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Original Article

Dentistry Section

Evaluation of Antimicrobial Efficacy of Titanium Dioxide on Dental Plaque Colonisers: An In-vitro Study

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ABSTRACT

Introduction: Denture Stomatitis (DS), a common inflammatory condition of the denture bearing mucosa amongst denture wearers is strongly related to poor denture hygiene. Various materials have been incorporated into denture base acrylic resins to have an antimicrobial effect. The antimicrobial efficacy of Titanium dioxide (TiO_2) on denture base acrylic resins has been demonstrated; however, studies are lacking in assessing the effective dose concentration of TiO_2 in inhibiting microorganisms present in denture plaque.

Aim: To investigate the effectiveness of TiO_2 coating on denture base acrylic resin in inhibiting colonisers of denture plaque.

Materials and Methods: The in-vitro study was conducted in the Department of Prosthodontics and Crown & Bridge and Department of Microbiology, Sri Ramachandra Institute of Higher Education and Research, Chennai, Tamil Nadu, India between August 2021 to September 2021. Stock solutions of various concentrations of TiO_2 were prepared to evaluate the effective dose concentration. Forty specimens of heat cure acrylic denture base resin were divided into two groups: an uncoated group (n=20) and TiO_2 coated group (n=20). They were further subdivided into 4 groups, namely specimens containing *Streptococcus sanguinis*, *Staphylococcus aureus*, *Candida albicans*, and specimens containing a mixture of the above organisms. After inoculating, the specimens were UV treated, incubated for 24 hrs, and then washed with phosphate buffer saline. Subcultures were obtained and microbial growth colonies were measured using automated bacterial colony counter. The obtained results were subjected to statistical analysis (SPSS software version 21) and Mann-Whitney U test to compare between the groups, p value of less than or equal to 0.05 ($p \leq 0.05$) was considered significant.

Results: Colonisers of denture plaque in the uncoated acrylic group was higher (mean:12973.33, 441998.33, 9126.66, and 13.3 CFU) when compared to the TiO_2 coated acrylic resin group (mean:1, 0.99, 1, and 1 CFU). The obtained results between the groups was statistically significant ($p \leq 0.05$).

Conclusion: TiO_2 coating on denture base acrylic resin can inhibit the growth of microorganisms associated with denture plaque.

Keywords: Acrylic resins, Biofilms, Complete denture, Dental Materials, Denture stomatitis

INTRODUCTION

Denture hygiene and overall maintenance of oral health is imperative in denture wearers as they are susceptible to a variety of diseases like aspiration pneumonia and DS [1,2]. Acrylics such as polymethyl methacrylate (PMMA) have been widely used for fabricating dentures. With a wide range of advantages like low cost, good aesthetics, ease of handling, hardness, and rigidity to withstand masticatory force, PMMA has been regarded as the best suitable material for fabrication of dentures [3]. However, PMMA is susceptible to microbial adherence thereby causing deterioration of its properties. Cleaning of dentures for debridement helps in alleviating denture plaque. Although various aids for cleaning dentures like denture cleaning solutions and tablets are available, mechanical debridement of denture plaque using mechanical cleaning with brushes has proven to be the most effective [4]. Geriatric individuals with limited dexterity and disability often find maintenance of oral hygiene and denture hygiene difficult. They especially experience difficulty in cleaning the intaglio surface and the interdental areas of the dentures [5].

Various studies have been conducted for modifying denture materials such as Teflon coatings on dentures to resist microbial adhesion on denture surfaces [6,7]. Titanium dioxide (TiO_2) coating has been used in a variety of medical products like catheters and dentures because of its anti-microbial activity, attributed to its high hydrophilic properties, and functional suitability to photocatalytic reactions [8-10].

Denture plaque comprises of variety of dense microorganisms and their metabolites [11]. Role of *S. sanguinis* and *C. albicans* in the denture plaque formation has been thoroughly investigated. Studies have been performed to evaluate the antimicrobial efficacy of TiO_2 on *C. albicans, S. sanguinis*, and *S. aureus* species and have successfully demonstrated the antimicrobial effectiveness of TiO_2 [8,10,12-16]. Previously performed studies only evaluated the antimicrobial efficacy against particular species but denture plaque comprises of mixture of organisms [8,10,16]. Studies are lacking to assess the antimicrobial efficacy of TiO2 on mixtures of microorganisms present in denture plaque. Hence the aim of this in-vitro study was to investigate the effect of TiO_2 coating on denture base acrylic resin in inhibiting colonisers of denture plaque. The null hypothesis was that no significant difference would be found between the TiO_2 coated acrylic resin group and uncoated acrylic resin group concerning antimicrobial activity.

MATERIALS AND METHODS

This in-vitro study was conducted in the Department of Prosthodontics and Crown and Bridge and Department of Microbiology, Sri Ramachandra Institute of Higher Education and Research, Chennai, Tamil Nadu, India between August 2021 to September 2021. Forty specimens of size 10×10×0.5 mm were processed by polymerising heat cure acrylic denture base resin (Dental products of India, Mumbai, India) [8]. Sample size was calculated as 40 with a power of 90% and alpha error 5% (G power software 3.1) [8].

Study Procedure

Wax patterns of the prescribed dimension were made and a plaster mold cavity was prepared. Powder and liquid were mixed according to the manufacturer's instructions and packed into the plaster mold.

The plaster mold was then cured in an acryliser until the curing cycle was completed (74°C for 2 hours followed by 100°C for 1 hour). The cured specimens were then trimmed and polished up to the equivalent of grit #1000 by wet abrasive paper. Stock solutions comprising of various concentrations of TiO_2 (Concentrations: 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256, and 512 mg/L) were prepared to identify the effective dose of TiO_2 in inhibiting microorganisms namely *S. sanguinis, S. aureus, C. albicans*, and specimens containing the mixture of the above organisms. The effective concentration was analysed using agar dilution method, with the Muller Hinton agar as the reference medium. The stock solutions containing various concentrations of TiO_2 were evaluated by adding TiO_2 to the pre-inoculated culture plates comprising of the above organisms. The solution containing 512 mg/L of TiO_2 was found to be effective in inhibiting the growth of microorganisms.

The acrylic specimens were divided into two groups: an uncoated group (n=20) [Table/Fig-1] in which the surfaces were finely polished and a TiO_2-coated group (n=20) [Table/Fig-2] in which the surfaces were coated with 512 mg/L of TiO_2 after polishing. In the coated group the specimens were treated with a primer (MONOBOND-S, Ivoclar Vivadent) after which TiO_2 was sprayed onto the plate using an air brush gun and then allowed to dry for 10 minutes at 70°C [12].

[Table/Fig-1]: Uncoated acrylic disks.

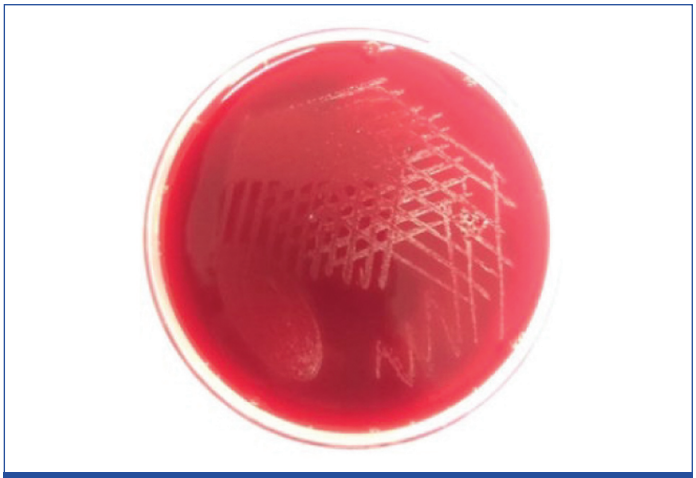
[Table/Fig-2]: Titanium dioxide coated disks.

Bacterial strains and growth conditions: *S. sanguinis* were maintained in Tryptic soy agar supplemented with 0.5% yeast and were precultured at 37°C for 24 hours. The tryptic soy agar broth containing the microorganisms were diluted to a concentration of 3.0×10^8 mL-1. *S. aureus* was maintained in tryptic soy agar broth and were incubated at 37°C for 12 hours. The final broth containing the organisms was diluted to a concentration of 3.0×10^8 mL-1. *C. albicans* were cultured in Sabouraud's dextrose broth containing 1% yeast extract at 37°C for 24 hours and the final broth was diluted to a concentration of 1.3×10^5 mL-1. The mixture of the above organisms was cultured in tryptic soy agar broth supplemented with 0.5% yeast extract at 37°C for 24 hours and the microorganisms were diluted to a concentration of 3.0×10^8 mL-1 [8,10,16].

Both the coated and uncoated groups were further subdivided into 4 groups (5 samples for each group) namely specimens containing *S. sanguinis, S. aureus, C. albicans,* and specimens containing the mixture of the above organisms. The coated and uncoated acrylic specimens were immersed in 500 µL of cultured *S. sanguinis, S. aureus*, *C. albicans*, and mixed suspensions. After inoculation, the specimens were incubated at 37°C for 24 hours and then washed with phosphate buffer saline. Subcultures were obtained from the specimens and the number of microbial growth colonies were evaluated using automated bacterial counter [Table/Fig-3-6].

STATISTICAL ANALYSIS

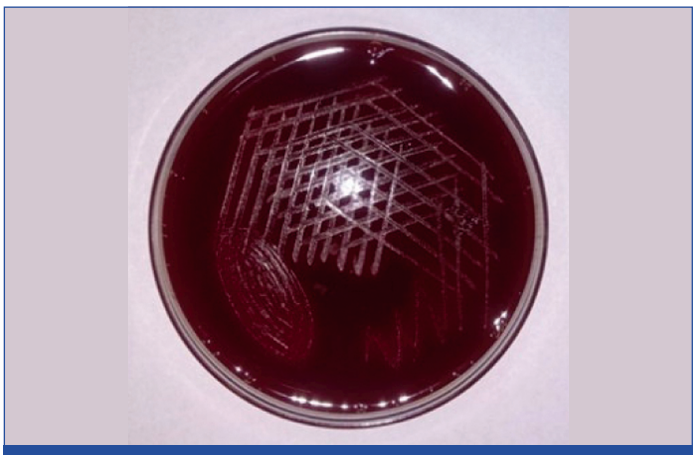
The obtained results were computed in a Microsoft Excel spreadsheet. The data were subjected to statistical analysis using SPSS software version 21. The data was non-normally distributed and Mann-Whitney U test was used to compare between the groups, ($p\leq0.05$) was considered significant.



[Table/Fig-3]: *Streptococcus sanguinis* growth culture.

[Table/Fig-4]: *Staphylococcus aureus* growth culture.

[Table/Fig-5]: *Candida albicans* growth culture.



[Table/Fig-6]: Mixture of organisms growth culture.

RESULTS

The results obtained from this study revealed that the growth of microbial colonies drastically reduced in the TiO_2 coated acrylic resin group when compared to the uncoated acrylic resin group. The mean and median *S. aureus, S. sanguinis, C. albicans*, and the mixed organisms counts in the TiO_2 coated acrylic resin group (Mean:1, 0.99, 1, and $1.\times10_3$) were reduced when compared to the uncoated acrylic resin group (Mean:12973.33, 441998.33, 9126.66, and $13.3 \times10_3$) as seen in [Table/Fig-7]. The difference in the microorganism count between the TiO_2 coated and uncoated acrylic resin were found to be significant statistically with a p-value of 0.05.

DISCUSSION

The most common oral mucosal lesion found in an elderly denture wearer is DS which is strongly related to denture plaque. Denture plaque is made up of a complex structure of microorganisms known as biofilm, that adhere to the intaglio surfaces of dentures [17]. The hydrophobic nature of early colonisers of denture plaque such as *S. sanguinis* and *S. mutans* help in initial adhesion of the microorganisms to the denture, that contributes to plaque formation [8]. These early colonisers of denture plaque namely *S. sanguinis* and *S. mutans* comprise of adhesins which aid in attachments of these microorganisms to the salivary pellicle and denture surfaces. They also exhibit coaggregation reactions which aid in attachment of others microorganisms [18]. Furthermore, in denture wearers with debilitating conditions and poor oral hygiene, the situation is complicated by colonisation of *C. albicans*. The highly hydrophobic nature of acrylic resin with added poor denture hygiene leads to accumulation of denture plaque, which causes DS [19].

The antimicrobial effectiveness of TiO_2 on denture base acrylics, silicones & medical devices has been widely studied. Arai T et al., stated that TiO_2 coating on denture base acrylic resin inhibited the adhesion of *S. sanguinis* and altered the hyphal form of *C. albicans* [8]. Abdulrazzaq Naji et al., from his experimental study concluded that denture base resin when modified with TiO_2 had an inhibitory effect on growth of microgranisms [13]. Anehosur GV et al., concluded from her experimental studies that TiO_2 incorporation in denture base acrylic resin exhibited an anti-microbial effect against *S. aureus* and *C. albicans* [16]. The antimicrobial effect of TiO_2 has been attributed to the hydrophilic and oxidative action of TiO_2. The hydrophilic nature of TiO_2 modified the hydrophobic nature of the acrylic disks and inhibited the attachment of microorganisms to its surface, preventing the formation of denture plaque [5]. Though TiO_2 effectively inhibits the growth of microorganisms; however, effective dose concentration at which the TiO_2 exhibits this anti-microbial property on single species of microorganisms and mixture of microorganisms is lacking. Hence, in this current study we assessed the concentration of TiO_2 which was effective in inhibiting the growth of microorganisms. The results of this present study concluded that 512 mg/L of TiO_2 coating on denture base acrylic resin inhibited the growth of *S. sanguinis, S. aureus, C. albicans*, and the mixture of the above organisms.

Literature suggests that TiO_2 when mixed with dentures possess a self-cleansing ability and inhibits the growth of microorganisms [20]. However, addition of TiO_2 to acrylic resin decreases the mechanical strength of the acrylic resin making its use unviable and unfavourable as a denture base material [20]. Paul L et al., in his invitro experimental study concluded that though incorporation of TiO_2 nanoparticles improved the impact strength of denture base acrylic resins, it cannot be incorporated into all denture base resins to reinforce the dentures as there was significant increase in tensile strength and the other mechanical properties were not assessed [21]. Antimicrobial efficacy of TiO_2 on individual species of microorganisms has been investigated by coating TiO_2 on acrylic resin without any change in the mechanical properties [20,22]. Hence in this study, TiO_2 coating on acrylic resin was used to investigate its effect on colonisers of denture plaque.

Amano D et al., reported the superior bonding ability of TiO_2 to acrylic resin when treated with a primer, hence in this study the acrylic specimens were treated with primer prior to application of TiO_2 to improve the bonding of TiO_2 to the acrylic resins [22].

Literature suggests that various authors have evaluated the antimicrobial effect of TiO_2 coating on denture base acrylic resin on specific microorganisms namely *S. sanguinis, C. albicans,* and *S. aureus* [8,10,13,16]. However, denture plaque that causes DS comprises a mixture of various microorganisms, Hence in our study, we evaluated the anti-microbial effect of TiO_2 coating on *S. sanguinis, S. aureus, C. albicans* and mixture of the above microorganisms resembling denture plaque. The obtained results were statistically significant thus rejecting the null hypothesis ($p \leq 0.05$). The results of this study were in accordance with the studies performed by Arai T et al., Naji SA et al., and Anehosur GV et al., where they concluded that TiO_2 inhibited the growth of microorganisms [Table/Fig-8] [8,10,13,16].

With the obtained results we can establish that, 512 mg/L of TiO_2 coating on the denture base acrylic resin inhibited the growth of microorganisms associated with denture plaque and can be used in geriatric patients for easy cleansing of dentures.

Group		*Streptococcus sanguinis*	*Staphylococcus aureus*	*Candida albicans*	Mixed organisms
Uncoated acrylic disks	Mean CFU	12973.33	441998.33	9126. 66	13.30
	SD	55.07	743051.24	6778.28	0.60828
	Median	13000	13015.00	12990.00	13.0000
TiO_2 coated acrylic disks	Mean$\times10^3$ CFU	1.0000	0.9967	1.00	1.0067
	SD	0.10000	0.10504	0.03	0.00577
	Median	1.0000	1.0000	1.00	1.0100
p-value (Mann- Whitney U test)		0.05	0.05	0.05	0.046

[Table/Fig-7]: Comparison of antimicrobial activity between Titanium dioxide (TiO_2) coated and uncoated acrylic disks.
*Statistically significant $p \leq 0.05$; SD: Standard deviation; TiO_2: Titanium dioxide; CFU: Colony forming unit

S. No.	Author's name and year	Place of study	Sample size	Organisms evaluated	Conc. of TiO2	Conclusion
1.	Arai T et al., 2009 [8]	Japan	60 acrylic plates (30 coated with TiO_2 & 30 uncoated)	*S. sanguinis, C. albicans*	-	TiO_2 coating inhibited the growth of *S. sanguinis* and altered the hyphal form of *C. albicans*
2.	Anehosur GV et al, 2012 [10]	India	75 PMMA-TiO_2 Samples	*S. aureus*	3 wt% of TiO_2	3 wt% of TiO_2 showed antimicrobial activity against *S. aureus*
3.	Anehosur GV et al., 2016 [16]	India	60 Specimens per group (Group1- 60 coated with TiO_2 & Group 2- 60 uncoated)	*C. albicans*	3 wt% of TiO_2	TiO_2 when combined with denture base material showed considerable antifungal activity

4.	Naji SA et al., 2018 [13]	Iran	Three groups- Denture base acrylic incorporated with Titanium Dioxide nanotubes (TNT) 0% (control), denture base acrylic incorporated with TNT 2.5% and denture base acrylic incorporated with TNT 5% by weight. (Sample size not mentioned)	*S. mutans*, Lactobacillus Acidophilus and *C. albicans*	2.5 wt% & 5 wt% of TiO_2	Modification of denture base acrylic resin with TiO_2 nanotubes can greatly improve its antimicrobial properties
5.	Present study	India	40 Specimens divided into TiO_2 coated denture base acrylic resin group (20) and uncoated denture base acrylic resin group (20).	*S. sanguinis*, *S. aureus*, *C. albicans* and mixed group comprising of mixture of the above organisms.	512 mg/L of TiO_2 coating	Heat cured denture base acrylic resin with a coating of 512 mg/L of TiO_2 positively inhibited the colonisers denture plaque.

[Table/Fig-8]: Comparative evaluation of similar studies [8,10,13,16].

Limitation(s)

Since the present study is an in-vitro study, the results of this study cannot not be directly applied to any clinical situation. To validate the present results, human clinical studies are required to investigate the effect of TiO_2 coating on dentures.

CONCLUSION(S)

Heat cured denture base acrylic resin with a coating of 512 mg/L of TiO_2 positively inhibited the colonisers of denture plaque. Future studies can be conducted to assess the surface roughness and mechanical properties of a denture coated with 512 mg/L of TiO_2.

REFERENCES

[1] Sumi Y, Kagami H, Ohtsuka Y, Kakinoki Y, Haruguchi Y, Miyamoto H, et al. High correlation between the bacterial species in denture plaque and pharyngeal microflora. Gerodontology. 2003;20(2):84-87.
[2] Okuda K, Kimizuka R, Abe S, Kato T, Ishihara K. Involvement of periodontopathic anaerobes in aspiration pneumonia. J Periodontol. 2005;76(11 Suppl):2154-60.
[3] Gad MM, Fouda SM, Al-Harbi FA, Näpänkangas R, Raustia A. PMMA denture base material enhancement: A review of fiber, filler, and nanofiller addition. Int J Nanomedicine. 2017;12:3801-12.
[4] Baysan A, Whiley R, Wright PS. Use of microwave energy to disinfect a long-term soft lining material contaminated with Candida albicans or Staphylococcus aureus. J Prosthet Dent. 1998;79(4):454-58.
[5] Kulak-Ozkan Y, Kazazoglu E, Arikan A. Oral hygiene habits, denture cleanliness, presence of yeasts and stomatitis in elderly people. J Oral Rehabil. 2002;29(3):300-04.
[6] Lopez-Lopez G, Pascual A, Perea EJ. Effect of plastic catheter material on bacterial adherence and viability. J Med Microbiol. 1991;34(6):349-53.
[7] Sela MN, Steinberg D, Klinger A, Krausz AA, Kohavi D. Adherence of periodontopathic bacteria to bioabsorbable and non-absorbable barrier membranes in vitro. Clin Oral Implants Res. 1999;10(6):445-52.
[8] Arai T, Ueda T, Sugiyama T, Sakurai K. Inhibiting microbial adhesion to denture base acrylic resin by titanium dioxide coating. J Oral Rehabil. 2009;36(12):902-08.
[9] Sekiguchi Y, Yao Y, Ohko Y, Tanaka K, Ishido T, Fujishima A, et al. Self-sterilizing catheters with titanium dioxide photocatalyst thin films for clean intermittent catheterization: Basis and study of clinical use. Int J Urol. 2007;14(5):426-30.
[10] Anehosur GV, Kulkarni RD, Naik MG, Nadiger RK. Synthesis and determination of antimicrobial activity of visible light activated TiO_2 nanoparticles with polymethyl methacrylate denture base resin against Staphylococcus aureus. J Gerontol Geriat Res. 2012;1(1):01-08.
[11] Nikawa H, Hamada T, Yamamoto T. Denture plaque—past and recent concerns. J Dent. 1998;26(4):299-04.
[12] Obata T, Ueda T, Sakurai K. Inhibition of denture plaque by TiO 2 coating on denture base resins in the mouth. J Prosthet Dent. 2017;118(6):759-64.
[13] Naji SA, Jafarzadeh Kashi TS, Pourhajibagher M, Behroozibakhsh M, Masaeli R, Bahador A, et al. Evaluation of antimicrobial properties of conventional poly (methyl methacrylate) denture base resin materials containing hydrothermally synthesised anatase TiO_2 nanotubes against cariogenic bacteria. Iran J Pharm Res. 2018;17(Suppl 2):161-72.
[14] El Shafie, El Shimy, El Sheredy. Antifungal effect of photocatalytic nano-titanium dioxide incorporated in silicone elastomer. Alex Dent J. 2019;44(2):52-60.
[15] Ramya G, Rajasekar A. Enhanced antibacterial effect of Titanium dioxide nanoparticles mediated grape seed extract on oral pathogens- Streptococcus mutans and Lactobacillus. J Evolution Med Dent Sci. 2021;10(22):1656-62.
[16] Anehosur GV, Naik MG, Kulkarni R, Nadiger RK. Antifungal effect on denture base materials using visible light activated titanium oxide synthesized by two different techniques. J Adv Oral Res. 2016;7(1):12-19.
[17] Ribeiro AB, de Araújo CB, Silva LEV, Fazan-Junior R, Salgado HC, Ribeiro AB, et al. Hygiene protocols for the treatment of denture-related stomatitis: local and systemic parameters analysis - a randomized, double-blind trial protocol. Trials. 2019;20(1):661.
[18] Jenkinson HF, Lala HC, Shepherd MG. Coaggregation of Streptococcus sanguis and other streptococci with Candida albicans. Infect Immun. 1990;58(5):1429-36.
[19] Morris IJ, Wade WG, Aldred MJ, Walker DM. The early bacterial colonization of acrylic palates in man. J Oral Rehabil. 1987;14(1):13-21.
[20] Cheng Y, Sakai T, Moroi R, Nakagawa M, Sakai H, Ogata T, et al. Self-cleaning ability of a photocatalyst-containing denture base material. Dent Mater J. 2008;27(2):179-86.
[21] Paul L, Ravichandran R, Kumar KH, Nair V, Janardanan K. Effect of titanium dioxide nanoparticles incorporation on tensile and impact strength in two different acrylic denture base resins - In vitro study. International Dental Journal of Student's Research. 2020;8(2):65-74.
[22] Amano D, Ueda T, Sugiyama T, Takemoto S, Oda Y, Sakurai K, et al. Improved brushing durability of titanium dioxide coating on polymethylmethacrylate substrate by prior treatment with acryloxypropyl trimethoxysilane-based agent for denture application. Dent Mater J. 2010;29(1):97-103.

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