IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 19, Issue 2 Ser.8 (February. 2020), PP 18-24
www.iosrjournals.org

Adherence of Candida Albicans To Surface Modified Denture Resin Surfaces With Polytetrafluoroethylene(PTFE) Polymer– An In Vitro Study

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Abstract:
Background*: PTFE is an anti-adherent and aesthetic material. The aim of this study was to evaluate, in vitro, the influence of PTFE coating on the biofilm formation between Polytetrafluoroethylene (PTFE) coated denture resin surfaces and uncoated denture resin surfaces.*
Materials and Methods*: Two groups were tested [Group 1: control, pure polymethylmethacrylate(PMMA); Group 2: modified PMMA with PTFE coating. Thirty resin specimens for each group were polymerized, and four experimental subgroups for each surface type were devised, consisting of 2 and 4 days of incubation in C. albicans suspension. After incubation one set for sample was taken for colony counting whereas other set was used for crystal violet staining and UV spectrophotometer assay.*
Results*: At day 2, the PTFE coated resin had statistically significantly lower levels of Candida than the uncoated group. The PTFE coated resin had statistically significantly lower levels of Candida accumulation at days 2 and 4 compared to the uncoated samples.*
Conclusion: *The research findings suggest that coating the denture resin surfaces with PTFE reduces biofilm adhesion to a larger extent and minimizes iatrogenic side effects.*
Key Word*: Biofilm formation, Polytetrafluoroethylene coating.*

Date of Submission: 30-01-2020 Date of Acceptance: 15-02-2020

I. Introduction

Removable dentures (RD) provide edentulous patients with the rehabilitation of masticatory and esthetic functions[1]. However, one consequence of the continual use of RD is the adhesion of microorganisms and biofilm formation[2] in the base of the prosthesis. Candida species are opportunistic pathogens that are frequently isolated from the oral cavity and its biofilms are often associated with oral candidosis[2,3,4].

Furthermore, studies have shown that the adhesive interactions between Candida species and oral bacteria such as Streptococcus mutans play a crucial role in microbial colonization of denture acrylic, which may lead to denture stomatitis[5]. Bacteria facilitate the adherence of Candida cells to the denture base and mucosa, mainly by extracellular polymer production as well as increased acidity, which creates optimal environmental conditions for yeast growth. Systemic alterations[6], poor oral hygiene and the surface characteristics of acrylic resin, such as hydrophobicity and surface roughness, have played a role in the development and maintenance of denture stomatitis. It has been demonstrated that moderately hydrophobic surfaces support cell adhesion whereas hydrophilic surfaces demonstrate limited cell adhesion[7]. In the case of surface roughness, it can be seen that rough surfaces provide a large surface area and may act as niches for microorganisms, thus favoring adhesion[8,9,10]. Furthermore, other factors, such as the salivary pellicle, may also alter the surface characteristics of the substratum involved in the adhesion process[11]. It has been demonstrated that the salivary pellicle may regulate specific interactions between cellular adhesion of C. albicans and receptors in the pellicle[12]. Studies have shown that surface modification could be considered a promising

approach to avoid denture stomatitis[13,14,2] because this modification could reduce the adhesion of microorganisms to the substratum[14,2].

PTFE (Polytetrafluoroethylene) is a polymer consisting of carbon and fluorine and is better known as Teflon. PTFE is a material characterized by a completely fluoridated chain. This chain is responsible for its physical and chemical characteristics. PTFE is an anti-adherent and aesthetic material that has excellent chemical inertia as well as good mechanical stability. Its modest mechanical properties can be improved using some filler. In order for PTFE to be coated, purpose-cleaned compressed air transports the atomized particles to the substrate and placed in a chamber furnace for heat treatment. PTFE is widely accepted in clinical use due to its nonreactive, heat-resistant and hydrophobic properties. It is made through a sintering process and two forms exist: classical PTFE, not microporous (Teflon) and expanded PTFE (ePTFE), microporous (GoreTex). ePTFE is characterized by orientated microfibrils, kept together by solid junctions[15].

II. Material And Methods

The study evaluated two groups (Group 1: PMMA, Group 2: PMMA with PTFE coating). Resin specimens in Group 1 were made of pure PMMA and had no surface coatings applied to the surfaces. Resin specimens were modified with PTFE coating for Group 2.

Fabrication of resin specimens:

60 PMMA samples were fabricated using DPI heat cure (Dental Products India, Mumbai, India) as per manufacturer's instructions. Test samples were prepared in an 11*5mm mold with highly polished surfaces to produce reproducible results. 30 samples per experimental group were prepared for the assay. The samples were washed with distilled water to remove any residual monomer and then stored in sterile distilled water for 24 hours.

PTFE coating of resin samples:

Ptfe coating was done on the resin samples through spray application using a siphon cup as directed by the manufacturer. A thickness of 25-30 microns dry film thickness was maintained.

METHODOLOGY:

Preparation of candida albicans culture:

Sabouraud dextrose broth (SD fine) was prepared using sterile water and autoclaved for use as a growth medium. The yeast was initially precultured in Sabouraud dextrose broth at 360C for 48 hours. 2 experimental sub groups for each surface type were devised, consisting of 2 and 4days of incubation in C. albicans suspension. Resins were added to separate test tubes. Care was taken to place the resin blocks with the smooth surface facing upward. The plates were then covered and placed in an incubator set at 360C to simulate the ambient temperature of the oral cavity. The plates were oscillated at a rate of 90 rpm to keep the suspension from settling. At 48 hours, the day 2 samples were removed from the incubator for fixing and staining. This procedure was repeated at 4th day also.

Surface adherence and crystal violet test:

Nonadherent yeast was removed by gently rinsing the blocks with phosphate-buffered saline (PBS). The blocks were then submerged in a PBS solution containing 1.5% glutaraldehyde (Sigma-Aldrich) for 1 hour to allow fixation of the adherent C. albicans. The resins were gently rinsed with sterile, deionized water to remove the fixing agent and allowed to air dry. The blocks were momentarily submerged in Gram's crystal violet (Sigma-Aldrich) and allowed to set on the table for 1 minute. The blocks were gently rinsed with sterile, deionized water from a squeeze bottle until the runoff was clear, dipped in Gram's iodine (Sigma-Aldrich), allowed to set for 1 minute, and then rinsed. This procedure was repeated for the remaining samples at days 4. The wash samples are subjected to colony count analysis and UV-visible spectrophotometer analysis for evaluating the no. of yeast colonies adhered and subsequent absorption of crystal violet stain.

Bioassay:

After incubation one set for sample was taken for colony counting whereas other set was used for crystal violet staining and UV spectrophotometer assay.

Microbial colony forming units (CFU's) assay:

Rose Bengal agar with Chloremphenicol was used for the CFU's estimation. The plates were counted after 48 hours of incubation.

Crystal Violet staining assay:

Initially PBS was prepared, autoclaved and stored. The culture broth was decanted carefully and allowed the resin to dry for 2 minutes. To that few drops of crystal violet [himedia] was added and allowed to stand still for

5 minutes. After 5 minutes the resin samples were washed in 3ml of PBS. The washed samples were dried and pictures were taken. The washed PBS samples were analyzed in UV spectrophotometer at 550nm for finding the absorbance. Growth phase of the yeast was quantified by UV-visible spectrophotometer at 660 nm.

Statistical analysis:

Descriptive statistics like mean, standard deviation and percentages were calculated. MANN-WHITNEY TEST was used to compute the significant difference among the groups using SPSS version 19 given the small sample sizes and lack of normality in the data.For all statistical analyses, a p-value <0.05 was considered statistically significant.

III. Result

W: With coating
WO: Without coating
1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30: Sample nos

Table 1: Growth curve and colony count for 48hours and 96 hours at 660 nm using UV-Visible spectrophotometer.

Sample Name	2^{nd} Day			4^{th} day		
	OD at 660nm	CFU's x 10^6	Percentage reduction in CFu's	OD at 660nm	CFU's x 10^6	Percentage reduction in CFu's
W1	1.342	378		2.273	494	
W2	1.298	423		1.436	354	
W3	1.299	420		1.488	368	
W4	1.340	380		2.220	484	
W5	1.339	381		2.168	476	
W6	1.302	410		1.541	378	
W7	1.305	408		1.593	389	
W8	1.325	391		1.855	424	
W9	1.328	390		1.899	435	
W10	1.314	398		1.805	418	
W11	1.332	388		2.010	442	
W12	1.339	382		2.168	476	
W13	1.310	400		1.751	406	
W14	1.334	386		2.064	459	
W15	1.308	405	49%	1.645	398	57.38%
WO1	1.276	849		1.919	1034	
WO2	1.256	723		1.795	956	
WO3	1.274	834		1.909	1030	
WO4	1.260	753		1.826	976	
WO5	1.271	824		1.889	1022	
WO6	1.259	744		1.814	968	
WO7	1.261	763		1.832	980	
WO8	1.273	830		1.898	1029	
WO9	1.262	770		1.844	984	
WO10	1.258	734		1.805	960	
WO11	1.270	817		1.882	1015	
WO12	1.263	780		1.850	990	

WO13	1.266	796		1.865	1001	
WO14	1.264	786		1.857	995	
WO15	1.268	807		1.874	1009	

Table 2: Crystal violet test at 48hours and 96 hours at 550 nm using UV-Visible spectrophotometer.

Sample Name	2nd Day	4th day
W16	0.243	0.342
W17	0.199	0.344
W18	0.236	0.342
W19	0.224	0.342
W20	0.229	0.342
W21	0.216	0.343
W22	0.239	0.342
W23	0.209	0.344
W24	0.218	0.343
W25	0.233	0.342
W26	0.221	0.343
W27	0.211	0.344
W28	0.203	0.344
W29	0.241	0.342
W30	0.213	0.343
WO16	0.291	0.454
WO17	0.367	0.503
WO18	0.331	0.482
WO19	0.355	0.497
WO20	0.307	0.462
WO21	0.329	0.478
WO22	0.348	0.487
WO23	0.309	0.464
WO24	0.360	0.501
WO25	0.302	0.460
WO26	0.321	0.472
WO27	0.351	0.490
WO28	0.318	0.470
WO29	0.339	0.485
WO30	0.299	0.457

TABLE 3: Comparison Of The Mean Distribution Of Optical Density At 2nd Day Between The Groups Using Mann-Whitney Test

	Minimum	Maximum	Mean	Std. Deviation	Mean diff	p value
With OD	1.30	1.34	1.32	.016	0.06	0.00*
Without OD	1.26	1.28	1.26	.006		

*significant

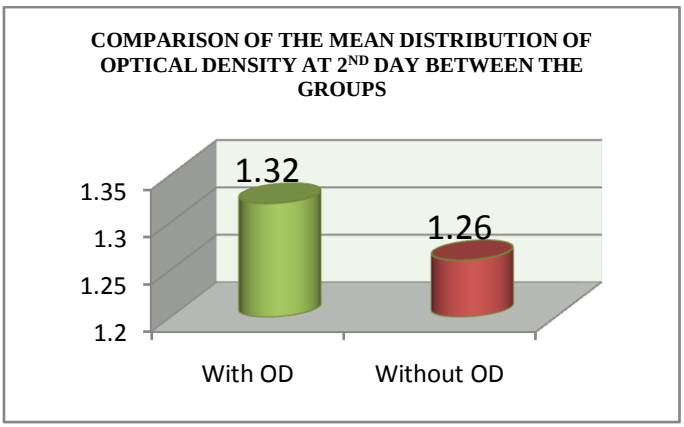


TABLE 4: Comparison Of The Mean Distribution Of CFU At 2nd Day Between The Groups Using Mann-Whitney Test

	Minimum	Maximum	Mean	Std. Deviation	Mean diff	p value
With OD	378.00	423.00	396.0	14.57	-391.3	0.00*
Without OD	723.00	849.00	787.3	39.16		

*significant

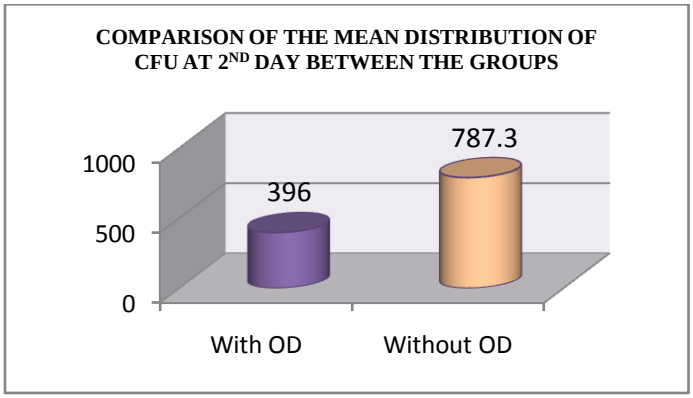


TABLE 5: Comparison Of The Mean Distribution Of Optical Density At 4th Day Between The Groups Using Mann-Whitney Test

	Minimum	Maximum	Mean	Std. Deviation	Mean diff	p value
With OD	1.44	2.27	1.86	.28	0.01	0.96
Without OD	1.80	1.92	1.85	.03		

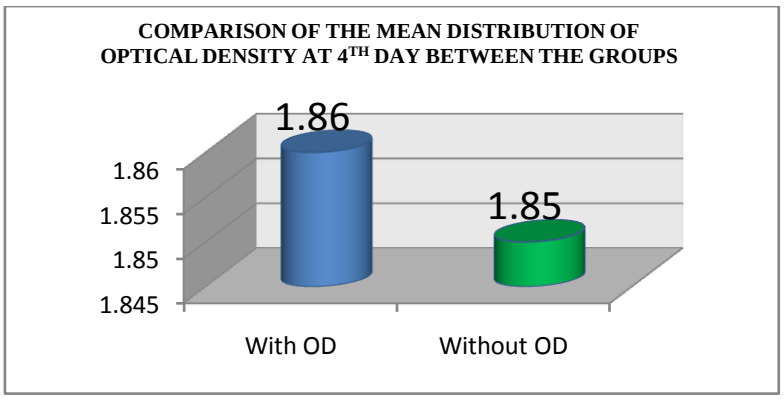


TABLE 6: Comparison Of The Mean Distribution Of Cfu At 4th Day Between The Groups Using Mann-Whitney Test

	Minimum	Maximum	Mean	Std. Deviation	Mean diff	p value
With OD	354.00	494.00	426.7	44.62	-569.9	0.00*
Without OD	956.00	1034.00	996.6	25.95		

*significant

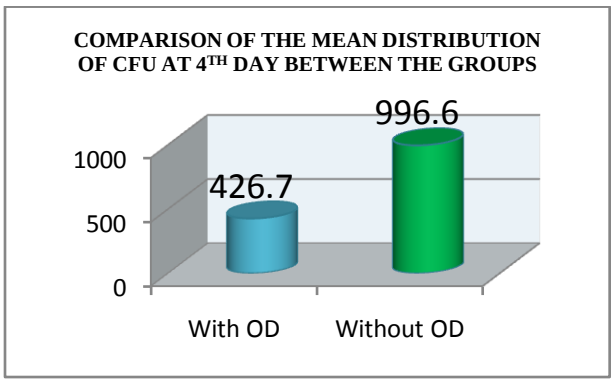


TABLE 7: Comparison Of The Mean Distribution Of Crystal Violet Test At 2nd Day Between The Groups Using Mann-Whitney Test

	Minimum	Maximum	Mean	Std. Deviation	Mean diff	p value
With OD	.20	.24	.222	.014	0.106	0.00*
Without OD	.29	.37	.328	.024		

*significant

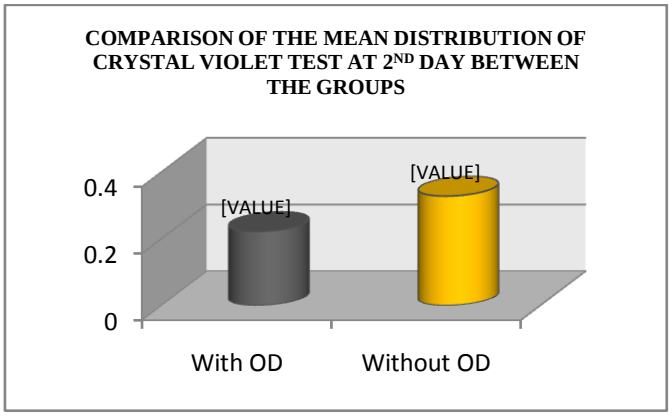
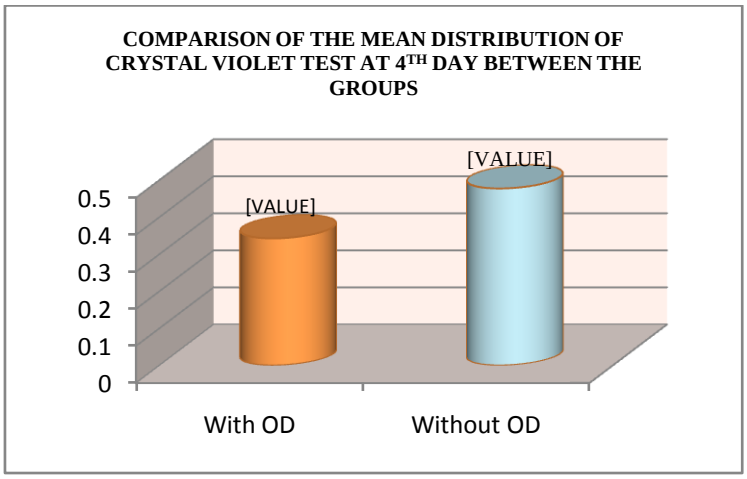


TABLE 8: Comparison Of The Mean Distribution Of Crystal Violet Test At 4th Day Between The Groups Using Mann-Whitney Test

	Minimum	Maximum	Mean	Std. Deviation	Mean diff	p value
With OD	.34	.34	.3428	.00	-0.13	0.00*
Without OD	.45	.50	.4775	.016		

*significant



Each group consisted of 15 samples had measurements of surface *Candida* taken at 2 time period (2 and 4 days). The mean distribution of optical density at 2nd and 4th day is shown in table 3 and table 5. Table 4 and 6 summarized the CFU between the groups on day 2 and 4. Table 7 and table 8 demonstrated the crystal violet test results on day 2 and 4. Multiple tests demonstrated that the PTFE coated resin surface had statistically significant lower levels of *Candida* than the uncoated group.

IV. Discussion

Coatings are traditionally used on dental materials to protect the material surface from degradation and the external stresses involved in mechanical and chemical factors, which could increase the surface roughness and porosity[16]. The development of coatings or sealants with antimicrobial and anti-adherent potential is attracting great interest in the scientific community[17]. Recently, Molino et al. observed that surface modification reduced interfacial protein adsorption, with the complete inhibition of adhesion and colonization by primary mouse myoblasts[18]. Since PTFE coatings can easily be applied to denture surfaces, this study evaluated the effectiveness of PTFE coatings in an attempt to reduce the adherence of *C.albicans.*

The result of the present study revealed that the PTFE coated samples had significantly lower amounts of adherent *C.albicans*on the resin surface compared to the uncoated group.

Many methods can be used to determine the extent of fungal adhesion to biomaterials and comparison of the various methods is difficult, as they present different limitations. For the purpose of the present study, surface area of adherent fungal colonization was measured, because growth of Candida involves multicellular strands and colony-forming units, thus restricting the ability to assay single cell forms. Staining the fungal cells provided sufficient contrast, and adherent Candida was easy to visualize by microscopy because of their large size and high refractivity.

The effects of saliva, pH, and the presence of a multitude of microorganisms that coexist within the oral environment and their possible association on Candida adhesion have been investigated. Research has shown that whole saliva contains factors for detachment of Candida cells to material surfaces. Whole saliva and secretory immunoglobulin A (sIgA) showed an inhibitory effect on the adherence of C. albicans to resin restorative material. Candida biofilms on oral surfaces and prosthetic devices may also contribute to increased resistance to antifungal agents and protection from the host defense mechanisms. Including these potential contributing factors may give new insights into the adhesion of C. albicans to surface modified denture resins. The results also show that patients with poor oral hygiene could benefit greatly by using these surface-modified resins to reduce the occurrence of denture stomatitis. This was an accelerated study to investigate methods of surface modification to reduce the adhesion of C. albicans on denture resin surfaces. The long-term effect of both of these techniques must be evaluated as to whether this positive effect could carry on semi-permanently throughout the life of the denture prior to clinical application.

V. Conclusion

The amount of C. albicans adhered to the resin surfaces reduced significantly with modification of surface charge by PTFE coating. Modification of surface characteristics of PTFE is an effective method in reducing adhesion of C. albicans to PMMA surfaces.

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Dr.RekhaRevannaetal. "Adherence of Candida Albicans To Surface Modified Denture Resin Surfaces With Polytetrafluoroethylene(PTFE) Polymer– An In Vitro Study". *IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)*, 19(2), 2020, pp. 18-24.