y, Miyamoto H.
High correlation between the bacterial species in denture plaque and pharyngeal microflora.

9) Ryu M, Ueda T, Saito T, Yasui M, Ishihara K, Sakurai K.
Oral environmental factors affecting number of microbes in saliva of complete denture wearers.

Self-cleaning effects of acrylic resin containing fluoridated apatite-coated titanium dioxide.
Gerodontology. Published online at April 15 2013. doi: 10.1111/ger.12052.

11) Kado D, Sakurai K, Sugiyama T, Ueda T.
Evaluation of cleanability of titanium dioxide (TiO2)-coated acrylic resindenture base.

12) Arai T, Ueda T, Sugiyama T, Sakurai K.
Inhibiting microbial adhesion to denture base acrylic resin by titanium dioxide coating.
13) Amano D, Ueda T, Sugiyama T, Takemoto S, Oda Y, Sakurai K.  
Improved brushing durability of titanium dioxide coating on polymethylmethacrylate substrate by prior treatment with acryloxypropyl trimethoxysilane-based agent for denture application.  

14) Shibata T, Hamada N, Kimoto K, Sawada T, Sawada T, Kumada H, Uemoto T, Toyoda M.  
Antifungal effect of acrylic resin containing apatite-coated TiO2 photocatalyst.  

Self-cleaning ability of a photocatalyst-containing denture base material.  

Cytocompatible antifungal acrylic resin containing silver nanoparticles for dentures.  

17) Jorge JH, Giampaolo ET, Vergani CE, Machado AL, Pavarina AC, Carlos IZ.  
Effect of post-polymerization heat treatments on the cytotoxicity of two denture base acrylic resins.  

18) Ebrahimi Saravi M, Vojdani M, Bahrani F.  
Evaluation of cellular toxicity of three denture base acrylic resins.  

Cytotoxicity of titanium and titanium alloying elements.


Legends

Table 1. Reaction of hamster oral mucosa to materials was measured by mucosal irritation test. Marked erythema (all exposure area: 2 points) was found with all animals in the positive control group. In contrast, no erythema was apparent in the other groups (0 point).

Table 2. Reaction of guinea pig skin to materials was measured by skin sensitization test. The reaction was scored with regard to focused on erythema (no erythema: 0 point, less than exposure area: 1 point, all exposure area: 2 points, beyond exposure area: 3 points and eschar formation: 4 points) and swelling (no swelling: 0 point, barely tactile thickness: 1 point, less than 1 mm thickness: 2 points, approximately 1 mm thickness: 3 points and more than 1 mm thickness: 4 points). In the positive control, marked erythema and swelling were apparent in all animals. Overall, there was no evidence of significant skin reaction in the ‘PMMA’, ‘primer-coated PMMA’ or ‘TiO2-coated PMMA’ groups.

Table 3. Reaction of rabbit skin to components eluted from materials was measured by intracutaneous test. The reaction was scored with regard to focused on erythema (no erythema: 0 point, less than exposure area: 1 point, all exposure area: 2 points, beyond exposure area: 3 points and eschar formation: 4 points) and swelling (no swelling: 0 point, barely tactile thickness: 1 point, less than 1 mm thickness: 2 points, approximately 1 mm thickness: 3 points and more than 1 mm thickness: 4 points). The each net score was obtained by subtracting the mean negative score from the mean extract score. Marked erythema and swelling were found in all positive control animals. No significant reaction was observed in any of the test groups (net scores: <<1.0, the acceptable limit of intracutaneous reaction), although minor erythema (less than exposure area: 1 point) was observed.

Fig. 1. An example of histological evaluation of hamster cheek pouch mucosa exposed to liquid of resin as positive control (A) and TiO2-coated PMMA (B) was exhibited. Tissue exposed to material
specimens for 24 hours were fixed with formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Cell poor fibrous connective tissue was covered by stratified squamous epithelium with hyperkeratosis. Abnormal finding was not observed TiO₂-coated PMMA case. The coagulation necrosis both epithelium and connective tissue layers, and pressure bulla were observed. Lymphoidocyte infiltration was also observed in the connective tissue on the positive control.
<table>
<thead>
<tr>
<th>Group</th>
<th>Mean score</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>With specimen</td>
<td>Without specimen</td>
</tr>
<tr>
<td>Positive control</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PMMA</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Primer-coated</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>TiO₂-coated</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td>24 hours</td>
<td>48 hours</td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td>----------</td>
<td>----------</td>
<td></td>
</tr>
<tr>
<td>Negative control</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Positive control</td>
<td>5.8</td>
<td>6.2</td>
<td></td>
</tr>
<tr>
<td>PMMA</td>
<td>0.2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Primer-coated</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>TiO₂-coated</td>
<td>0.2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td>Solvent</td>
<td>Mean score</td>
<td></td>
</tr>
<tr>
<td>---------------</td>
<td>---------</td>
<td>------------</td>
<td>----------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>With extract</td>
<td>Without extract</td>
</tr>
<tr>
<td>Positive control</td>
<td>Saline</td>
<td>5.93</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Oil</td>
<td>5.18</td>
<td>0</td>
</tr>
<tr>
<td>PMMA</td>
<td>Saline</td>
<td>0.04</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Oil</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>Primer-coated</td>
<td>Saline</td>
<td>0.11</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Oil</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>TiO$_2$-coated</td>
<td>Saline</td>
<td>0.04</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Oil</td>
<td>0.33</td>
<td>0.11</td>
</tr>
</tbody>
</table>
Figure 1