Acta Scientific Dental Sciences (ISSN: 2581-4893)

Volume 7 Issue 2 February 2023

Research Article

Comparative Evaluation of The Effect of Surface Sealant on the Surface Topography and Microbial Adhesion of Long-Term Provisional Fixed Implant Prosthesis- *Invitro* Study

Sivasankari S[1], Divya Krishnan[2]*, Jayakrishnakumar S[3], Vidhya J[4], Hariharan Ramakrishnan[5], Vallabh Mahadevan[6] and Azhagarasan NS[7]

[1] *Prosthodontist, Ragas Dental College, Chennai, India*

[2] *Reader, Department of Prosthodontics and Implantology, Ragas Dental College and Hospital, Chennai, India* ***Orcid:*** 0000-0002-4303-3318

[3] *Professor and HOD, Department of Prosthodontics, Ragas Dental College and Hospital, Chennai, India*

[4] *Ragas Dental College and Hospital, Chennai, India*

[5] *Professor, Department of Prosthodontics, Ragas Dental College, Chennai, India*

Orcid: 0000-0003-4466-5744

[6] *Professor, Ragas Dental College and Hospital, Chennai, India*

[7] *Professor Emeritus, Ragas Dental College, Chennai, India*

***Corresponding Author:** Divya Krishnan, Department of Prosthodontics and Implantology, Ragas Dental College and Hospital, Chennai, India.

DOI: 10.31080/ASDS.2023.07.1573

Received: January 03, 2023

Published: January 20, 2023

Abstract

Purpose: To evaluate the effect of surface sealant on the surface topography and microbial adhesion to two different long term provisional materials for fixed implant prosthesis.

Materials and Methods: Twenty samples of Heat cure PMMA resin samples (n = 10) and CAD/CAM PMMA resin samples (n = 10) of dimension 12 x 10 x 2.5 mm were prepared. One side of the test sample was considered as uncoated (side A) and opposite side was coated with NANOCOAT surface sealant and considered as coated (side B). The heat cure PMMA resin samples were designated as uncoated (Group IA) and coated (Group IB); the CAD/CAM resin samples were designated as uncoated (Group IIA) and coated (Group IIB). Surface roughness of the test samples were evaluated using surface profilometer and microbial adhesion analysis was done using the spread plate pure culture technique. The results were tabulated and statistically analyzed using Wilcoxon signed ranks test, Mann Whitney U test, Paired t-test and Independent t- test using the SPSS version 2.0 software

Results: Mean surface roughness of Group IA and Group IB was 39.783 nm and 22.960 nm. Mean surface roughness of Group IIA and Group IIB was 27.2130 nm and 18.617 nm. Mean microbial adhesion of Group IA and Group IB was 371 CFU/ml and 248.10 CFU/ml. Mean microbial adhesion of Group IIA and Group IIB was 130.70 CFU/ml and 80.800 CFU/ml. Reduction in surface roughness was noticed after coating with the surface sealant in both Heat cure PMMA and CAD /CAM PMMA resin groups, decrease is more in CAD/CAM PMMA resin samples but was statistically insignificant ($P > 0.05$). Highly significant reduction in microbial adhesion was noticed after coating with surface sealant in both the groups with CAD/ CAM PMMA resin group showing a higher significant reduction ($P < 0.001$).

Conclusion: CAD/CAM PMMA resin with sealant application had significantly reduced the microbial adhesion of Streptococcus mutans.

Keywords: Polymethylmethacrylate; Sealant; Adhesion; Resin; Colony Forming Units

Introduction

Provisional restorations are an acrylic progenitor of the final restoration in all aspects, except the material from which it is fabricated. They are designed to assist in the determination of therapeutic effectiveness of a specific treatment plan, form and function of the planned definitive prosthesis [1]. Interim restorations are necessary to maintain tooth position, esthetics, withstand the functional forces of mastication without fracture or displacement, maintain stability of inter and intra-arch relationships and protect the prepared tooth and periodontal tissues [2].

Citation: Divya Krishnan*., et al.* "Comparative Evaluation of The Effect of Surface Sealant on the Surface Topography and Microbial Adhesion of Long-Term Provisional Fixed Implant Prosthesis- *Invitro* Study". *Acta Scientific Dental Sciences* 7.2 (2023): 164-177.

In complex fixed prosthodontics, like implant therapy, the crucial role of the long term provisionals is to maintain the health of the periodontal tissues. This is accomplished by ensuring that the gingival contours do not impinge on the periodontal tissues and the prosthesis should be smooth, highly polished and thereby maintaining marginal integrity. Lower surface roughness of provisional restoration is very important to maintain the periodontal health since high surface roughness causes bacterial adhesion. Provisional restorations are mechanically polished by using abrasive materials of varying grit size, abrasive pastes and aluminium oxide particles containing liquid polish [1,2].

Polymethylmethacrylate resin materials have gained immense popularity as a long-term provisional restoration due to their adequate strength, ease of handling, manipulation, repairability and stability in the oral environment. However, a main disadvantage is inadequate mechanical properties due to the possibility of surface and subsurface voids, lack of marginal integrity, poor color stability, dimensional changes, susceptibility to fracture, residual monomer and increased risk of plaque accumulation and caries [3,5-7].

Recently, thermoplastic resin materials that are fabricated by indirect technique have been introduced as interim restoration. Because these materials require special equipment and are technique sensitive, they have been used with rapid prototyping including both liquid-based stereolithography and powder-based 3-dimensional printing alone [4].

Currently computer aided design and computer aided manufacturing (CAD/CAM) PMMA resin-based pre-polymers with highly cross-linked structure have been introduced as a material for provisional restorations. They are milled from pre polymerized PMMA billets which are polymerised under high pressure and temperature. CAD/CAM PMMA resins have proven to be less porous and thereby less likely to harbor microbes such as streptococcus species and candida albicans. Improved optical properties, color stability and reduced residual monomer content with superior surface properties and increased flexural strength are a few of the factors favoring CAD/CAM resins over conventional heat cure PMMA resins [2-6].

The threshold level of surface roughness of dental prosthesis for plaque accumulation is defined to be 0.2 µm [6]. Oral microbial species causing caries e.g., streptococcus mutans, actinomyces species, lactobacillus species and those causing periodontitis e.g., porphyromonas gingivalis, actinobacillus actinomycetemcomitans, prevotella intermedia adhere only to non-shedding surfaces [7,8]. Hence surface roughness and surface free energy are crucial for bacterial adhesion, retention and colonization [6].

Proliferation of the initially adhering microorganisms accounts for the major part of the microbial mass increase during early plaque formation which may explain the importance of surface roughness in initial plaque formation [9]. Subgingivally, the impact of surface roughness is not much pronounced. The pocket by itself offers a shelter, which limits the impact of surface roughness on the subgingival plaque composition [10]. Bacterial adhesion and colonization also depend on the bacterial species. Streptococci are one of the most common "early colonizing bacteria [11]. Hence streptococcus mutans species were used in the current study.

Recently, surface sealant agents have been developed to eliminate surface defects and increase stain and wear resistance. Nano-coat is a revolutionary, light cured, protective nano filled varnish which effectively seals the outer layer of direct and indirect composite restorations to create a smooth and glossy surface [6]. However, reports in the literature evaluating the use of nano-coat as protective coatings for CAD/CAM PMMA long term provisional prosthesis and the microbial adhesion are lacking.

In view of the above, the present invitro study was conducted to compare and evaluate the effect of surface sealant agent on the surface topography and microbial adhesion to Heat cure PMMA resin and CAD/CAM PMMA resin materials. The null hypothesis of the present study is that with the application of a surface sealant (NANOCOAT) there will be no significant difference in the surface roughness and microbial adhesion to long term provisional implant restorations used in this study.

Materials and Methods

Group sample preparation

A stereolithography (STL) file was virtually designed using the MESHMIXER software (a 3D design software used for 3D printing) to the required dimensions (12mm x 10mm x 2.5 mm) to obtain the CAD/CAM milled PMMA test samples (n = 10). The specifications for designing the STL file includes Chord height - 0.001 mm and Angular tolerance 15 degrees.

Using the data from STL file format, dry milling of a 98 mm diameter and 10mm thickness round pre-sintered CAD/CAM PMMA block (Bloomden Bioceramics, china), (shade A2) was done in the CNC unit (Yenadent D15, TURKEY) to fabricate ten test samples (Figure 1). The ten test samples were subjected to finishing and polishing procedures using acrylic trimmers and aluminium oxide abrasive papers (120,200,320,400 grits) followed with pumice applied to a rag wheel. The dimensions (12mm x 10mm x 2.5mm) of the samples were verified using digital vernier caliper and finally stored in labeled boxes. A custom-made addition silicone putty mold was obtained using one of the CAD/CAM milled PMMA sample (12mm x 10mm x 2.5mm) for the fabrication of the heat-cure PMMA resin test samples.

Figure 1: Subtractive milling of CAD/CAM PMMA resin blocks.

Wax patterns were fabricated using modelling wax which was melted and poured into the silicone mold. After the wax had been cooled down, the wax patterns were retrieved and measured with a digital caliper to ensure uniform dimension (12 x 10 x 2.5 mm). The wax patterns were invested in a dental flask using type II dental plaster. A two pour technique was followed for flasking the wax specimens. Adhering to the manufacturer's instructions, the polymer (DPI tooth moulding powder) and the monomer was proportioned (3:1), hand mixed for the polymerization of the Heat cure PMMA resin employing the compression molding technique. The samples were retrieved from the dental flask and subjected to finishing and polishing procedures using acrylic trimmers and sand papers (120,200,320,400 grits). The dimensions (12mm x 10mm x 2.5mm) of the samples were verified using digital vernier caliper. A total of ten Heat cure PMMA resin samples were obtained in a similar manner. Inclusion criteria of samples from both the groups included selection of samples with correct dimensions, well finished samples, samples which are having uniform contact on a flat surface like glass. Defective samples which didn't met the inclusion criteria from both groups were discarded. A total of 15 samples each for both groups were prepared and the best of ten samples were selected. All the samples were prepared by same single operator.

The finished and polished samples were stored in distilled water for 24hrs at 37ºc (ADA specification no 12, 1975) to reduce residual monomer from the samples and finally stored in labelled boxes. Twenty test samples obtained were grouped. One side of the individual test sample was considered as uncoated sample (side A). The other side of the same sample was coated with NANOCOAT surface sealant and considered as coated sample (side B).

NANOCOAT surface sealant (Prevest Denpro, India) is a nano filled light cure, self-adhesive varnish which seals the outer layer of restorations to provide a smooth finish. Nano-coat was applied to one side of Heat cure PMMA test samples and CAD/CAM PMMA test samples in one layer and one direction. It was applied immediately (within 1 minute after dispensing) on the surfaces to be coated using micro applicator. Nanocoat was applied in a sterile environment and light cured with a visible light curing unit for 60 seconds (>500 mW/cm^2).

Based on this the test samples were designated into the following groups, Group IA: The uncoated side of Heat cure PMMA resin samples, Group IB: The coated side of Heat cure PMMA resin samples, Group IIA: The uncoated side of CAD/CAM PMMA resin samples, Group IIB: The coated side of CAD/CAM PMMA resin samples (Figure 2). Using a 2D stylus profilometer the surface roughness values of the samples were evaluated.

The labelled samples from each group were placed on the specimen platform. A pick-up with a stylus was traversed over each sample at a constant velocity. The tip, attached to a cantilever was drawn across each sample in the X direction. The stylus radius and force were 2 µm and 1mg respectively and speed was set 150 µm/sec. The scan length and scan duration selected for thickness mea-

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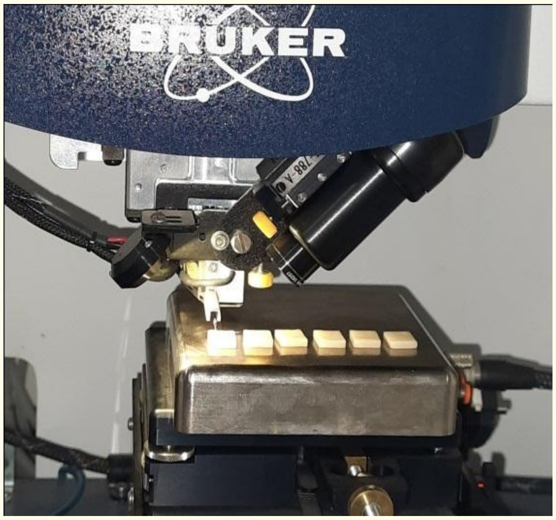

Figure 2: Measurement of surface roughness of uncoated Heat cure PMMA resin samples (Group IA) and CAD/CAM PMMA resin samples (Group IIA).

surement were 100 µm and 10s respectively. Vertical movements of the cantilever were registered in a digital signal, and a profile of the surface of each sample was recorded. For each sample 3 measurements were made, the surface roughness value was calculated and the arithmetic mean value of total samples were used for statistical analysis.

Microbial study

For evaluating the microbial adhesion (Figure 3-6) quantitative evaluation of microbial adhesion (streptococcus mutans) was carried out.

Post the surface roughness test evaluation the samples were sterilized in an UV Chamber and were immersed in 70% ethanol (70ml of Absolute ethanol is mixed with 30ml Distilled water) for 15min and air dried. Nutrient Broth Medium (Himedia) was used as a sterility-testing medium and for the preparation of the inoculum suspension of young colonies of Streptococcus mutans. 2ml of autoclaved nutrient broth preparation was used for inoculum preparation. Mueller - Hinton Agar medium, a microbiological growth medium commonly used for antibiotic susceptibility testing was selected as the culture medium. Inoculation of streptococcus mutans (ATCC 25175) (Himedia, Mumbai): was completed. The

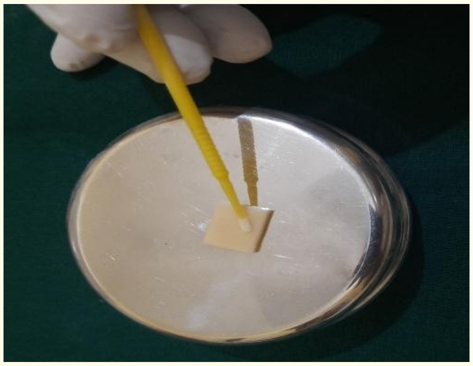

Figure 3: Coating of one side of all the test samples with NANOCOAT surface sealant.

Figure 4: Light curing of NANOCOAT surface sealant.

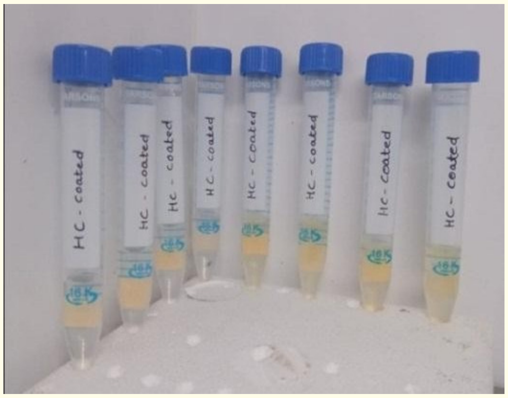

Figure 5: Immersion of sealant coated Heat cure PMMA resin samples in nutrient broth containing streptococcus mutans (ATCC 25175).

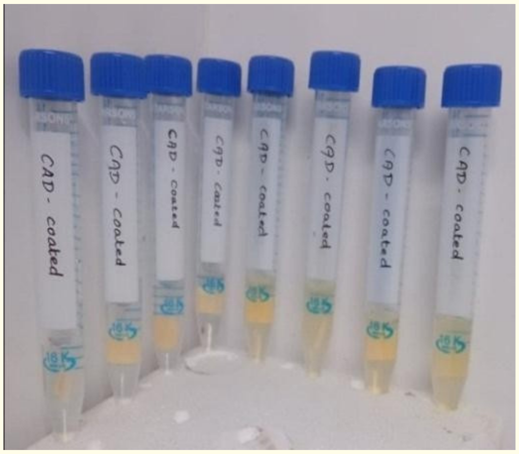

Figure 6: Immersion of coated CAD/CAM PMMA resin samples in nutrient broth containing streptococcus mutans (ATCC 25175).

test bacterial strains ATCC 25175 Streptococcus mutans (Himedia, Mumbai) were revived from glycerol stock maintained at - 20°C. The overnight young subculture of the test strains was suspended in 2ml nutrient broth (Himedia, Mumbai) to match the turbidity of 0.5Mc Farland suspension of standard inoculum suspension of 10^8 CFU/ml. The sterilized samples were then immersed in the nutrient broth containing 10^8 CFU/ml Streptococcus mutans (ATCC 25175). The unbound Streptococcus mutans cells were removed from the samples by washing thrice with phosphate-buffered saline (PBS). The samples were placed in sterile tubes with 3ml of Nutrient broth (HiMedia, Mumbai India) for 48h. The adherent Streptococcus mutans were dislodged from the sample surfaces by gentle scraping. The suspension was centrifuged at 2,000 rpm for 10 min. The pellet suspension was utilized for determining the total colony forming unit. Colony count was determined by spread plate - pure culture technique, by spreading 100 µl of the respective pellet suspension onto Mueller Hinton agar surface and the plates were incubated at 37°C for 24hours. The colonies were counted using a digital colony counter and the total colony forming unit was calculated using the formula: Total colony forming unit, CFU/ml = Total number of colony counted/ Dilution factor.

Statistical analysis

The results obtained were tabulated and subjected to statistical analysis using the statistical software SPSS V20. (Los Angeles, California). The data was analyzed for surface roughness using the Wilcoxon signed ranks test and Mann Whitney U test. For analyzing the microbial adhesion, the paired t-test and independent t- test were used. $P < 0.05$ was considered significant.

Results

The present study was to compare and evaluate the effect of surface sealant on the surface topography and microbial adhesion to long term provisional fixed partial denture materials- an invitro study. A total of twenty samples of dimension 12*10*2.5 mm were prepared for evaluating surface roughness and microbial adhesion analysis. Heat cure PMMA resin samples (n = 10) and CAD/CAM PMMA resin samples (n = 10) were fabricated.

The basic data of mean surface roughness and mean microbial adhesion of each group was determined (Table 1-12) and statistically analyzed using Wilcoxson signed ranks test, Mann- Whitney U test, paired t-test and independent t-test (Table 13-16).

The mean surface roughness of uncoated Heat cure PMMA and CAD/CAM PMMA resin samples was found to be 39.7383 nm and 27.2130 nm respectively. The mean surface roughness of coated Heat cure PMMA and CAD/CAM PMMA resin samples was found to be 22.9600 nm and 18.6173 nm respectively. On comparative evaluation of the mean surface roughness of uncoated (Group IA) and coated (Group IB) Heat cure PMMA resin samples, the uncoated samples exhibited higher surface roughness (39.738) than the coated samples (22.9600) but the difference was statistically insignificant ($P > 0.05$). On comparative evaluation of the mean surface roughness of uncoated (Group IIA) and coated (Group IIB) CAD/CAM PMMA resin samples, the uncoated samples exhibited higher surface roughness (27.2130) than the coated samples (18.6173). But the difference was statistically insignificant ($P > 0.05$). On comparative evaluation of the mean surface roughness of uncoated (Group IA) Heat cure PMMA resin samples and uncoated (Group IIA) CAD/CAM PMMA resin samples, the uncoated Heat cure PMMA resin samples exhibited higher surface roughness (39.7383) than the CAD/CAM PMMA resin samples (27.2130), but the difference was statistically insignificant ($P > 0.05$). On comparative evaluation of the mean surface roughness of coated (Group IB) Heat cure PMMA resin samples and coated (Group IIB) CAD/CAM PMMA resin samples, the coated Heat cure PMMA resin samples exhibited higher surface roughness (22.9600) than the coated CAD/CAM

	Surface roughness values (nm)			
S.no	**Uncoated heat cure Group IA**	**Uncoated Cadcam Group IIA**	**Coated heatcure. Group IB**	**Coated Cadcam Group IIB**
1	26.39	18.42	21.72	18.12
2	60.8133	39.6233	13.3833	11.14333
3	132.9533	29.3433	17.7	29.87333
4	20.03667	23.35	34.11333	16.41667
5	25.25	41.8	16.64	14.12
6	24.7833	36.35667	21.96	19.72
7	26.57	16.4633	30.07607	24.11333
8	33.11	21.25333	20.23	15.04
9	16.1466	31.28667	27.88333	13.40677
10	31.33	14.2333	25.8933	24.22
Mean surface roughness values	39.738	27.2130	22.960	18.6173

Table 1: Basic data to evaluate mean surface roughness of test samples.

Inference: For Group IA test samples, the maximum surface roughness was 132.95 nm and the minimum surface roughness was 16.15 nm. The group mean surface roughness was 39.738 nm. For Group IA test samples, the maximum surface roughness was 41.80 nm and the minimum surface roughness was 14.23 nm. The Group mean surface roughness was 27.2130 nm. For Group IB test samples, the maximum surface roughness was 34.11 nm and the minimum surface roughness was13.38 nm. The Group mean surface roughness was 22.960 nm. For Group IIB test samples, the maximum surface roughness was 29.87 nm and the minimum surface roughness was 11.14 nm. The Group mean surface roughness was 18.6173 nm.

Group		**Mean**	**Standard deviation**	**P value**
Heatcure PMMA resin	Uncoated	39.7383	34.924	0.241
	Coated	22.9600	6.473	
Cadcam PMMA resin	Uncoated	27.2130	9.906	0.139
	Coated	18.6173	5.874	

Table 2: Comparative evaluation of the mean surface roughness of uncoated (Group IA) and coated (Group IB) Heat cure PMMA and uncoated (Group IIA) and coated (Group IIB) CAD/CAM PMMA resin samples using Wilcoxson signed ranks test.

Inference: On comparative evaluation of the mean surface roughness of uncoated (Group IA) and coated (Group IB) Heat cure PMMA resin samples, the uncoated samples exhibited higher surface roughness than the coated samples. But the difference in surface roughness was not statistically significant ($P > 0.05$).

On comparative evaluation of the mean surface roughness of uncoated (Group IIA) and coated (Group IIB) CAD/CAM PMMA resin samples, the uncoated samples exhibited higher surface roughness than the coated samples. But the difference in surface roughness was not statistically significant ($P > 0.05$).

S.no	Point a	Point b	Point c	Surface roughness (nm)
1	19.51	22.16	23.49	21.72
2	17.48	14.09	8.58	13.38333
3	13.76	24.06	15.28	17.7
4	50.19	17.81	34.34	34.11333
5	16.79	20.51	12.62	16.64
6	24.56	17.47	23.85	21.96
7	27.09	13.3	49.84	30.07667
8	21.32	18.51	20.86	20.23
9	25.71	27.65	30.29	27.88333
10	36.14	20.98	20.56	25.89333
Mean value				22.960

Table 3: Basic data of surface roughness of coated Heat cure PMMA resin samples (Group IB)

Inference: For GroupIB test samples, the maximum surface roughness was 34.11 nm and the minimum surface roughness was 13.38 nm. The Group mean surface roughness was 22.960 nm.

S.no	Point A	Point B	Point C	Surface roughness (nm)
1	21.23	16.26	16.87	18.12
2	9.79	9.08	14.56	11.14333
3	23.54	34.11	31.97	29.87333
4	19.93	14.35	14.97	16.41667
5	13.79	14.77	13.8	14.12
6	22.24	22.3	14.62	19.72
7	29.19	25.63	17.52	24.11333
8	14.15	20.69	10.28	15.04
9	11.72	15.5	13	13.40667
10	30.13	22.36	20.17	24.22
Mean value				18.6173

Table 4: Basic data of surface roughness of coated CAD/CAM PMMA resin samples (Group II B)

Inference: For Group II B test samples, the maximum surface roughness was 29.87 nm, and the minimum surface roughness was 11.14 nm. The Group mean surface roughness was 18.6173 nm.

Group		Mean	Standard deviation	P value
Heat cure PMMA resin	Uncoated	39.7383	34.924	0.241
	Coated	22.9600	6.473	

Table 5: Comparative evaluation of the mean surface roughness of uncoated (Group IA) and coated (GroupIB) Heat cure PMMA resin samples using wilcoxson signed ranks test.

(P > 0.05); No significant difference.

Inference: On comparative evaluation of the mean surface roughness of uncoated (Group IA) and coated (Group IB) Heat cure PMMA resin samples, the uncoated samples exhibited higher surface roughness than the coated samples. But the difference in surface roughness was not statistically significant (P > 0.05).

Group		Mean	Standard deviation	P value
CAD/CAM PMMA Resin	Uncoated	27.2130	9.906	0.139
	Coated	18.6173	5.874	

Table 6: Comparative evaluation of the mean surface roughness of uncoated (Group II A) and coated (Group II B) CAD/CAM PMMA resin samples using wilcoxson signed ranks test.

(P > 0.05); No significant difference.

Inference: On comparative evaluation of the mean surface roughness of uncoated (Group II A) and coated (GroupIIB) CAD/CAM PMMA resin samples, the uncoated samples exhibited higher surface roughness than the coated samples. But the difference in surface roughness was not statistically significant (P > 0.05).

Group		Mean	Standard deviation	P value
Uncoated	Heat cure PMMA	39.7383	34.924	0.545
	CAD/CAM PMMA	27.2130	9.906	

Table 7: Comparative evaluation of the mean surface roughness of uncoated (Group IA) Heat cure PMMA resin samples and CAD/CAM PMMA resin samples (Group IIA) using Mann-Whitney U test.

(P > 0.05); No significant difference.

Inference: On comparative evaluation of the mean surface roughness of uncoated Heat cure PMMA resin samples (Group IA) and CAD/CAM PMMA resin samples (Group II A), the uncoated Heat cure PMMA resin samples exhibited higher surface roughness than the CAD/CAM PMMA resin samples. But the difference in surface roughness was not statistically significant (P > 0.05).

Group		Mean	Standard deviation	P value
Coated	Heat cure PMMA	22.960	6.4738	0.131
	CAD/CAM PMMA	18.6173	5.874	

Table 8: Comparative evaluation of the mean surface roughness of coated Heat cure PMMA resin samples (Group I B) and coated CAD/CAM PMMA resin samples (Group II B) using Mann-Whitney U test.

(P > 0.05); No significant difference

Inference: On comparative evaluation of the mean surface roughness of coated Heat cure PMMA resin samples (Group I B) and CAD/CAM PMMA resin samples (Group II B), the Heat cure PMMA resin samples exhibited higher surface roughness than the CAD/CAM PMMA resin samples. But the difference in surface roughness was not statistically significant (P > 0.05).

S.NO	Colony forming units (CFU/ml)
1	348
2	358
3	339
4	371
5	407
6	390
7	363
8	382
9	341
10	375
Mean value	371.0

Table 9: Basic data to evaluate the mean microbial adhesion of streptococcus mutans to uncoated Heat cure PMMA resin samples (Group III A).

Inference: For Group IIIA test samples, the maximum microbial adhesion was 407 CFU/ml and the minimum microbial adhesion was 339 CFU/ml. The mean microbial adhesion was 371.0 CFU/ml.

S.NO	Colony forming units (CFU/ml)
1	142
2	117
3	128
4	115
5	131
6	155
7	120
8	132
9	109
10	158
Mean value	130.70 CFU/ml

Table 10: Basic data to evaluate the mean microbial adhesion of streptococcus mutans to uncoated CAD/CAM PMMA resin samples (Group IV A).

Inference: For Group IVA test samples, the maximum microbial adhesion was 158 CFU/ml and the minimum microbial adhesion was 109 CFU/ml. The mean microbial adhesion was 130.70 CFU/ml.

S.NO	Colony forming units (CFU/ml)
1	255
2	279
3	208
4	241
5	259
6	248
7	287
8	233
9	240
10	231
Mean value	248.1

Table 11: Basic data to evaluate the mean microbial adhesion of streptococcus mutans to coated Heat cure PMMA resin samples (Group III B).

Inference: For Group IIIB test samples, The maximum microbial adhesion was 287 CFU/ml and the minimum microbial adhesion was 208 CFU/ml. The mean microbial adhesion was 248.1 CFU/ml.

S.NO	Colony forming units (CFU/ml)
1	78
2	92
3	65
4	71
5	95
6	84
7	80
8	77
9	87
10	79
Mean value	80.800

Table 12: Basic data to evaluate the mean microbial adhesion of streptococcus mutans to coated CAD/CAM PMMA resin samples (Group IV b).

Inference: For Group IVB test samples, the maximum microbial adhesion was 95 CFU/ml and the minimum microbial adhesion was 65 CFU/ml. The mean microbial adhesion was 80.800 CFU/ml.

Group		Mean	Standard deviation	P value
Heat cure PMMA	Un coated	371.00	21.396	0.000**
	Coated	248.10	23.283	

Table 13: Comparative evaluation of the mean microbial adhesion of streptococcus mutans to uncoated (Group IIIA) and coated (Group IIIB) Heat cure PMMA resin samples using paired T- test.

(P < 0.001**); highly significant at level 1.

Inference: On comparative evaluation of the mean microbial adhesion of streptococcus mutans to uncoated (Group IIIA) and coated (Group IIIB) Heat cure PMMA resin samples, the uncoated Heat cure PMMA resin samples exhibited higher microbial adhesion than the coated Heat cure PMMA resin samples which has high statistical significance (P < 0.001**).

Group		Mean	Standard deviation	P value
CAD/CAM PMMA	Uncoated	130.70	16.640	0.000**
	Coated	80.800	9.114	

Table 14: Comparative evaluation of the mean microbial adhesion of streptococcus mutans to uncoated (Group IVA) and coated (Group IVB) CAD/CAM PMMA resin samples using paired T- test.

(P < 0.001**); highly significant at level 1.

Inference: On comparative evaluation of the mean microbial adhesion of streptococcus mutans to uncoated (Group IVA) and coated (Group IVB) CAD/CAM PMMA resin samples, the uncoated CAD/CAM PMMA resin samples exhibited higher microbial adhesion than the coated CAD/CAM PMMA resin samples which has high statistical significance (P < 0.001**).

Group		Mean	Standard deviation	P value
Uncoated	Heat cure PMMA	371.00	21.396	0.000**
	CAD/CAM PMMA	130.70	16.640	

Table 15: Comparative evaluation of the mean microbial adhesion of streptococcus mutans to uncoated Heat cure PMMA resin samples (Group IIIA) and CAD/CAM PMMA resin samples (Group IVA) using Independent t-test.

(P < 0.001**); highly significant at level 1.

Inference: On comparative evaluation of the mean microbial adhesion of streptococcus mutans to uncoated Heat cure PMMA resin samples (Group IIIA) and CAD/CAM PMMA resin samples (Group IVA), the uncoated Heat cure PMMA resin samples exhibited higher microbial adhesion than the CAD/CAM PMMA resin samples which has high statistical significance (P < 0.001**).

Group		Mean	Standard deviation	P value
Coated	Heat cure PMMA	248.10	23.283	0.000**
	CAD/CAM PMMA	80.800	9.114	

Table 16: Comparative evaluation of the mean microbial adhesion of streptococcus mutans to coated Heat cure PMMA resin samples (Group IIIB) and CAD/CAM PMMA resin samples (Group IVB) using Independent t-test.

(P < 0.001**); highly significant at level 1.

Inference: On comparative evaluation of the mean microbial adhesion of streptococcus mutans to coated Heat cure PMMA resin samples (Group III B) and CAD/CAM PMMA resin samples (Group IV B), the coated Heat cure PMMA resin samples exhibited higher microbial adhesion than the CAD/CAM PMMA resin samples which has high statistical significance (P < 0.001**).

PMMA resin samples (18.6173), but the difference was statistically insignificant ($P > 0.05$).

The mean microbial adhesion of uncoated Heat cure PMMA and CAD/CAM PMMA resin sample was found to be 371.0 CFU/ml and 130.70 CFU/ml respectively. The mean microbial adhesion of coated Heat cure PMMA and CAD/CAM PMMA resin sample was found to be 248.1 CFU/ml and 80.800CFU/ml respectively. On comparative evaluation of the mean microbial adhesion of streptococcus mutans to uncoated (Group IA) and coated (Group IB) Heat cure PMMA resin samples, the uncoated Heat cure PMMA resin samples exhibited higher microbial adhesion than the coated Heat cure PMMA resin samples which has high statistical significance ($P < 0.001$). On comparative evaluation of the mean microbial adhesion of streptococcus mutans to uncoated (Group IIA) and coated (Group IIB) CAD/CAM PMMA resin samples, the uncoated CAD/CAM PMMA resin samples exhibited higher microbial adhesion than the coated CAD/CAM PMMA resin samples which has high statistical significance ($P < 0.001$). On comparative evaluation of the mean microbial adhesion of streptococcus mutans to uncoated (Group IA) Heat cure PMMA resin samples and uncoated (Group IIA), CAD/CAM PMMA resin samples, the uncoated Heat cure PMMA resin samples exhibited higher microbial adhesion than the CAD/CAM PMMA resin samples which has high statistical significance ($P < 0.001$).

On comparative evaluation of the mean microbial adhesion of streptococcus mutans to coated (Group IB) Heat cure PMMA resin samples and coated (Group IIB) CAD/CAM PMMA resin samples, the coated Heat cure PMMA resin samples exhibited higher microbial adhesion than the CAD/CAM PMMA resin samples which has high statistical significance ($P < 0.001$).

Discussion

Provisional or interim fixed dental restorations are commonly used in prosthodontics procedures usually between tooth preparation and placement of the definitive restoration. Fabricating an ideal provisional restoration is very important to maintain periodontal health, to protect the pulp, to minimize the migration or supra-eruption of abutments and to assess the form and function of the definitive prosthesis and for prosthetically guided tissue healing in order to achieve an acceptable emergence profile [13].

Major limitations of conventional PMMA provisionals such as the presence of residual monomer content (self-cure - up to 5% and Heat cure 0.2% to 0.4%), poor mechanical and surface properties, dimensional instability, polymerization shrinkage and susceptibility to fracture have prompted researchers to seek for newer materials [13]. Computer aided design and computer aided manufacturing (CAD/CAM) PMMA resins have been gaining popularity over the last decade and have reduced several obstacles towards making of provisional restorations. Despite the upper hand in the presence of superior surface properties compared to acrylic PMMA provisionals, CAD/CAM restorations are milled with rotary instruments coated with diamond abrasive particles of varying grit size and such rotary instruments create a rather high initial surface roughness on the restoration.

This high, initial surface roughness may lead to increased wear of adjacent teeth, can also affect staining, patient comfort and esthetics. It also causes plaque accumulation and bacterial adhesion around irregularities of tooth surface and gingival margin, leading to the risk of gingivitis and/or secondary caries [14,15]. Bollen., *et al.* [7]. reported that among the microbes causing plaque accumulation on long term provisional restorations, streptococcal species are predominant primary colonizers on tooth enamel and represent 42% to 48% of the microbial flora of denture plaque. Surface roughness provides niches in which microorganisms are protected from shear forces and oral hygiene measures, thus providing the entrapped microbial cells time to attach irreversibly to a surface. The resultant increase in microbial numbers immobilized at the surface has been shown to advance the maturation rate of plaque, which in turn may lead to an increase in the prevalence of dental caries, gingival and periodontal diseases, and denture- induced stomatitis in the adjacent oral tissues [16].

Hence it is of clinical importance that patients need to have a smooth surface restoration to deter the formation of biofilm. The initial high surface roughness of the provisional restorations can be reduced through finishing and polishing [17]. Several authors including Glaucio serra., *et al.* [18], Al-Rifaiy., *et al.* [19], Al-Kheraif., *et al.* [20], Pesun., *et al*. [21]. have reported on the importance of finishing and polishing of acrylic resins. Berger., *et al*. [15]. in 2006 reported a study showing a statistically significant difference in surface roughness between different acrylic resin materials before and after polishing. Here, conventional polishing resulted in greatest decrease in surface roughness [15]). Rizzante., *et al*. [22]. reported that finishing and polishing greatly influences the surface topography of restorations. However conventional finishing and polishing do not provide a completely smooth surface, the use of resins as surface sealant agents to coat the dental prosthetic materials can fill microstructural defects through the capillary effect, thereby improving surface properties.

Several authors Sahin O., *et al*. [6], Dede Do., *et al*. [23], Azuma A., *et al*. [13], Albin Ameer., *et al*. [3], Perez Davidi., *et al*. [21], Kamonwanon P., *et al*. [34], Leglerdphol., *et al*. [28], Fathi HM [29], Santos M., *et al*. [30], Catelan A., *et al*. [31], Bagis B., *et al*. [32], Perez., *et al*. [33], Ali A., *et al*. [34], and Dickinson GL., *et al*. [35] have reported on the use of surface sealant agents as protective coating on acrylic denture base materials. However, studies evaluating the effect of surface sealants on the surface roughness of CAD/CAM PMMA provisional restorative materials are lacking. Also, studies evaluating the effect of surface sealant agents on reducing quantitative microbial adhesion to long term provisional are very few.

In the present study, a protective surface sealant agent, Nanocoat was used to modify the surface topography of two different PMMA interim materials (Heat cure PMMA and CAD/CAM PMMA) in an attempt to reduce the surface roughness and streptococcus mutans adhesion to long term provisional fixed implant prosthesis [24,25].

The present study reveals a decrease in the surface roughness of the Heat cure PMMA and CAD/CAM PMMA resin samples after coating with the surface sealant. This can be attributed to the fact that the surface sealant fills the micro fissures and micro defects that form after finishing/polishing of the material. Similar results have been reported by Sahin O., *et al*. [6] in 2016, on comparison between coated Heat cure PMMA resin samples and coated CAD/CAM PMMA resin samples, the surface roughness had decreased more in the CAD/CAM PMMA resin samples. However, in the current study the decrease in the surface roughness after application of the sealant was found to be statistically insignificant. The results were in accordance with another study conducted by Sahin., *et al*. [20] in 2016 wherein the effect of different surface sealants on the surface roughness and color stability of denture base materials was evaluated. Another study conducted by Fathi HM., *et al*. [28] investigated the effect of three different nanocryl coating agents on the surface roughness and thickness of heat cure PMMA resin after simulating tooth brushing with three different dentifrices. The results were that the nanocryl sealants did not demonstrate any significant improvement in reducing the surface roughness.

Further in the study the application of the sealant revealed a decrease in the mean microbial adhesion in both Heat cure PMMA and CAD/CAM PMMA resin samples. Statistically there was a high significant difference within uncoated and coated heat cure PMMA resins ($P<0.001$) and within uncoated and coated CAD/CAM PMMA resin ($P < 0.001$). On comparing between the coated Heat cure PMMA and coated CAD/CAM PMMA resins, it was shown that coated CAD/CAM PMMA resin had a higher resistance to microbial adhesion and the difference was statistically highly significant ($P < 0.001$). The result concurred that the application of the sealant did help in reducing the microbial colonization of streptococcus mutans. The bacterial adhesion was noticed more in Heat cure PMMA resin samples in comparison to CAD/CAM PMMA samples. This can be attributed to the fact stated by Al–Diwari., *et al*. [3] that CAD/CAM material are milled from pre polymerised PMMA billets that are polymerised under high temperature and pressure. Hence, they are less porous and less likely to harbor virulent microorganisms [3] The results were also in accordance with a study conducted by Michael Perez Daividi., *et al*. [36] in 2007 wherein the effect of liquid-polish coating (Biscover, Bisco dental) on *in vivo* biofilm accumulation on various PMMA provisional restorations was evaluated and the study revealed that the liquid polish coatings significantly reduced early biofilm formation.

Studies on the effect of sealants on plaque adhesion or microbial adhesion have been conducted in the past on denture base resin materials and conventional provisional restorative materials, but few are present on the effect of sealants on newer materials like CAD/CAM provisional implant restorations.

The null hypothesis adopted for surface roughness was partially rejected because there was a difference in surface roughness between Heat cure PMMA and CAD/CAM PMMA samples though it was statistically insignificant ($P > 0.05$). However, null hypothesis for microbial adhesion was rejected since there was significant decrease in adhesion after coatings on both groups ($P < 0.001$).

The present study had certain limitations. The effect of Nanocoat application modifying the surface wettability of provisional resin needs to be evaluated. Surface hardness, wear resistance, color stability after the application of surface sealant needs to be evaluated. Mechanical properties such as flexural strength and fracture resistance following the application of Nanocoat must be studied. Salivary protein adsorption assay can be carried out since adherence of oral bacteria to various surfaces is mediated by adsorption of salivary proteins. Quantitative microbial adhesion measurement of other early colonizing bacteria and at various time intervals needs to be evaluated. The longevity of coating needs to be evaluated.

Conclusions

There is a significant decrease in microbial adhesion of CAD/CAM PMMA resin than Heat cure PMMA acrylic resin following addition of a surface sealant-Nanocoat.

Clinical Significance

Clinical use of CAD/CAM PMMA resin with surface sealant is highly recommended for long term fixed implant provisional restorations.

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Citation: Divya Krishnan*., et al.* "Comparative Evaluation of The Effect of Surface Sealant on the Surface Topography and Microbial Adhesion of Long-Term Provisional Fixed Implant Prosthesis- *Invitro* Study". *Acta Scientific Dental Sciences* 7.2 (2023): 164-177.