Improving Polymethyl Methacrylate Resin Using Novel Nano-Ceramic Coating

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THESIS
Submitted as partial fulfillment of the requirements for the degree of Master of Science in Oral Sciences in the Graduate College of the

University of Illinois at Chicago, 2018

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ACKNOWLEDGEMENT

I would first like to thank my thesis advisor Dr. Bin Yang at University of Illinois / College of Dentistry. The door to Prof. Yang office was always open whenever I needed help or guidance.

I would also like to thank the experts who were involved in this research project: Dr. Christos Takoudis, Dr. Cortino Sukotjo, Dr. Stephen Campbell, Dr. Kent L. Knoernschild, Dr. Christine Wu, Dr. Wei Li, Dr. Judy Yuan, Arghya Kamal Bishal and Su Huang without their passionate participation and valuable input, this research could not have been successful.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - INTRODUCTION</td>
<td></td>
</tr>
<tr>
<td>1.1 Background &amp; Significance</td>
<td>1</td>
</tr>
<tr>
<td>1.2 Specific Aims</td>
<td>3</td>
</tr>
<tr>
<td>2 - REVIEW OF LITERATURE</td>
<td></td>
</tr>
<tr>
<td>2.1 Edentulism</td>
<td>4</td>
</tr>
<tr>
<td>2.2 PMMA in Dentistry</td>
<td>5</td>
</tr>
<tr>
<td>2.3 Advantages of PMMA</td>
<td>5</td>
</tr>
<tr>
<td>2.4 Limitation of PMMA</td>
<td>5</td>
</tr>
<tr>
<td>2.5 <em>Candida albicans</em></td>
<td>6</td>
</tr>
<tr>
<td>2.6 Titanium Dioxide</td>
<td>6</td>
</tr>
<tr>
<td>2.7 Atomic Layer Deposition</td>
<td>7</td>
</tr>
<tr>
<td>2.8 Previous Studies</td>
<td>9</td>
</tr>
<tr>
<td>3 - MATERIAL AND METHODS</td>
<td></td>
</tr>
<tr>
<td>3.1 PPMA specimen fabrications</td>
<td>13</td>
</tr>
<tr>
<td>3.2 TiO₂ coating of PMMA specimens</td>
<td>13</td>
</tr>
<tr>
<td>3.3 PMMA and TiO₂-PMMA surface characterization</td>
<td>14</td>
</tr>
<tr>
<td>3.4 Three-point binding test</td>
<td>14</td>
</tr>
</tbody>
</table>
3.5 Wear resistance by brushing test 15
3.6 Chemical and mechanical challenge test 15
3.7 Antimicrobial (Candida attachment/Biofilm formation) 16

4 – RESULTS 18
5 – DISCUSSION 20
6 – CONCLUSIONS 25
7 – FIGURES 26
8 – CITED LITERATURE 35
9 – VITA 42
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>FIGURES</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a: Atomic layer deposition coating (ALD) mechanism</td>
<td>26</td>
</tr>
<tr>
<td>1b: Growth rate of TiO$_2$ using TDEAT and O$_3$</td>
<td>27</td>
</tr>
<tr>
<td>2: Flexure strength of PMMA and TiO$_2$-PMMA</td>
<td>28</td>
</tr>
<tr>
<td>3: Surface roughness of PMMA and TiO$_2$-PMMA before and after brushing test.</td>
<td>29</td>
</tr>
<tr>
<td>4: XPS spectrum result of PMMA and TiO$_2$ coated PMMA</td>
<td>30</td>
</tr>
<tr>
<td>5: XPS spectrum result of TiO$_2$-PMMA and TiO$_2$-PMMA after brushing test</td>
<td>31</td>
</tr>
<tr>
<td>6: XPS spectrum result of mechanical and chemical test</td>
<td>32</td>
</tr>
<tr>
<td>7a: The adherence of <em>C. albicans</em> on PMMA and TiO$_2$-PMMA</td>
<td>33</td>
</tr>
<tr>
<td>7b: Biofilm formation of <em>C. albicans</em> on PMMA and TiO$_2$-PMMA</td>
<td>34</td>
</tr>
</tbody>
</table>
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALD</td>
<td>Atomic Layer Deposition</td>
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<tr>
<td>PMMA</td>
<td>Poly (methyl methacrylate)</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
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<tr>
<td>SPSS</td>
<td>Statistical product and service solutions</td>
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<td>TiO$_2$</td>
<td>Titanium dioxide</td>
</tr>
<tr>
<td>XPS</td>
<td>X-ray photoelectron spectroscope</td>
</tr>
</tbody>
</table>
SUMMARY

Objectives: Poly methyl methacrylate (PMMA) has been broadly used in dentistry. Unfortunately, PMMA has poor surface wear resistance, is relatively porous, and promotes adhesion of complex oral biofilms associated with oral mucositis and systemic co-morbidities. The Aim of this study is to improve the surface physical and chemical characteristics of PMMA by developing a novel, thin film, nano-ceramic coating process, and to analyze the resulting coated surface.

Methods: Atomic Layer Deposition (ALD) technique was developed to deposit a titanium dioxide (TiO$_2$) nano-ceramic thin film on PMMA. The surface wettability for both coated and uncoated PMMA was determined by water contact angle. Wear resistance was performed using a mechanical tooth-brushing device with a load of 50g for 6000 strokes after 5-month water storage. Chemical and mechanical challenge test was performed by using sonication in 3.8% sodium perborate (Polident denture cleanser) for 1 hour with aged specimens. X-ray photoelectron spectroscopy (XPS) was utilized before and after the brushing test and challenge test to analyze the PMMA surface chemical composition. The mechanical strength of coated and uncoated PMMA was measured by three-point bending with a universal testing machine (n=10). Surface microbial interactions were evaluated using \textit{C. albicans} attachment and biofilm formation test.
**Results:** Nano-ceramic TiO$_2$ coating (30 nm-thick) was successfully deposited on PMMA at 65°C. After coating, water contact angle decreased from 67° to less than 5°, indicating formation of a super-hydrophilic surface. After brushing test, the ceramic coating remained intact. XPS analysis revealed no loss of TiO$_2$ from coated samples following brushing and denture cleanser sonication for 1 hour. There was no statistically significant difference in mechanical strength (MPa) (Mean±SD) between PMMA (139.37±11.34) and TiO$_2$-PMMA (160.65±37.07). *C. albicans* attachment decreased by 63% on the coated PMMA surface. *C. albicans* biofilm formation decreased by 53% on the coated PMMA surface.

**Conclusions:** ALD is a promising technique to modify surface properties of PMMA and resulted in a stable adherent thin film. By depositing a TiO$_2$ nano-ceramic coating, PMMA surface properties may lead to significantly reduced microorganism adhesion and easier pathogen removal from PMMA. For patients who wear dentures, reducing the oral microbial biofilm burden using a TiO$_2$ coated PMMA surface could positively impact their oral and systemic health.

**Key words:** POLYMETHYL METHACRYLATE RESIN, nano-ceramic coating, surface characterization, *C. Albicans* attachment
1. INTRODUCTION

1.1 Background and Significance

Poly methyl methacrylate (PMMA) is the most commonly used material for the fabrication of dentures, maxillofacial, and implant supported prostheses. Despite its suitability as a denture base material, PMMA has poor wear resistance and it is porous. The hydrophobic surface of PMMA facilitates the initial adhesion of *Candida albicans* due to their hydrophobic properties. As a result, acrylic resin denture base surfaces provide hard, non-shedding niches for microbial adherence with subsequent formation of bacteria denture plaque that harbors potential oral pathogens. This material is susceptible to surface degradation, surface roughness changes and infection with microorganisms that contributes to a denture plaque and biofilm formation. Furthermore, denture plaque has been associated with stomatitis, peri implantitis and increased risk for systemic diseases such as aspiration pneumonitis and systemic candidiasis. These observations underscore the importance of controlling and preventing microbial adherence, oral biofilm formation on PMMA surfaces, and diffusion into the volume of acrylic material.

There are published guidelines regarding the cleaning and the maintenance of complete dentures, but all *in vitro* studies have not achieved effective cleaning and disinfection techniques. Currently, plaque/biofilm on removable dentures can be reduced by mechanical (brushing and ultrasonic devices) and chemical methods (alkaline peroxide and hypochlorite, citric acids, enzymes and glutaraldehyde). However, these methods are ineffective at disinfecting acrylic resin denture base material and do not prevent short-
term biofilm recolonization by *Candida species* \(^{13}\). Innovation in resin cleaning techniques or innovation in resin surface chemistry could lead to effective maintenance regimens that improve edentulous patient oral health.

Titanium dioxide film and particles have multiple effects, benefits and applications. Titanium dioxide film/particles can inactivate *C. albicans* after 30-90 min of UV light (315-400 nm) exposure. Reduction in *C. albicans* has been shown to be more than 90\%. \(^9,\) \(^{14,15}\) Under UV light irradiation, strong oxidative species including hydroxyl (OH\(^-\)), superoxide free radicals (O\(_2\)\(^-\)), or hydrogen peroxide (H\(_2\)O\(_2\)) are created from photoexcited TiO\(_2\). These aggressive oxidative activities cause irreversible damage to the cell surface, destroying the organic compounds inside, and result in the inactivation of bacteria or fungi. \(^{16}\) An additional benefit from TiO\(_2\) is its super hydrophilic properties, “water sheathing effect” \(^{17}\) This allows debris to be removed with water. Many researchers have also demonstrated the biocompatibility of TiO\(_2\) as a surface and as a bio-sensor for orthopedic and dental applications. \(^{18-20}\) In summary, these properties demonstrate TiO\(_2\) antimicrobial activity, cleansability and biocompatibility.

Limited evidence exists regarding the modification of acrylic resin surfaces to provide a more hydrophilic, less porous surface that minimizes microorganism accumulation and is more readily cleansed and disinfected.
1.2 Specific Aims

The purpose of this study is to develop and evaluate a novel technique that was developed by the authors to process a thin film nano-ceramic coating (TiO$_2$) on the surface of acrylic resin denture base material using an atomic layer deposition (ALD) mode at low temperatures. This technique takes advantage of in-situ precipitated nanoparticles and nanostructures to improve surface mechanical properties, surface wettability, and potentially eliminate diffusion of pathogens into the acrylic. This ceramic coating may provide improved hydrophilic surface properties, reduced *Candida albicans* adherence and biofilm formation, cleansability and wear resistance of PMMA without altering its mechanical properties.

The objectives of this study were to (1) deposit a novel thin film ceramic (TiO$_2$) coating on acrylic resin denture material without altering the mechanical properties of acrylic resin bulk; (2) determine the surface characteristics of the ceramic coating – chemical composition, roughness and morphology, wettability evaluation; (3) evaluate wear resistance of this ceramic coating with tooth brush abrasion; 4) determine the adherence of the ceramic coating to PMMA using ultrasonic and chemical challenges combined with water storage; and 5) investigate the attachment and biofilm formation of *C. albicans* to denture-base materials after the surface modification with TiO$_2$ ceramic coating compared to uncoated PMMA control.
2. REVIEW OF LITERATURE

2.1 Edentulism

There are many factors associated with complete and partial edentulism such as: age, disease, accidents, smoking and alcoholism. Based on the World Health Organization (WHO) classification, fully edentulous patients satisfy WHO criteria for being: physically impaired\textsuperscript{23}, disabled and handicapped.\textsuperscript{23-25} Douglas et al\textsuperscript{26} suggested that number of completely edentulous patients in the US was not declining. Douglas’s study suggested that the decrease in complete edentulism is around 10% per decade but that will be offset by the increase in adults over the age of 55 years\textsuperscript{26}. They suggested that the number of fully edentulous patients in the United State would increase from 33.6 to 37.9 million by 2020, this increase can be justified by increase in life expectancies and the increase in population growth.\textsuperscript{26}

Slade et al\textsuperscript{27} studied the edentulism in the United State. They studied population trends in U.S. adults over 15 years of age using five national cross sectional surveys (1957 to 1958, 1971 to 1975, 1988 to 1998, 1999 to 2002, and 2009 to 2012).\textsuperscript{27} These surveys included 155,524 individuals. The authors found that the prevalence of edentulism declined from 18.9% at the 1957 to 1958 to 4.9% in the most recent age cohort\textsuperscript{27}. They reported the rate of edentulism in the United States in 2010 at 12.2 million. As might be expected, edentulism was less common in high income population, and states with higher levels of poverty showed higher levels of edentulism. Slade et al projected that the rate of decline would slow to 2.6% by 2050; however, they also concluded that rate of decline in edentulism may be offset by increased population growth and aging.\textsuperscript{27}
2.2 Comorbidity and Edentulism

According to Felton 2016, tooth loose and complete edentulism is associated with many systemic comorbid conditions such as: risk of malnutrition and obesity, increased risk of COPD related events, poorly maintained removable dentures may be associated with increases in pneumonia related hospitalizations. Also, studies suggested that edentulism is an independent predictor of cardiovascular disease mortality. Educating patients and caregivers of the potential long-term harmful effect of tooth extraction and of poorly maintained dental prostheses is an important step to minimize the negative outcome of tooth loose.

2.3 PMMA in Dentistry

PMMA was first introduced by Rohm and Hass in 1936 as a denture base material and quickly became the top choice material. It can cover the residual ridge, restore the lost gingival tissue and also support the artificial teeth. Currently it’s used in dentures, orthodontic appliances, maxillofacial, implant retained fixed and removable prostheses.

2.4 Advantages of PMMA

The wide use of PMMA is due to its favorable mechanical and physical properties such as hardness and rigidity, good performance under masticatory forces in addition to ease of handling, good esthetics, low cost also it’s easy to repair in case of fracture.
2.5 Limitation of PMMA

Despite resin suitability for the fabrication of dentures, PMMA is porous and vulnerable to surface degradation and surface coarseness changes which results in increase microorganism retention and color discoloration, also PMMA denture base material has poor wear resistance. The hydrophobic surface of PMMA facilitates the initial adhesion of yeast especially *C. albicans* due to their hydrophobic properties. As a result, acrylic resin denture base surfaces provide home for microbial adherence and subsequent formation of denture plaque harboring various potential pathogens.\(^{30}\)

2.6 *Candida albicans*

There are over 700 bacterial species have been detected in human mouth.\(^{31}\) Even if some of them are good bacterial which can help with the digestion of food. Still, some can cause problems such as cavities and periodontal diseases. Compare with bacterial cells, a relatively few fungal species appear in oral environment. Among all the common yeast, for example, *C. glabrata, C. tropicalis, C. parapsilosis, C. albicans, C. krusei, C. kefyr, and C. guilliermondii, C. albicans* is the most commonly encountered oral fungi in human mouths.\(^{32}\) *C. albicans* is a fungus that is normally present on the skin and in mouth. *C. albicans* becomes an infectious agent when there are some changes that allows it to grow out of control. *C. albicans* prevalence in the oral cavity of 45-65% of healthy individuals. Prevalence of *C. albicans* in denture users increases to 60-100%. *C. albicans* biofilms are frequently associated with the denture stomatitis and candidiasis. Patients may
display numerous symptoms such as burning sensation, alteration of taste, and swallowing difficulties.

The prevalence increases in denture users can be explained by the fact that Candida has affinity for the acrylic surface of dentures due to their hydrophobic properties also dentures lessening the flow of $O_2$ and saliva to the basic tissue leading to anaerobic environment that helps yeast overgrowth. Surface roughness of the prostheses is another important factor and there are evidences showed that significantly higher number of *Candida albicans* on rough surfaces than on smooth surfaces.

Another very important factors that the non-shedding surface of the denture that means that the ability to remove adherent microbes through self-renewal of surface layers does not occur.

### 2.7 Evidence-Based Guidelines to Clean Dentures

Based on the ACP guidelines to clean dentures, PMMA can be cleaned using

1- Mechanical Brushing with denture creams and pastes but *Candida* appears to be resistant to mechanical debridement and the abrasiveness of denture pastes is of concern.

2- Soaking and brushing with commercially available denture cleaners but none of the in vivo trials demonstrated that any of the methods were bactericidal.

3- Ultrasonic cleaning with BioSonic Enzymatic and Ultra-Kleen solutions, while ultrasonic cleaning improved kill rates of bacteria, neither of these two solutions tested were completely bactericidal.
4- Alternative denture cleaning methods with Microwave irradiation of dentures immersed in sterile water at 650 Watts. However, the long-term effects of this technique have not been investigated. Additionally, boiling of a denture base has been shown to deform the base, rendering it unusable.28

2.8 Titanium Dioxide

Titanium dioxide (TiO$_2$) is considered as an inert and safe material and has been used in many applications for decades. TiO$_2$ particles can inactive C. albicans after 30-90 min exposure under UVA light (315-400 nm).33-35 Titanium dioxide generate free radicals under UV light. These free radicals cause damage to the cell surface, destroying the organic compounds inside, and result in the inactivation of bacteria or fungi.36 Titanium dioxide as a self-cleaning and self-disinfecting material were widely used not only as inactivate reagent but also as anticorrosion material.37 Last but not the least, many researchers have proven that TiO$_2$ is a biocompatible material and has a promising future for bio-sensor orthopedic and dental applications such as dental implants.38,40 Its relative cell proliferation rate was over 92% and the toxicity grade was 0 or 1 class.40 There are many applications of TiO$_2$ in our daily life, one of the main benefits for titanium dioxide is its defiance to staining (UV) so it’s used in products such as paints and coatings, due to its hydrophilic properties it can be used for anti-fogging coatings and self-cleaning windows. It has disinfecting properties making it suitable for applications such as medical devices, food preparation surfaces, air conditioning filters, also its used in the Pharmaceuticals and cosmetics industries.40
2.9 Atomic Layer Deposition (ALD)

Atomic Layer Deposition (ALD) is a nano-thin film deposition technique that belongs to the class of Chemical Vapor Deposition (CVD) and evolves from Atomic Layer Epitaxy (ALE) in 1900s by Tuomo Suntola. According to the patent published in 1970s, the first ALD was to deposit ZnS film for electroluminescent display panels. Later on, the deposition of Al₂O₃ was studied intensively and become the most popular reaction in ALD. The principle behind the ALD technique is the repeated utilization of separate, saturating gas-solid surface reactions. Due to the limiting amount of surface sites, the binary reactions that happen on the surface were limited. These self-limiting reactions provide the accurate control of deposition thickness to angstrom or monolayer level. In an atomic layer deposition process, the number of deposition cycles is the key parameter to determine the total thickness of the film. Each ALD cycle consist of pulse of reactant A, purge of by-product, pulse of reactant B and purge of by-product. Because of the purge steps, which evacuate the non-reacted reactants and the gaseous reaction by-product, the two reactants will not able to in contact with each other. These sequential reactions lead to a conformal, high aspect ratio nano-thin film independent of the surface geometry.

2.10 Previous Studies on Improving PMMA

Azuma in 2012 studied the effect of Hydrophilic surface modification of acrylic denture base material by silica coating and its influence on Candida albicans
adherence. Azoma’s study was designed to modify the PMMA surface using silica in alcohol as a solvent which was applied to the PMMA surface using a non-woven cloth and evaluate the effect of the coating on *candida albicans* adherence. They found that the mean water contact angle was significantly decreased in the coated group, that means the surface became more hydrophilic and the *candida albicans* on the coated group was significantly lower than on the non-coated group. 

Azuma’s study suggested that Hydrophilic surface minimized C. adherence however they didn’t study stability of the coating on the PMMA also they didn’t study the effect of the coating process of the physical properties of PMMA. Specially that they used alcohol as solvent for the silica. And we know from previous studies alcohol is not recommended on PMMA. Since it causes degradation, color changes and decrease in the physical properties of PMMA.

Mirizadeh *et. al.* in 2017 tried to find a solution to the same issue of the denture base being colonized by bacteria and candida species. Instead of working on surface modification, they designed the study to incorporate an antimicrobial monomer into the denture PMMA and study its effect on the antimicrobial and the mechanical properties. They found out that the reduction in number of *E coli, S aureus, and C albicans* was beyond 99% which is really impressive however the flexural strength was significantly deceased.

Kazunari Mori, *et al* 2015 evaluated the clinical appearance (color, gloss, and surface roughness) of TiO₂ coating on polymethyl methacrylate (PMMA) resin dentures. A spraying method, using air brushes, was used to generate thin TiO₂ coating. The PMMA
Specimens were pre-treated with a primer agent that was sprayed for 2 s using an airbrush gun. TiO$_2$ coating agent, was then sprayed onto the substrate for 2 s and dried in an oven for 10 min at 70 °C. The specimens were divided into PMMA, primer-coated PMMA, and TiO$_2$-coated PMMA. Color values were measured using a reflective colorimeter. Glossiness of each specimen was measured with a gloss meter. Surface morphology was assessed using field emission scanning electron microscopy. They found that the titanium dioxide coating on denture base materials was achieved; the thin coating increased the degree of surface glossiness, while maintaining the color of the resin denture base without increasing the surface roughness.\textsuperscript{46}

Masashi Tsuji, \textit{et al} 2016 investigated the biocompatibility of TiO$_2$ coated acrylic produced by spray coating technique using airbrush gun. To verify the biocompatibility of the tio2 coated acrylic resin. Samples were fabricated and polished. The TiO$_2$-coating agent was sprayed onto the specimens using an airbrush gun. Specimens were then divided into 3 groups PMMA, primer coated PMMA, TiO$_2$ coated PMMA. Samples were evaluated for biological safety using a hamster oral mucosa irritation test, a guinea pig skin sensation test and a rabbit intracutaneous test. They found that TiO$_2$ coated denture base resin did not cause irritation to the oral mucosa, nor did it cause skin sensation. TiO$_2$ coating had no deleterious effects on the tissues. This study concluded what previous studies had presented regarding the biocompatibility of TiO$_2$.\textsuperscript{47}

In 2009, Kaariainen successfully deposited TiO$_2$ and Al$_2$O$_3$ between PMMA and Ti/TiC
as intermediate layers via Atomic Layer Deposition. Before deposition, PMMA substrate was subject to plasma gas treatment. The adhesion of each lm was measured by pull-off test. Results show that Al₂O₃ lm with 33 nm of thickness and TiO₂ lm with 50 nm of thickness provided higher adhesion strength.
3. MATERIALS AND METHODS

3.1 PMMA specimen fabrication

PMMA disk specimens (2cm x 2cm x1mm) were fabricated using Lucitone 199® (DENTSPLY Intl, York, PA, US) according to the manufacture’s instruction. PMMA specimens were serially polished by an ECOMET Polisher/Grinder with silicon carbide grinding paper from grit P800 to P4000. To obtain a good bonding between PMMA and TiO₂, pretreatment is an important step. Before ALD deposition, PMMA specimens were pre-cleaned in 5% NaOH solution for 10 minutes and in DI water for one hour in an ultrasonic bath at room temperature. Specimens were then dried by nitrogen gas.

3.2 TiO₂ coating of PMMA specimens

Forty of the PMMA specimens were subjected to the TiO₂ nano thin film coating technique. The film was deposited on PMMA specimen at 65°C using a custom-made ALD system (Fig. 1a). Titanium dioxide films were grown from tetrakis (dimethylamido) titanium (TDMAT) (Sigma-Aldrich, 99.999%) and ozone (O₃). Ozone was generated by a custom-built ozone generator from O₂ (99.999% purity) and injected into the reaction chamber as needed. TDMAT was vaporized from the source and kept at 65 °C. One ALD deposition cycle consisted of one 0.5s of TDMAT pulse, 10s of Ar purge, 1s of ozone pulse, and 15 seconds of Ar purge. Overall, 300 deposition cycles created the film thicknesses of 30 nm. The film thickness was measured using spectral ellipsometry (Model M-44, J. A. Woollam Co.) (Fig.1b).
3.3 PMMA and TiO$_2$-PMMA surface characterization

Surface roughness of coated and non-coated samples were characterized qualitatively and quantitatively using an optical profilometer (NewView 6300 3D optical surface profiler from Zygo Corporation, Middlefield, CT, USA). A sessile drop method was performed for water contact angle measurements of samples before and after coating. A micro-syringe (Hamilton, Reno, NV, USA) was used to place a 5µl deionized water droplet on the test specimen surfaces. The water contact angles were measured by a goniometer (CA Goniometer, Rame-Hart NRL, Succasunna, NJ, USA). Three measurements were made for each of 10 specimens to calculate mean and standard deviations for contact angle for treated and untreated groups.

3.4 Three-point bending test

The flexure strength of two groups, TiO$_2$-PMMA and PMMA was conducted by three-point bending. There were ten specimens for each group. The specimen dimensions were: L x B x H= (7±0.1) × (3±0.1) × (1±0.1) mm. A universal testing machine (ReNew Model 1125 Upgrade Package, MTS Systems Corporation, Eden Prairie, MN) was used at a crosshead speed of 1mm/min. The measurements of flexural strength and Young’s Modulus were computerized using TestWork4 software. Independent T test was performed to compare the mean flexural strength of PMMA vs. TiO$_2$ coated PMMA groups.

3.5 Wear resistance by brushing test
The PMMA (n=10) and two TiO$_2$-PMMA (n=10) specimen groups were brushed under a mechanical device (MSet Nucci ME; Sao Carlos, SP, Brazil) with an Oral-B Sensi-Soft Manual Toothbrush, and a 50g load was applied to each toothbrush. A drop of liquid soap (Erva-Doce; JOB-Qumicaprodutos para limpeza Ltda, Ribeirao Preto - SaoPaulo, Brazil) was used as an auxiliary hygiene agent for the brushing test. Linear tooth-brushing strokes were applied with a speed of 350 strokes per minute at room temperature. For group TiO$_2$-PMMA, after 3000 cycles of linear brushing, the samples were rinsed and stored in DI-water at 37±1°C for 5 months, followed by another 3000 cycles of brushing. Surface roughness evaluation was conducted with a NewView 6300 3D optical surface profiler and X-ray photo-electron spectroscopy (Kratos AXIS- 165, Kratos Analytical Ltd, Manchester, UK) was performed to analyze the surface chemical composition before and after brushing.

3.6 Chemical and mechanical challenge test

Denture cleanser challenge tests were performed to examine the interface adherence and stability between TiO$_2$ coating and PMMA. TiO$_2$ coated PMMA specimens were randomly divided into four groups with three specimens each. 1) TiO$_2$-PMMA; 2) TiO$_2$-PMMA sonicated in DI water for 1 hour; 3) TiO$_2$-PMMA samples soaked in 3.8% sodium perborate (Polident daily care, GlaxoSmithKline Consumer Healthcare, Mississauga, ON) solution for 4 hours; 4) TiO$_2$-PMMA samples sonicated in 3.8% sodium perborate for 1 hour. Specimens were examined with XPS for surface chemical composition before and after treatment.
3.7 Antimicrobial (Candida attachment/Biofilm formation)

*C. albicans* (ATCC 90028) was grown on Sabouraud Agar (SDA) for 2 days. Each suspension consists of one single colony of *C. albicans* with 5ml of Sabouraud Dextrose Broth (SDB) solution. The suspensions were cultured overnight at 37°C. Afterwards, cells were harvested and washed twice with phosphate-buffered saline (PBS), and then centrifuged for 5 minutes. Cells were re-suspended in appropriate fresh broth to an optical density (OD) of 0.095 at 625 nm.

To assess *C. albicans* attachment, PMMA and TiO$_2$-PMMA specimens (n = 5) were placed in 6 well polystyrene microplates, and 5mL of cell suspensions (10$^4$ cells/mL) were added to each well. The plate was incubated for 6 hours at 37°C in an orbital shaker to promote the attachment of *C. albicans* on PMMA surfaces. After 6-hours incubation, PBS was used to wash off the non-adherent of *C. albicans* cells from PMMA surfaces. The suspensions that contain detached *C. albicans* were vortexed, diluted and spread on SDA. After incubation for 12h at 37°C, plate counting method was used to calculate the number of viable *C. Albicans*.\textsuperscript{49,50} The attachment test was performed twice with five specimens each time to validate the repeatability.

To assess *C. albicans* biofilm formation on PMMA surfaces, 3 mL of SDB was added to each microplate well. The plates were then incubated at 37°C for 12 hours under aerobic conditions. After 12-hours incubation, the cultured plates were removed from the incubator and PBS was used to wash the wells. Samples were sonicated in DI water for 30 seconds to stop the aggregation of biofilm cells. The suspensions that contained
detached *C. albicans* were vortexed, diluted and spread on SDA. After incubation for 12h at 37°C, plate counting method was used to calculate the number of viable *C. albicans*. The biofilm formation test was performed twice with five specimens each time to validate the repeatability.
4. RESULTS

TiO$_2$ films were successfully demonstrated on PMMA denture base material by the ALD deposition technique (Fig. 1a). A growth rate of 0.1nm/cycle was obtained in this study. The deposition was conducted using 300 cycles, which resulted in approximately 30nm-thick TiO$_2$ on PMMA specimens (Fig. 1b).

The mean water contact angle (WCA) of PMMA substrate without coating was 67$^\circ$. After TiO$_2$ coating the WCA was less than 5$^\circ$. The mean (standard deviation: SD) flexural strength of uncoated PMMA was 139.4 (11.3) MPa, and 160.7 (37.1) MPa for the TiO$_2$-PMMA coated group. Statistical analysis (independent T test) revealed no significant difference between the two groups ($p < 0.05$) (Fig.2).

The surface roughness change of PMMA and TiO$_2$-PMMA was measured after 6000 linear brushing cycles and 5 months of storage. The mean (SD) PMMA roughness was 0.12 (0.02) $\mu$m whereas TiO$_2$ PMMA was 0.08 (0.03) $\mu$m. The mean (SD) surface roughness of PMMA specimens increased to 3.23 (0.95) $\mu$m after 6000 strokes brushing. However, the mean (SD) surface roughness for TiO$_2$-PMMA samples was 0.17 (0.03) $\mu$m after brushing and resulted in no significant change before and after brushing for TiO$_2$-PMMA. (Fig. 3).

Chemical composition of the PMMA group and TiO$_2$ coated PMMA group were examined by XPS before surface properties testing (Fig. 4). Ti peak was still observed on TiO$_2$-PMMA specimen surfaces after the brushing test (Fig. 5).

For the mechanical and chemical challenge test, the TiO$_2$ samples including (1) TiO$_2$-PMMA; (2) TiO$_2$-PMMA sonicated in DI water for 1 hour; (3) TiO$_2$-PMMA soaked in
Polident solution for 4 hours; (4) TiO$_2$-PMMA sonicated in Polident solution for 1 hour all continued to retain the TiO$_2$ thin film on the PMMA surface after cleanser treatment. The pre-cleanser treatment Ti peak with binding energy of 458.5 eV (Figure 4) was also detected for each group after mechanical and chemical cleanser exposure (Fig. 6).

Reduction in the mean (SD) number of viable attached cells of *C. albicans* was demonstrated in Fig. 7. ($p < 0.05$) between PMMA control group: $7.38 \times 10^4 (0.7 \times 10^4)$ CFU/ml; TiO$_2$-PMMA: $2.76 \times 10^4 (0.4 \times 10^4)$ CFU/ml. Reduction in the mean (SD) number of viable attached biofilm film cells of *C. albicans* was demonstrated in Fig. 7. ($p < 0.05$) between PMMA control group: $79.56 \times 10^4 (1.82 \times 10^4)$ CFU/ml; TiO$_2$-PMMA: $35.11 \times 10^4 (0.1 \times 10^4)$ CFU/ml. The *Candida* reduction reached 63-77% for the attachment test (Fig. 7a) and 56% for biofilm formation (Fig. 7b).
5. DISCUSSION

This study demonstrated that the ALD technique can be used to deposit TiO$_2$ on PMMA surfaces and thereby change surface chemistry, microbial adherence and wear resistance while not adversely altering the flexural strength of the PMMA material. The clinical relevance of this technique relates to its straightforward application at low temperatures in a dental laboratory environment well below the PMMA glass transition temperature and melting point (160°C) that could lead to deformation or structural changes of the denture base material such as undesirable mechanical properties and compromised dental esthetics. Furthermore, the TiO$_2$-PMMA coated samples reduced the microbial biofilm. Resistance to wear under the toothbrush testing cycles, and resistance to denture cleanser degradation further demonstrated the clinically important potential of this material for improved supportive professional care and improved patient care of the prosthesis. The result could improve patient health. The TiO$_2$ deposition philosophy is a paradigm shift from the current denture standard of a highly polished surface finish to one of a highly improved and stable surface chemistry.

In this study, the flexural strength for PMMA and TiO$_2$-PMMA samples were carried out by 3-point bending. Statistical analysis (independent T test) reveals that there is no significant difference between groups PMMA and TiO$_2$-PMMA. The mean flexural strength for both groups is consistent with Balos’s work in 2015. This indicates that the thin film TiO$_2$ coating and process do not change the mechanical properties of the PMMA bulk. The application of a low temperature ALD technique is the key to successfully maintaining the mechanical properties and dimensional stability of PMMA denture base
material.

The ALD technique takes advantage of in-situ precipitated nanoparticles and nanostructures on the substrate to improve surface mechanical properties, surface wettability, and may eliminate diffusion of pathogens into the acrylic. The mean water contact angle of uncoated PMMA samples (67°) was reduced to less than 5° after nano-ceramic coating, leading to a super-hydrophilic surface. These results were consistent with Azuma et al. (2012)\textsuperscript{,46} who coated acrylic denture base material with silica, and demonstrated that more hydrophilic denture surfaces reduced \textit{C. albicans} adherence. In contrast, other studies demonstrated that \textit{Candida} attaches to hydrophobic surfaces and forms a biofilm.\textsuperscript{47,48} Therefore, deposition of TiO\textsubscript{2} and concomitant increasing surface wettability may be an essential factor that reduces, inhibits or prevents the initial attachment of \textit{Candida} on denture base resin.

After 6000 cycles of brushing and 5 months of storage, the surface roughness (Ra) change for PMMA and TiO\textsubscript{2}-PMMA was measured (Fig. 5) before and after brushing. The mean surface roughness of the PMMA specimens increased dramatically from less than 0.12 \( \mu \text{m} \) to more than 3.23 \( \mu \text{m} \) after the brushing test. In contrast, the TiO\textsubscript{2} - PMMA group, had no significant change in the mean surface roughness before (0.08 \( \mu \text{m} \)) and after brushing test (0.17 \( \mu \text{m} \)) (Fig. 3). Abuzar, Menaka A., \textit{et al.}\textsuperscript{49} suggested that the standard surface roughness of PMMA denture base material should be no more than 0.2 \( \mu \text{m} \). Da Silva \textit{et al.}\textsuperscript{50} reported that a lower surface roughness value can reduce \textit{C. albicans} biofilm accumulation on PMMA. This study demonstrated the TiO\textsubscript{2} coating was able to dramatically improve the surface wear resistance of PMMA (Fig. 3). As
shown in Fig. 5, the titanium peak can still be detected by XPS after 6000 cycles of brushing and 5 months of water storage without significant change in roughness (Fig, 3). This further indicated that the surface wear resistance of PMMA was improved by thin film ceramic coating and may provide long-term protection for dentures.

The thin film ALD technique allows adhesion to the substrate surface as a result of a gas-solid surface chemical reaction bonding to the substrate that leads to a strong bonding interface between the TiO$_2$ coating material and PMMA substrate surface. To examine the adhesion strength between TiO$_2$ (inorganic material) and PMMA (organic material), XPS was used to detect TiO$_2$ on the PMMA substrate after both mechanical and chemical challenge testing. Results suggest the TiO$_2$ adhesion to PMMA is stable. As shown in Fig. 6, the Ti peak was observed for all coated samples after testing which confirmed that the deposited TiO$_2$ is still detected on the PMMA substrate after mechanical and chemical challenge testing. The ALD process provided a physical, mechanical attachment between PMMA and TiO$_2$, as well as a chemical bond between the two materials. The amorphous TiO$_2$ surface coating obtained by ALD coating exhibited anti-corrosion behavior under aggressive chemical (3.8% perborate) and physical (sonication cleansing) treatment.

The attachment of C. albicans on PMMA surfaces is an initial step for microbial colonization.\textsuperscript{51,52} With increasing surface roughness, the accumulation of microorganisms may increase and further promoting microbial colonization.\textsuperscript{51,52} In addition, the hydrophobicity of denture base material may affect the attachment and later formation of C. albicans.\textsuperscript{49} Ramage et al.\textsuperscript{53} reported that within the first 8 hours,
candida attached to the surface, biofilm formation began and gradually became mature. After 10 hours, the growth rate of C. albicans was stable with the substrate being fully covered by biofilm.⁵⁴

In this study, antimicrobial activity was observed with the TiO₂-PMMA surface in comparison with PMMA. A significant decrease in the number of viable, attached biofilm cells of C. albicans was observed with the TiO₂-PMMA (2.76 x 10⁴ CFU/ml) coating compared to the PMMA control group (7.38 x 10⁴ CFU/ml). The C. albicans reduction rate (Fig. 7a) reached 63-77% for the attachment test and 56% for biofilm formation (Fig. 7b). The low surface roughness of TiO₂ coated PMMA and its super-hydrophilicity may lead to less microbial adherence. The improved hydrophilicity of the sample surface reduces the initial attachment of C. albicans. In addition, TiO₂ coating provided a smooth, low porosity surface and superior wear resistance, which may further minimize the diffusion of pathogens in to the PMMA, adherence of biofilm, and colonization of microorganisms on and within PMMA.

This pilot study evaluated the surface chemistry of PMMA materials with a 30nm surface coating. The influence of differing thickness of TiO₂ on the properties tested in this study is unknown. Future studies that evaluate the influence of greater or lesser thickness of ALD applied TiO₂ are indicated. Furthermore, the TiO₂ deposition was on small uniformly shaped specimens that do not resemble the overall dimensions and surface area of a complete denture prosthesis. Additional studies are warranted to evaluate the parameters necessary for successful deposition in clinically relevant situations.
C. albicans was the only organism tested in this study. Although it is associated with pathosis, additional microbes are also associated with biofilm formation that were not included in this study. Future studies are indicated that further assess their adhesion on the TiO$_2$ surface. Also, we stored samples in DI which is different from saliva, The effect of artificial saliva on the bonding between TiO$_2$ and PMMA was not studied in this project and finally the thermocycling effect on the Nano ceramic coating was not investigated.

The ALD technique represents a change in prosthesis fabrication technique that would also include a final step of TiO$_2$ coating after finishing and polishing. Adjustment of the prosthesis after coating would certainly remove the applied layer. An additional layer could be applied after adjustment with prosthesis insertion, and could even be applied following months or years of denture service and wear. Regardless of timing of application, any technique that reduces denture biofilm will have the potential for improvement of oral and systemic health.
6. CONCLUSION

A novel TiO$_2$ ceramic film was successfully applied at low temperatures on PMMA surface to improve PMMA surface properties, overcome many recognized limitations, while retaining resin usefulness and mechanical properties. TiO$_2$ improved wettability, resistance to wear, surface smoothness, and resistance to microbial adherence. Under the conditions of this study, the following conclusions may be drawn:

1. Atomic layer deposition resulted in TiO$_2$ layering at a predictable linear rate.
2. Flexural strengths of PMMA and TiO$_2$ PMMA were not significantly different.
3. Mechanical brushing of the TiO$_2$-PMMA surface did not significantly alter surface roughness, whereas the same brushing conditions created roughness 30 times greater (3um vs 0.1um) for untreated PMMA.
4. Brushing and denture cleansing did not remove the TiO$_2$ coating.
5. *C. albicans* adherence and initial growth toward biofilm formation was significantly reduced with TiO$_2$ coating.
6. Ease of application and improved behavior suggests future *in-vitro* research leading to clinical application is indicated.
7. FIGURES

Figure 1a: Atomic layer deposition coating (ALD) mechanism
Figure 1b: Growth rate of TiO₂ from TDEAT and O₃
Figure 2: Flexure strength of PMMA and TiO$_2$-PMMA.
Figure 3: Surface roughness of PMMA and TiO$_2$-PMMA before and after brushing test.
Figure 4: XPS spectrum result of PMMA and TiO$_2$ coated PMMA. Ti peak was observed on TiO2-PMMA but there is no Ti peak noticed on PMMA.
Figure 5: XPS spectrum result of TiO$_2$-PMMA and TiO$_2$-PMMA after 3000 cycles of brushing and water storage for 5 months then another 3000 cycles of brushing (6000 cycles total).
Figure 6: XPS spectrum result of 1) TiO$_2$-PMMA (control) and after mechanical and chemical aging challenge test: 2) TiO$_2$-PMMA sonicated in DI water for 1 hour (Sonication); 3) TiO$_2$-PMMA soaked in Polident solution for 4 hours (Polident soak); 4) TiO$_2$-PMMA sonicated in Polident solution for 1 hour (Polident sonication).
Figure 7a: The adherence of *C. albicans* on group PMMA and group TiO$_2$-PMMA
Figure 7b: Biofilm formation of *C. albicans* on group PMMA and group TiO$_2$-PMMA
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