COMPARATIVE EVALUATION OF THREE SURFACE SEALANTS COATED ON COMPOSITE RESIN AGAINST STREPTOCOCCUS MUTANS ADHESION – AN INVITRO STUDY

A Dissertation submitted
in partial fulfillment of the requirements for the degree of

MASTER OF DENTAL SURGERY

BRANCH – IV

CONSERVATIVE DENTISTRY AND ENDODONTICS

THE TAMILNADU DR. MGR MEDICAL UNIVERSITY
CHENNAI – 600 032
2017-2020

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation titled " **COMPARATIVE EVALUATION OF THREE SURFACE SEALANTS COATED ON COMPOSITE RESIN AGAINST STREPTOCOCCUS MUTANS ADHESION – AN INVITRO STUDY"** is a bonafide and genuine research work carried out by me under the guidance of **Dr.P.SHAKUNTHALA, Associate Professor,** Department Of Conservative Dentistry and Endodontics, TamilNadu Government Dental College and Hospital, Chennai -600003.

F.AMALA AARTHI

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This is to certify that **Dr. F.Amala Aarthi**, Post Graduate student (2017-2020) in the Department Of Conservative Dentistry and Endodontics, Tamil Nadu Government Dental College and Hospital, Chennai-600003 has done this dissertation titled **" COMPARATIVE EVALUATION OF THREE SURFACE SEALANTS COATED ON COMPOSITE RESIN AGAINST STREPTOCOCCUS MUTANS ADHESION – AN INVITRO STUDY "** , under my direct guidance and supervision in partial fulfillment of the regulations laid down by the TamilNadu Dr.M.G.R Medical University Chennai- 600032, for M.D.S., Conservative Dentistry and Endodontics (Branch IV) Degree Examination .

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DECLARATION

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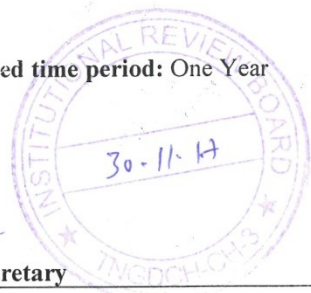
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And

Mrs. Dr.P. Shakunthala aged 48 years working as **Associate Professor** in Department of Conservative Dentistry & Endodontics at the college, having residence address No.17/23, Jeevanantham II Cross Street, Avadi. Chennai -71.

And

Mrs. Dr. F.AMALA AARTHI aged 34 years currently studying as **Post Graduate student** in Department of Conservative Dentistry & Endodontics, Tamil Nadu Government Dental College and Hospital, Chennai 3 (herein after referred to as the PG student and co - investigator).

Whereas the PG student as part of her curriculum undertakes to research on " **COMPARATIVE EVALUATION   OF THREE SURFACE SEALANTS COATED ON COMPOSITE RESIN AGAINST STREPTOCOCCUS MUTANS ADHESION – AN INVITRO STUDY** " for which purpose the Principal Investigator shall act as principal investigator and the college shall provide the requisite infrastructure based on availability and also provide facility to the PG student as to the extent possible as a Co-investigator.

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ABSTRACT

AIM AND OBJECTIVE:

1. To evaluate the initial adhesion of clinical and MTCC strains of Streptococcus mutans isolated from the dental composite resins finished with **Caulk one –step micropolisher**; dental composite resins coated with **three commercially available surface sealants**, and dental composite resins finished with **Mylar strip**

2. To correlate the above finding to the surface characteristics of the coating material.

MATERIALS AND METHODS

110 resin composite discs made of filtek Z 250 (8 mm x 1 mm) and where randomly divided into five groups.

GROUP 1 - Discs finished with Mylar Strip alone (n = 22).

GROUP 2 – Discs finished and polished with Caulk Micropolisher (n = 22).

GROUP 3 – Permaseal applied over finished and polished Discs (n = 22).

GROUP 4 - Optiguard applied over finished and polished Discs (n = 22).

GROUP 5 – G Coat plus applied over finished and polished Discs (n = 22).

Two representative discs from each group were taken for SEM and profilometric analysis respectively. The Streptococcus mutans adhesion to the respective groups where done on Clinical strain and MTCC strains at 6 hour and 18 hour incubation by spread plate methods.

RESULTS

PS and OG reduced the surface roughness of composite resin discs finished and polished with Caulk Micropolisher. The reduction in the Streptococcus mutans adhesion to composite resins finished and polished with Caulk Micropolisher in PS and OG were comparable or even lesser than the control Mylar Group. The Streptococcus mutans adhesion increased at 18 hours but not statistically significant. The Clinical and Standard strains of Streptococcus mutans performed similarly except for Standard strains at 18 hours.

CONCLUSION

The results of the present study encourages the use of surface sealants PS and OG after finishing and polishing procedure of composite resin to improve surface smoothness and to decrease the initial Streptococcus mutans adhesion.

ABBREVIATIONS USED

PS	Permaseal
OG	Optiguard
GIC	Glass Ionomer Cement
RMGIC	Resin Modified Glass Ionomer Cement
SEM	Scanning Electron Microscopy
AFM	Atomic Force Microscopy
TEM	Transmission Electron Microscopy
MTCC	Microbial Type Culture Collection
ATCC	American Type Culture Collection
CLSI	Clinical and Laboratory Standards Institute

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INTRODUCTION

Finishing and polishing is one of the most important steps for the success of a composite restoration. A well finished and polished composite restoration has great esthetic results, helps maintain good oral hygiene and improves long-term results[32,50]. An improperly done finishing and polishing has rough surface which in turn decrease the gloss and has an unesthetic appearance as well as increase the chances for bacterial biofilm accumulation. This eventually leads to increased risk of caries and periodontal inflammation[11].

Increased surface roughness facilitates the colonization of cariogenic microflora and cariogenic biofilm formation leading to secondary caries. Restoration surface biodegradation, secondary caries, and periodontal inflammation are the leading causes for replacement of restoration. Secondary caries was the reason for failure in 23% after 10 years follow –up in teeth restored with composite restoration. A significant higher proportion of Streptococcus mutans at the cavity margins of the resin composite restorations were observed in the study[56].

Other studies discussed that there is a correlation between marginal deterioration and secondary caries[53,61]. Because of the high technical sensitivity of posterior resin composites, and it was proposed to reduce the marginal disintegration by technical means. Histological studies describe the secondary caries lesion in two parts: an outer lesion formed on the surface of the tooth adjacent to the restoration and a wall lesion which is formed due to leakage between the restoration and the tooth[39]. The adhesion of early colonizers such as streptococcus is the main critical step in biofilm formation[26,51]. Streptococcus mutans is responsible for initiation and progression of caries[12]. These microbes adhere to surface of the composite restoration and the surrounding tooth

surface through the initial physiochemical interaction. This interaction between the bacteria and the restoration principally depends initially on physical forces such as Brownian motion, Vander Waals attraction forces and hydrophobic interactions. From the bacterial aspect, the presence of bacterial capsule, fimbriae, ability to form biofilm, environmental factors, bacterial hydrophobicity, bacterial surface charge are the factors determining bacterial adhesion. From the material aspect, the bacterial adhesion depends on surface roughness, surface configuration, surface hydrophobicity[69].Previous studies on Streptococcus mutans adhesion have used the standard strains which may have lost few of the inherent properties such as the production of slime layer. Though it is suggested that the presence of the pellicle layer apparently masks any difference among materials, with regard to surface properties and biocompatibility, bacterial interaction bound to occur overtime[28]. The increase of Streptococcus mutans adhesion on the composites is appreciably more in first 24 hours later stabilizes according to the environment changes such as salivary flow. Studies suggest that a roughness value of 0.2μm is an optimal surface finish where there is least bacterial adhesion[11].The need for a smooth, glossy surface of the composite restoration is essential. Many methods are available to improve polishing and finishing of composites. Regardless of the restorative material, Mylar polishing system revealed the smoothest surface and the lowest adhesion of S. mutans as compared to Caulk one step and Sof-Lex multi-step polishing systems[63]. One step polishing system performed better than the multi-step system by enhancing the surface smoothness[67].

Application of surface sealants over the finished composite restoration is one such means which was considered as an alternative over the use of polishing discs[29]. Surface sealants are polymerizable mainly containing unfilled resins and other light molecular weight monomers[42,58]. The surface sealants act by penetrating the structural

microdefects on the surface of the finished and polished restoration accentuates surface smoothness making it less compliant for biofilm formation[18,21,40]. Upon polymerization, the integrity of the previously weakened surface is substantially enhanced[35].

The ideal properties of the surface sealants are low viscosity, high flow rate and high wettability properties[10,62]. The surface roughness, hydrophobicity and surface free energy of dental restoration are the factors affecting the adhesion of oral bacteria to the surface[38]. Studies suggest that the surface sealants maintain surface smoothness, improve wear resistance, and ensure good marginal sealing of the composite restoration[35]. Thus, the use of certain surface sealants may be warranted in the clinical scenario to improve surface characteristics. Surface sealants used in composite restorations undergo hydrolytic degradation[10]. Though several studies on surface sealants on its role in wear resistance, marginal integrity, aesthetics has been investigated[35,49], correlation of surface smoothness achieved by surface sealants to the Streptococcus mutans adhesion is dealt scarcely. Initial Streptococcus mutans adhesion on the dental composite coated with surface sealants is less known in the literature[14]. Permaseal, Optiguard and G Coat Plus were the three commercially available sealants chosen in the study. Permaseal and Optiguard are two unfilled surface sealants. G Coat plus is a nanofilled surface sealant which is mainly used as a coating agent for GIC, RMGIC. Hence, in the present study, the adhesion of clinical and standard strains of Streptococcus mutans to composites coated with three surface sealants at 6 hour and 18 hour incubation were to be studied to evaluate factors associated.

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AIM

To evaluate the adhesion of clinical and standard strains of Streptococcus mutans on the dental composite resins coated with three commercially available surface sealants and correlate the above finding to the surface characteristics of the coating material.

OBJECTIVE

1. To compare the number of colony forming units of clinical and MTCC strains of Streptococcus mutans isolated from the dental composite resins finished with **Caulk one –step micropolisher**; dental composite resins coated with **three commercially available surface sealants**, and dental composite resins finished with **Mylar** strip.

2. To evaluate the effect of the surface characteristics of each group using Scanning Electron Microscopy and Profilometric analysis.

3. To compare the adhesion of Streptococcus mutans to composite resins at two incubation period, 6 hours and 18 hrs.

REVIEW OF LITERATURE

SURFACE SEALANTS PERFORMANCE IN IN VITRO STUDIES

Lambrechts P, Van herle G [40] (1982) investigated the retention of glazes on polished composite surfaces. Sections of three differently resin-coated composites have been prepared in vitro and examined by scanning electron microscopy (SEM). All samples showed an interrupted interstice between glaze and composite. The results suggest the use of glazing materials seems unsuitable for a permanent gloss on composite surfaces.

Dickinson[23] et al (1990) evaluated the effect of surface penetrating sealant on the wear rate of posterior composite resins. The experimental surface -penetrating sealant prepared for this study. The application of the surface sealant to one-half of the restorations was done on a random basis. The mean loss of materials for the unsealed restorations using the calibrated casts was 26.4 µm, but only 14.1 µm for the surface sealant at the end of one year. They suggested the application of a low-viscosity surface-penetrating sealant enhances the wear resistance of posterior composite resins.

Shinkai K[61] et al (1994) investigated the effects of sealants on the wear of various luting agents in conjunction with composite resin inlays. The specimens were subjected to a three-body wear test for 400,000 cycles. The wear values of both composite resin and luting agents were determined by profilometric tracings to the nearest 2 µm. There were no statistically significant differences between the treated and untreated groups as it related to the wear of the luting agents. The wear values of composite resin inlays treated with the sealant, however, were significantly lower than those without treatment, regardless of the type of luting agent used for cementation.

Kawai[35] et al (1995) investigated whether surface-penetrating sealant designed to increase the wear resistance and marginal integrity of posterior composites. The surface-penetrating sealant (Fortify, BISCO) was an unfilled resin which contained hydrofurfural to increase its wetting characteristics. The in vitro wear testing was carried out. After finishing the composite restoration, its surface and surrounding enamel were subjected to a 10 second acid etch followed by washing and drying. The surface-penetrating sealant was applied to half of the specimens of each composite type. They inferred that the surface penetrating sealants having low viscosity and high wettability, wets the internal surfaces of microstructural defects as small a 1-2 μm in width. Upon polymerization, the integrity of the previously weakened surface is substantially enhanced.

Miyazaki[49] et al (1996) investigated the change in flexural strength and fracture toughness of light-cured glass ionomer cements after long-term immersion in water, and to investigate the effect of surface coatings on their properties. Specimens were subjected to the 3 point bending at 0.5 mm/min after storage in 37°C water for the periods of 1 h, 24 h, 1 wk, 1 month, and 6 months. The surface protection of the resin-modified glass ionomer cement has some effect on the mechanical properties during early setting reactions, and it is desirable that the cement should be protected from direct water contact for at least 1 h after cement mixing.

Pereira[53] et al (2006) tested the ability of commercially available composite surface sealers to penetrate and seal a controlled gap formed in all-enamel margin, Class V in vitro resin composite restorations in human bicuspids was examined. A fluorescent red dye (Rhodamine B) was incorporated to a variety of commercially available composite surface sealers. The teeth were restored with microfilled composite, finished, polished,

and sealed. The teeth were thermocycled and then immersed in an aqueous solution of a green fluorescent dye (Dextran-Fluorescein) to evaluate the sealing ability and penetration of surface sealers into the controlled gaps formed at unbonded margins and later imaged with confocal microscopy. The results showed that all sealers leaked, and use of a dentin-bonding agent to seal the gap performed better than the commercial sealing products. The application of re-bonding technique could be considered appropriate in order to reduce microleakage in resin composite restorations and this effect might be material-dependent. The application of Optiguard on the microhybrid and packable composite restorations significantly provided better sealing among the surface sealants at the cervical margins.

Cilli [14] et al (2009) evaluated the influence of two surface sealants (BisCover/Single Bond) and three application techniques (unsealed/conventional/co-polymerization) on the roughness of two composites (Filtek Z250/Z350) after the toothbrushing test. Each sample were subjected to three random roughness readings at baseline, after 100,000 (intermediate), and 200,000 (final) toothbrushing strokes. At any brushing stage, sealed composites presented superior performance when compared with unsealed composites.

Biazuz [10] et al (2015) studied the correlation between water sorption, solubility and surface roughness of commercial surface sealants for restorations. Five disc-shaped specimens (15 mm diameter X 1 mm high) were made from the surface sealants Natural Glaze (DFL) and Permaseal (Ultradent) and were light cured according to the manufacturer's instructions. The specimens were finished with 1500-grit SiC paper. Water sorption (WS) and solubility (SL) were assessed as recommended by the ISO 4049/2000 and were expressed in $\mu g/mm3$. Surface roughness was evaluated before and after WS and SL, and was expressed in μm as R1 (before WS and SL) and R2 (after

WS and SL). It was obtained from three parallel measurements along a 4mm length. Data were analyzed using T-test and paired T-test (α=0.05). Surface sealants used in composite restorations undergo hydrolytic degradation; however, this degradation seems not to interfere on surface roughness of these materials.

CLINICAL STUDIES ON SURFACE SEALANTS

Dickinson[24] et al (1993) did a clinical trial on surface sealants. 13 patients with a total number of 62 class I and class II cavity were selected, composite filling were done. Surface penetrating sealants were applied. Characteristics evaluated at each recall included – Color matching ability, interfacial staining, secondary caries, wear, marginal integrity, surface texture. Direct clinical evaluations were conducted at baseline, six months, 1, 2, 3 and 5 years. No secondary caries were detected at any recall. The mean wear of the sealed restorations were about half of those that did not receive additional treatment. At baseline 100 percent of the sealed and 97 percent of the unsealed samples were rated alfa. One unsealed sample was rated bravo. Five years after baseline, 92 percent of the sealed restorations were rated alfa—while only 67 percent of the unsealed restorations retained that rating. No difference were observed in surface texture between the groups at five years. The surface penetrating sealant's effectiveness could be enhanced if the material was reapplied twice a year. Such a recommendation can be based upon the probability that the adjacent material probably is lost to wear over a two year period.

Tekçe[65] et al (2018) evaluated the clinical performance of one-step self-etch adhesives over two years with and without the application of a surface sealant. Each patient received four Class I restorations, which included a 2-hydroxyethyl methacrylate (HEMA)–containing and HEMA-free one- step self-etch adhesive system with and

with- out surface sealant. The surface sealant application was not effective with regard to changes in color matching and surface texture, it improved the marginal adaptation of the dentin adhesive and the marginal discoloration of a HEMA-free adhesive.

STREPTOCOCCUS MUTANS ADHESION

An and Friedman (1998) in their review of the mechanisms of bacterial adhesion to biomaterial surfaces and the factors affecting the adhesion. The process of bacterial adhesion includes an initial physicochemical interaction phase (phase one) and a late molecular and cellular interaction phase (phase two) , which is a complicated process affected by many factors, including the characteristics of the bacteria themselves, the target material surface, and the environmental factors, such as the presence of serum proteins or bactericidal substances.

Banas [7](2004) in his article states that the main virulence factors associated with cariogenicity include adhesion, acidogenicity, and acid tolerance. Each of these properties works coordinately to alter dental plaque ecology. The ecological changes are characterized by increased proportions of S. mutans and other species that are similarly acidogenic and aciduric. The selection for a cariogenic flora increases the magnitude of the drop in pH following the fermentation of available carbohydrate and increases the probability of enamel demineralization. Mei et al (2011) studied the influence of surface roughness on streptococcal adhesion forces to composite resins. Polishing and grinding were applied to obtain smooth (roughness 20 nm), moderately rough (150 nm) and rough (350 nm) surfaces of two orthodontic, light-cured composites. Adhesion forces between Streptococcus sanguinis and Streptococcus mutans on the composite surfaces were measured using atomic force microscopy in absence or presence of a salivary conditioning film. Initial adhesion forces were measured as well adhesion after 120 s of

contact, as longer contact times are known to result in stronger adhesion forces ("bond-strengthening"). Streptococcal adhesion forces after bond-strengthening were significantly stronger than upon initial contact, irrespective of the composite type. Salivary conditioning films significantly decreased the surface roughness of the composites, as well as the streptococcal adhesion forces. Yet, also in the presence of a conditioning film, rougher composite surfaces exerted stronger adhesion forces, irrespective of composite type or bacterial strain.

Beyth N [9] et al (2008) tested the hypothesis that bacteria-composite surface interaction causes changes in surface-topography. Resin composite disks were polymerized between two glass slides. Streptococcus mutans cells were brought in contact with and grown on the disks for 1 day, 1 week or 1 month. The disks were analyzed using atomic force microscopy. One-month-aged composite specimens were assayed for changes in micro-hardness and bacterial outgrowth. S. mutans outgrowth was accelerated following direct contact with the surface of aged composites, with no changes in micro-hardness.

Kantorski KZ[34] et al (2009) evaluated the surface roughness and in vitro adherence of Streptococcus mutans to indirect aesthetic restorative materials that were uncoated with saliva. Restorative materials were evaluated according to material type: (1) microparticulate feldspathic ceramic; (2) leucite-reinforced feldspathic ceramic; (3) microhybrid resin composite and (4) microfilled resin composite. Roughness analysis (Ra, n = 10) were performed using a roughness analyser. Adhesion tests (n = 10) were carried out in 24-well plates; colony-forming units (CFU/mL) were evaluated. The microhybrid and microfilled resin composites were similar and the leucite-reinforced feldspathic ceramic was rougher and presented higher bacterial adherence than the microparticulate feldspathic ceramic.

Lassila [41] et al (2009) analysed the adhesion of Streptococcus mutans (S. mutans) to a short glass fibers reinforced semi-IPN polymer matrix composite resin. The effect of surface roughness on adhesion was also studied. Three direct composite resins (Z250, Grandio and Nulite), resin-modified glass ionomers (Fuji II LC), amalgam (ANA 2000), fiber-reinforced composite (FRC) (everStick and Ribbond), and pre-fabricated ceramic filling insert (Cerana class 1) were tested in this study. Enamel and dentin were used as controls. The specimens (n=3/group) with or without saliva were incubated in a suspension of S. mutans allowing initial adhesion to occur. For the enumeration of cells on the disc surfaces as colony forming units (CFU) the vials with the microbe samples were thoroughly Vortex-treated and after serial dilutions grown anaerobically for 2 days at +37°C on Mitis salivarius agars (Difco) containing bacitracin. Bacterial adhesion was also evaluated by using scanning electron microscopy. Surface roughness (Ra) of the materials was also determined using a surface profilometer. . Composite FC resin and other commercial restorative materials showed similar adhesion of S. mutans, while adhesion to dentin and enamel was significantly higher.

Shemesh[60] et al (2010) analysed the molecular modifications occurring during *in vitro* biofilm development of *Streptococcus mutans* on several different dental surfaces. Using DNA-microarray technology, differentially expressed genes of *S. mutans* were identified, reflecting the physiological state of biofilms formed on the different biomaterials tested. Eight selected genes were further analyzed by real time RT-PCR. To further determine the impact of the tested material surfaces on the physiology of the bacteria, secretion of AI-2 signal by *S. mutans* embedded on those biofilms were tested. The results demonstrate that gene expression of *S. mutans* differs in biofilms formed on tested surfaces, which manifest the physiological state of bacteria influenced by the type of surface material they accumulate onto. Moreover, the stressful

circumstances of adjustment to the surface may persist in the bacteria enhancing intercellular signaling and surface dependent biofilm formation.

Mei[47] et al (2011) studied the influence of surface roughness on streptococcal adhesion forces to composite resins. Polishing and grinding were applied to obtain smooth (roughness 20 nm), moderately rough (150 nm) and rough (350 nm) surfaces of two orthodontic, light-cured composites. Adhesion forces between Streptococcus sanguinis and Streptococcus mutans on the composite surfaces were measured using atomic force microscopy in absence or presence of a salivary conditioning film. Initial adhesion forces were measured as well adhesion after 120 s of contact, as longer contact times are known to result in stronger adhesion forces ("bond-strengthening"). Streptococcal adhesion forces after bond-strengthening were significantly stronger than upon initial contact, irrespective of the composite type. Salivary conditioning films significantly decreased the surface roughness of the composites, as well as the streptococcal adhesion forces. Yet, also in the presence of a conditioning film, rougher composite surfaces exerted stronger adhesion forces, irrespective of composite type or bacterial strain.

Soliman [63] et al (2019) compared the surface roughness and bacterial adhesion to bulk fill resin composites polished with different systems. Filtek Z350 XT (Incremental-fill resin compo- site), Filtek Bulk-fill Posterior (Bulk-fill resin composite), and Tetric N Ceram (Bulk-fill resin composite) were used as resin composites. The polishing systems used in this study were Sof-Lex multi-step, PoGo one step, and Mylar strip. Scanning electron microscope (SEM) was used to examine the surface roughness and adhesion of Streptococcus mutans standard strain to bulk-fill resin composites. Regardless of the restorative material, Mylar polishing system revealed the smoothest surface and the

lowest adhesion of S. mutans as compared to Pogo one step and Sof-Lex multi-step polishing systems.

SURFACE ROUGHNESS, FINISHING AND POLISHING OF COMPOSITE RESINS

Satou[59] et al (1991) studied the adherence of Streptococcus sanguis and S. mutans to seven restoratives in the presence and absence of an artificial salivary pellicle. The physicochemical surface characteristics of the bacteria and of the restoratives were also measured, together with the effect of salivary coating of restoratives. Zeta potential or water contact angle of S. mutans cells and s. sanguis cells was calculated. Zeta potential of uncoated resins showed positive correlation between the no. of adherent S. mutans and S. sanguis cells. The surface hydrophobicity was decreased upon saliva coating, whereas the zeta potential was slightly increased. The number of adherent cells of both S. sanguis and S. mutans to saliva-coated restoratives were decreased after the coating.

Perez[54] et al (2009) evaluated the surface roughness of composite materials after different finishing/polishing protocols. Two nanofilled resin composites, one resin-modified glass ionomer cement and one conventional glass ionomer cement were used. The finishing/polishing methods were divided into five groups: G1 (compression with Mylar matrix), G2 (finishing with diamond burs), G3 (Sof-Lex, 3M Dental Products), G4 (BisCover, BISCO, after diamond burs) and G5 (BisCover after Sof-Lex). The surface roughness was evaluated using a 3-D scanning instrument with two parameters considered (Ra and Rz). The results showed that BisCover (BISCO) was capable of reducing surface roughness and provided polished surfaces for all materials, enhancing smoothness over already polished surfaces (Sof-Lex, 3M Dental Products) and achieving polishing after finishing with diamond burs.

Gonçalves[27] et al (2012) evaluated the surface roughness of restorative composite resins after polishing with aluminum oxide discs and applying an adhesive layer. The following composite resins were used: Filtek Z250 (hybrid) and Filtek Supreme XT (nanofilled) G1-Z250/CO – control, did not receive any treatment; G2-Z250/SL – the specimens underwent finishing and polishing with Sof-Lex discs; G3- Z250/ADE, application of an adhesive layer on the top of the specimen and light curing for 20 seconds. Groups G4, G5 and G6 followed the same treatment sequence, but using Filtek Supreme XT. It was concluded that the SofLex discs performed better for the surface treatment of the composites resins tested, producing similar values of surface roughness for both composites.

Loupes [42]et al (2012) subjected the specimens to simulated toothbrushing using a 200 g load and 250 strokes/min to simulate 1 week, 1, 3 and 6 months and 1 and 3 years in the mouth, considering 10,000 cycles equivalent to 1 year of toothbrushing. Oral-B was used. The tested surface-penetrating sealants do not seem able to improve the surface roughness performance of a nanofiller composite resin, highlighting the potential of the nanofill technology in obtaining an adequate surface roughness without the use of any sealant.

Zimmerli[70] et al (2012) carried out a study on the performance of surface sealants and conventional polishing after ageing procedures. Eighty circular composite restorations were performed on extracted human molars. After standardised roughening, the restorations were either sealed with one of three surface sealants - Lasting Touch (LT), BisCover LV (BC), G-Coat Plus (GP) or a dentin adhesive Heliobond (HB)) or were manually polished with silicon polishers (MP) (n=16). The average roughness (Ra) and colourimetric parameters (CP) (L*a*b*) were evaluated. The specimens underwent an

artificial ageing process by thermocycling, staining (coffee) and abrasive (toothbrushing) procedures. SEM evaluation showed clear alterations after ageing in all coating groups. Surface sealants and dentin adhesives have the potential to reduce surface roughness but tend to debond over time. Surface sealants can only be recommended for polishing provisional restorations.

Dede and Omur[20] (2015) evaluated the effect of sealant agents on the surface of various denture tooth materials. Disk-shaped specimens were prepared for each type of denture tooth material. The specimens were assigned to 4 groups, according to the surface treatment, 3 surface sealant groups and a conventional laboratory polishing technique (control group). Palaseal and Optiglaze sealant agents provided smoother and more color-stable denture tooth surfaces than the conventional polishing technique.

Ansuj et al (2016) [4] evaluated the surface roughness of nanofilled composite resin submitted to different smoothing and finishing techniques. specimens were made with the Z350 XT composite resin (3M ESPE) and then divided into five study groups according to the smoothing and finishing method applied – G1 polyester strip; G2 composite spatula; G3 brush cleaned with absolute alcohol and dried; G4 brush cleaned with absolute alcohol, dried and moistened with Single Bond (3M ESPE); and G5 brush cleaned with absolute alcohol, dried and moistened with Natural Glaze surface sealant (DFL). The surface roughness of the specimens was measured using a profilometer. The lowest surface roughness was observed in control group. Natural Glaze group had lower surface roughness values compared to the other test groups, and presented values similar to those of the control group.

Dede[19] et al (2016) studied the effect of sealant agents on the surface roughness and color stability of 4 nanohybrid composite resin material and divided into 4 surface

treatment groups: 1 conventional polishing (control) and 3 different sealant agent. Scanning electron microscope images revealed rougher surfaces with conventionally polished groups compared with test groups. The surface treatment technique significantly affected the Ra values of the composite resins tested .

SURFACE ROUGHNESS ANALYSIS

Hannig[28] et al (1999) did an in vivo study describing the ultrastructural pattern of early plaque formation on various dental materials. Test pieces of amalgam, casting alloys, titanium, ceramics, glass polyalkenoate cement, composite resins, unfilled resins, and bovine enamel were attached to the buccal and lingual surfaces of the upper first molars in 3 subjects using removable intraoral splints. Specimens were exposed to the oral environment over a period of 24 h and subsequently processed for transmission electron microscopic evaluation. Electron microscopic observations revealed distinct differences in early biofilm formation between buccally and lingually mounted test pieces. These findings may be ascribed to the presence of the pellicle layer, which apparently masks any difference among materials, with regard to surface properties and biocompatibility.

Kakaboura A[33] at al (2007) compared various roughness and topography measurement methods to characterize the surface quality in several types of resin composites. The materials evaluated were of three categories: i) hybrid: TPH Spectrum; ii) reinforced microfill: Micronew and iii) microhybrid: Synergy Duo, Esthet-X, Point.4 and Palfique Estelite polished with Soflex discs. Atomic Force Microscopy (AFM) gave 3-D images and micro-roughness (Ra) of Group 2. Surface optical gloss at 60° was determined for Group 3. Specimens of each material were also studied by scanning electron microscopy. Moreover, the AFM method showed higher capability to distinguish

surface roughness compared with the 2-D profilometry and to reveal more detailed definition of surface texture than the examination under SEM.

Magni et al (2008)[44] evaluated the marginal integrity of class V restorations through an SEM observation and a microleakage test. A coating agent was applied on five polished and five non-polished restorations of each group. No coating was used on the remaining specimens. No gaps were detected after coating. The restorative materials did not differ in interfacial gaps. Immediate polishing increased the gaps of uncoated restorations ($p < 0.05$). The microleakage decreased with coating, except for occlusal wall of polished flowable composite restorations.

Tajima[64] **et al (2009)** studied the effects of coating root dentin surfaces with adhesives and the prevention of root dentin demineralization. Root dentin surface was ground with #600 SiC, and then either a single coat of Clearfil SE Bond (SE), Clearfil Tri- S Bond (TS), G-Bond (GB), Hybrid Bond (HB-1), or two coats of HB (HB-2) were applied. Specimens were immersed in an artificial demineralizing solution, then sectioned through the center of the root and polished. Thickness of the coating layer and depth of the demineralized dentin layer were observed under a confocal laser scanning microscope (CLSM). Nanohardness values of the coating layer and underlying dentin were measured using a nanoindentation tester. All obtained data were statistically analyzed. Dentin demineralization was not observed in the surface coating groups with the exception of HB-1, and nanohardness of the underlying dentin was comparable to that of normal dentin. Based on the results obtained, it seemed that coating root dentin surfaces with an adhesive material is a promising good practice to prevent demineralization.

ARMAMENTARIUM

For Composite Disc Preparation (FIG 1,2)

❖ Filtek Z250 (3M ESPE, St. Paul, MN, USA, A2 shade)

❖ Teflon cylindrical molds (8mm x 1mm)

❖ Teflon coated instrument

❖ Glass Slides

❖ Mylar strips (0.002 G)

❖ Light curing unit (Ivoclar Bluephase N MC)

❖ Caulk micropolisher (Dentsply)

❖ Contraangle micromotor hand piece (NSK, Japan)

❖ Microtip brushes

❖ Permaseal (Ultradent Products Inc, USA)

❖ Optiguard (Kerr, USA)

❖ G Coat plus(GC) Japan

For Microbiological Analysis (FIG 3,4,5,6)

❖ Laminar air flow (Accuma Max)

❖ Streptococcus mutans MTCC 497

❖ Clinical strain of Streptococcus mutans

❖ Mutans Sanguis Agar (HiMedia Pvt Ltd, India)

❖ Micropipette (Tarsons, India)

❖ Sterile disposable petriplates (HiMedia laboratories Pvt Ltd, India)

❖ Sterile Brain Heart Infusion Broth (HiMedia laboratories Pvt Ltd, India)

❖ 0.5 Mcfarland standard (HiMedia laboratories Pvt Ltd, India)

- ❖ 24 well tissue culture plate (Bio Globus)

- ❖ Eppendorf tubes (Tarsons, India)

- ❖ Sterile microtips (Tarsons, India)

- ❖ Digital colony counter (Deep vision, India)

For surface characteristics assessment (FIG 7,8)

- ❖ Scanning Electron Microscopy (TESCAN VEGA3 SBU)

- ❖ Surface Profilometer (Taylor Hobson)

Fig-1: Materials used in the preparation of Composite Resin discs along with three Surface Sealants
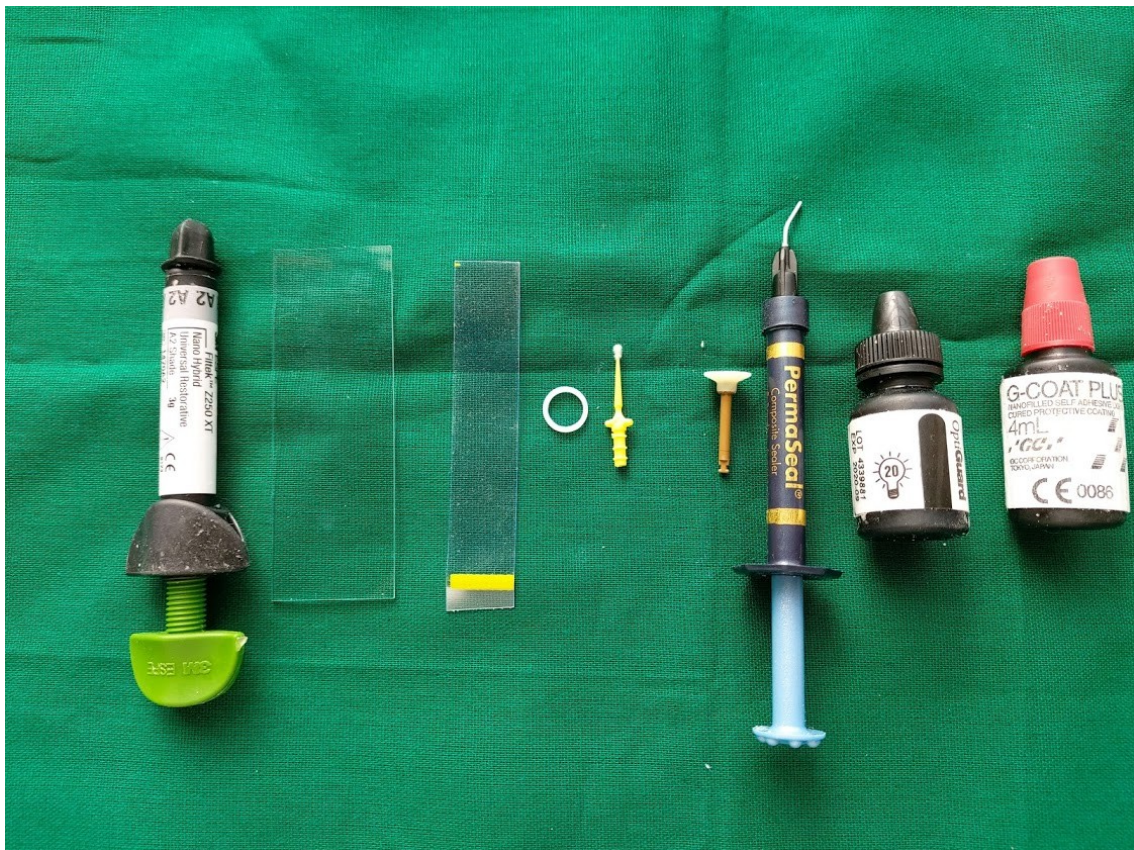
Fig-2: Light curing unit (Ivoclar Bluephase N MC)

Fig-3: Laminar air flow (Accuma Max)

Fig-4: Sterile Mutans sanguis agar plate   **Fig -5: 24 well tissue culture plate**
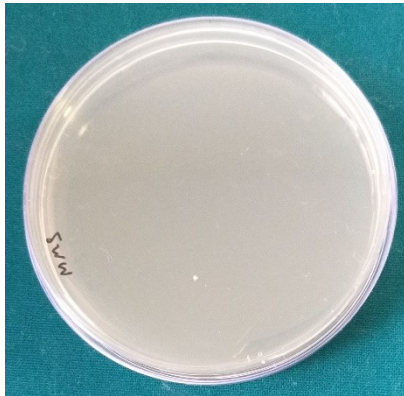
Fig-6: Digital Colony Counter

Fig-7: SEM (TESCAN VEGA3 SBU)

Fig-8: Surface Profilometer (TAYLOR HOBSON)

TABLE 1 - LIST OF MATERIALS USED

Material	Manufacturer	Description	Application method	Composition
Filtek Z250	3M ESPE, St. Paul, MN, USA	Microhybrid composite resin	Light cure for 20 seconds	Bis-GMA, UDMA, Bis-EMA, TEGDMA, zirconia, silica
PermaSeal (PS)	Ultradent Product Inc.	Unfilled surface sealant	Apply a thin layer for 5 sec, air thin, and light cure for 20 seconds	Bis-GMA, TEGDMA, DMAEMA
OptiGuard (OG)	Kerr Corp., Orange, CA, USA	Unfilled surface sealant	Apply in a thin layer, blow lightly, and light cure for 20 seconds	TEGDMA, 2,20-ethylenedioxydiethyl dimethacrylate, photoinitiators, stabilizers
G Coat Plus (GC)	GC, Japan	Nanofilled surface sealants	Apply a thin layer, air-thin by blowing a gentle stream of air, and light cure for 10 seconds	Methyl methacrylate, Methacryloyloxydecyl dihydrogen phosphate, Silanated colloidal SiO_2.

METHODOLOGY

SPECIMEN PREPARATION

Teflon cylindrical molds of dimension (8 mm X 1 mm) were custom made for the study. The mold was placed over the mylar strip on a glass slide. Filtek Z 250 was filled in the mold space using Teflon coated instrument and another mylar strip and a glass slide was placed over it. Glass slide was pressed to obtain even surfaces on both the sides and excess material was squeezed out fig 9. The tip of the curing unit (IvoclarBluephase N MC) was placed close to the glass slide and irradiated for 20 seconds, the same procedure was repeated on the other side fig 10. 110 specimens were prepared and placed atroom temperature in a dry place for 24 hours. The specimens were later separated from the mold and randomly divided into 5 groups, (n = 22 in each group) based on the finishing and polishing methods fig 15.

GROUP 1 - Discs finished with Mylar Strip alone (n = 22).

GROUP 2 – Discs finished and polished with Caulk Micropolisher (n = 22).

GROUP 3 – Permaseal applied over finished and polished Discs (n = 22).

GROUP 4 - Optiguard applied over finished and polished Discs (n = 22).

GROUP 5 – G Coat plus applied over finished and polished Discs (n = 22).

G1(Mylar), the surfaces of the disc were finished by the mylar strip alone as above mentioned procedure. In G2 (Caulk), both the surfaces were finished and polished with Caulkmicropolisher, the flat portion of the polishing disc was used as per the product description fig11. The discs were rinsed and air thinned. In the surface sealants group G3

(PS), G4(OG), G5(G Coat Plus), the discs are polished with micropolisher, rinsed and dried gently. Following the procedure, a thin coat of the specified surface sealants corresponding to each group was applied to the surfaces precisely and airthinned fig 12-14. The coated surfaces were roofed with the Mylar strip and glass slide. Subsequently, it was light cured by positioning the light guide tip of curing light (IvoclarBluephase N MC) across the glass slide and same procedure repeated on the other side of the disc. The surface sealants were applied according to the manufacturer's recommendations (Table 1). After storing for 24 hours at room temperature, the mylar strips were removed from the specimens.

Surface characterization

One depictive specimen of each group was sent for thescanning electron microscope (SEM). Specimens were mounted in aluminium stubs, sputter-coated with platinum, and analysed using a SEM TESCAN VEGA3 SBU. The images were obtained in 500x magnification. Another depictive specimen from each group was analysed using a previously calibrated profilometer(Taylor Hobson) at a stylus speed of 0.1 mm/sec, a cutoff of 0.8 mm, and a range of 600 μm. The surface roughness value Ra of each specimen was the average of the readings recorded by the stylus.

Bacterial strains used for this study:

I.   Standard strain:

Streptococcus mutans **MTCC 497 (Serogroup C, original Source: carious dentine)**

Freeze-dried culture of the *Streptococcus mutans* MTCC 497 procured from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology & Gene bank (IMTECH), Chandigarh.

II.   Clinical strain:

Stock culture of an oral isolate of *S. mutans* isolated from the unstimulated saliva of a patient with dental caries that had been identified, confirmed and maintained in the Department of Microbiology, SreeBalaji Dental College & Hospital, Chennai was used for the study.

This strain was revived on Mutans sanguis agar and used for the study.

Preparation of Mutans Sanguis Agar:

- **Ingredients**

  Caesin enzymatic hydrosylate   :   15 g

  Yeast extract   :   5 g

  L-cystine   :   0.2 g

  Sodium sulphite   :   0.1 g

  Sodium chloride   :   5 g

  DiSodium phosphate   :   0.8g

  Sodium bicarbonate   :   2 g

  Sodium acetate   :   12 g

  Sucrose   :   50 g

  Agar   :   12 g   (pH 7.3±0.2)

The dehydrated medium Fig 16 was procured from HiMedia Pvt Ltd, India. Ninety eight grams of the dehydrated medium was suspended in 1000 ml of distilled water. The medium was dissolved completely by boiling. The medium was sterilized by

autoclaving at 121ºC at 15 lbs pressure for 15 minutes. 20 ml of the medium was poured into sterile disposable petriplates Fig 15 (HiMedia laboratories Pvt Ltd, India). Sterility check was performed for each lot by incubating a representative plate at 37ºC. The plates were stored at 4º C until use.

Brain Heart Infusion Broth (BHIB)Fig 16:

Ingredients		Gms/ Lt
Peptic digest of animal tissue	:	10.000
Calf brain, infusion (solids)	:	12.500
Beef heart, infusion (solids)	:	5.000
Dextrose	:	2.000
Sodium chloride	:	5.000
Disodium phosphate	:	2.500

Final pH (at 25°C) 7.4±0.2

Thirty seven grams of the dehydrated medium was suspended in 1000 ml of distilled water. The medium was dissolved completely by boiling. The medium was sterilized by autoclaving at 121ºC at 15 lbs pressure for 15 minutes Fig 19. The medium was cooled to 45 - 50º C and 5 ml of the medium was poured into sterile test tubes. Sterility check was performed for each lot by incubating a representative plate at 37ºC.

Revival of *Streptococcus mutans*:

The freeze dried culture of *Streptococcus mutans* (MTCC 497) was reconstituted with 500 µl of sterile saline. Ten microliters of the reconstituted bacterial culture was pipetted out using sterile microtips (Tarsons, India.), micropipette (Eppendorf Research,

Germany) and was seeded on sterile Mutans sanguis agar plates. The inoculum was streaked using sterile Hi-FlexiLoop 4 (HiMedia laboratories Pvt Ltd, India) on the agar surface for isolation. The plates were incubated at 37˚C with 5% CO_2 in a candle jar for 24 hours. After incubation, the colony morphology of *S. mutans* was observed Fig 20.

Inoculum preparation - *S. mutans*:

Isolated colonies of *S. mutans* from Mutans Sanguis agar plate cultures were suspended in sterile Brain Heart Infusion Broth (BHIB) in individual test tubes and the cell densities were adjusted to 1.5 x10^8cfu/ml using 0.5 Mcfarlandstandard (HiMedia laboratories Pvt Ltd, India).

Grouping:

The specimens in each group were further divided into 4 subgroups (Subgroup**A**(Clinical): 6 hrs incubation; Subgroup**A**(Clinical) : 18 hrs incubation; Subgroup**B**(MTCC): 6 hrs incubation ,Subgroup**B**(MTCC) 18 hrs incubation) , 5 specimens in each subgroup.

TABLE 2 – GROUPS AND SUBGROUPS

Clinical *S.mutans*	Clinical *S. mutans*
SubgroupA: 6 hrs incubation	Subgroup A: 18 hrs incubation
Group 1: Mylar.	Group 1: Mylar.
Group 2: Caulk.	Group 2: Caulk.
Group 3:Permaseal.	Group 3:Permaseal.
Group 4: Optiguard,	Group 4: Optiguard,
Group 5: G Coat Plus	Group 5: G Coat Plus

<div style="border:1px solid black; padding:10px; width:45%; float:left;">

MTCC *S. mutans*

Subgroup B: 18 hrs incubation

   Group 1: Mylar.

   Group 2: Caulk.

   Group 3:Permaseal.

   Group 4: Optiguard,

   Group 5: G Coat Plus

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Subgroup B: 6 hrs incubation

   Group 1: Mylar.

   Group 2: Caulk.

   Group 3:Permaseal.

   Group 4: Optiguard,

   Group 5: G Coat Plus

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The discs were transferred aseptically into the respectively labelled wells in 24 well microtitreplate. One mL of sterile BHIB was added to each well. 10 µl of the *S. mutans* inoculum was added to each well. The 24 well tissue culture plates were incubated at 37°C for 6 hours and 18 hours.

After incubation (6 hours and 18 hours), the discs were aseptically removed from the wells and were washed twice with sterile saline to remove the non-adherent cells. Then, the discs were transferred into the respectively labelled Eppendorf tubes containing 1 mL of sterile saline (1 disc/ Eppendorf tube). The Eppendorf tubes were vortexed at constant speed to mechanically detached the cells adherent to the discs.

Estimation of colony count:

Colony count was performed in the Microbiology laboratory of SreeBalaji Dental College & Hospital using standard Microbiological method, spread plate method.

Ten µl of the sample from the respectively labelled Eppendorf tubes was pipetted out using sterile microtips (Tarsons, India.) and micropipette (Eppendorf Research, Germany) and was seeded on to sterile Mutans Sanguis plates. The inoculum was

uniformly seeded on the agar surface by spread plate method. One plate was incubated aerobically overnight at 37˚C in a candle jar with 5% CO_2.

The colony count was performed using a digital colony counter (Deep vision, India) and the number of colony forming units (cfu) was calculated and tabulated using the formula.

Cfu/ml = N x dilution factor X 100. (N = no. of colonies)

where dilution factor = 100
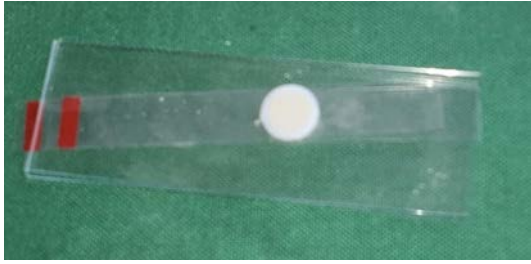
Fig-9: COMPOSITE DISC
PREPARATION IN TEFLON MOLD
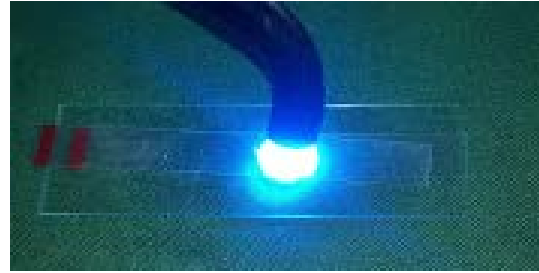
Fig-10: LIGHT CURING FOR 20
SECONDS

Fig-11: BOTH SURFACES OF
COMPOSITE DISC ARE POLISHED
WITH CAULK MICROPOLISHER IN
G2,G3,G4&G5

Fig-12: PS SURFACE SEALANT WAS
APPLIED IN GROUP 3, AND LIGHT
CURED FOR 20 SECONDS

Fig-13:OG SURFACE SEALANT WAS APPLIED IN GROUP 4, AND LIGHT CURED FOR 20 SECONDS	Fig-14: G COAT PLUS SURFACE SEALANT WAS APPLIED IN GROUP 5, AND LIGHT LIGHT CURED FOR 20 SECONDS

Fig-15: DISCS ALLOCATION IN EXPERIMENTAL GROUP

Fig-16: MUTANS SANGUIS AGAR
MEDIA
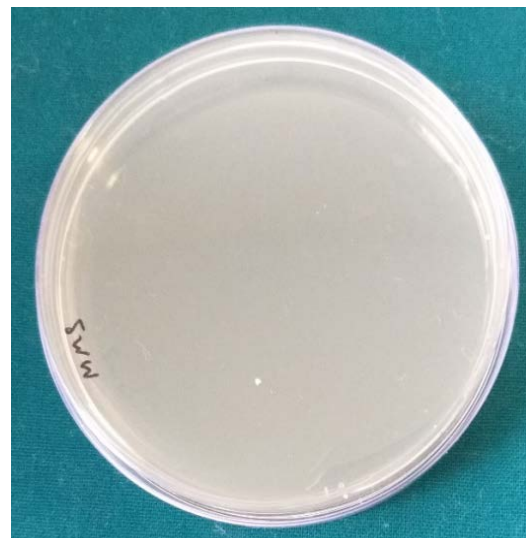
Fig-17: MUTANS AGAR CULTURE
PLATE
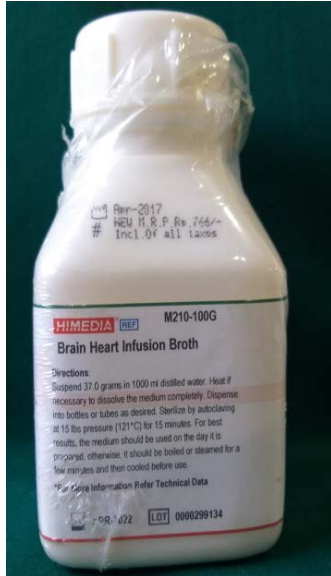
Fig-18: BRAIN HEART INFUSION BROTH

Fig-19: Sterile BHIB

Fig-20: COLONIES OF STREPTOCOCCUS MUTANS

PROCEDURAL FLOWCHART

Mutans Sanguis Agar was prepared.

Ten microliters of the respective bacterial culture was reconstituted and the inoculum was prepared.

The inoculum was streaked on the agar surface for isolation. The plates were incubated at 37˚C with 5% CO_2 in a candle jar for 24 hours

After incubation, the colony morphology of *S. mutans* was observed and inoculum was prepared by suspended in sterile Brain Heart Infusion Broth (BHIB) in individual test tubes and the cell densities were adjusted.

The discs were transferred aseptically into the respectively labelled wells in 24 well microtitre plate.

One mL of sterile BHIB was added to each well. 10 μl of the prepared *S. mutans* inoculum was added to each well of the 24 well tissue culture plate.

The 24 well tissue culture plates were incubated at 37°C for 6 hours and 18 hours in accordance to the respective subgroups.

After incubation (6 hours and 18 hours), the colony forming units are retrieved aseptically from discs and transferred to respectively labelled Eppendorf tubes containing 1 mL of sterile saline.

The inoculum was uniformly seeded on the agar surface by spread plate method. One plate was incubated aerobically overnight at 37˚C in a candle jar with 5% CO_2.

The colony count was performed using a digital colony counter and the number of colony forming units (cfu) was calculated

STATISTICAL ANALYSIS

The datas were evaluated and tabulated in Excel sheet, analysed using SPSS software (Version 25). The data were checked for normality and homogeneity of variance using Shapiro- Wallis test and Levine's test respectively.

The datas were found to be normally distributed $p > 0.05$, but the variances were not homogeneous in nature. Hence, One way ANOVA followed by GH post hoc test was employed to detect the statistical significant difference among 5 groups for four different parameters.

RESULTS

Table-3   Summarizes the raw data of the number of colony forming units of Clinical Strains and Standard Strains at 6 hr and 18 hr incubation.

Table-4   Represents the descriptive data

Table-5a   Describes the test of normality and test of variance
              5 b test of homogeneity

Table -6a   Signifies the ANOVA ,
              6 b Post -Hoc GH test

Table-7   Characterises the Mean Surface Roughness value Ra

Fig 21-25   Profilometric Analysis of the groups

Fig 26(i-v)   Illustrates the Scanning Electron Microscope Images of the groups.

Fig 27-29   Illustrates Streptococcus mutans colony forming units in respective Subgroups

TABLE 3: RAW DATA OF THE NUMBER OF COLONY FORMING UNITS OF CLINICAL STRAINS AND MTCC STRAINS AT 6 HR AND 18 HR INCUBATION.

GROUPS	CLINICAL STRAINS		MTCC STRAINS	
	A 6 HRS	A 18 HRS	B 6 HRS	B 18 HRS
G1	13000	14000	13850	20000
G1	15000	20000	18200	8000
G1	14300	13300	13000	10400
G1	14200	22400	17100	9200
G1	12500	14250	20100	9600
G2	24000	49800	54000	40400
G2	29200	54600	70000	23200
G2	26000	20400	80800	40800
G2	24000	26800	40000	18600
G2	32000	32000	53200	19200
G3	8000	14900	14800	14600
G3	15000	7900	15400	12400
G3	12200	10900	12600	9000
G3	8400	8100	12000	10400
G3	10400	6900	17200	10400
G4	12000	14200	12000	8000
G4	15200	14600	10000	6000
G4	16800	13000	14000	9200
G4	17800	9000	18000	9400
G4	13200	13600	10800	6800
G5	42000	34000	46400	48000
G5	35600	25800	41800	32000
G5	29200	26600	41600	22400
G5	31200	26800	50000	24000
G5	33200	30100	41200	20000

TABLE 4 : DESCRIPTIVE DATA

STRAIN	GROUPS	DESCRIPTION		STATISTIC	STD. ERROR
A 6 Hrs (Clinical)	**G1**	Mean		13800	457.165
		95% Confidence Interval for Lower Bound		12530.71	
		Mean	Upper Bound	15069.29	
		5% Trimmed Mean		13805.56	
		Median		14200	
		Variance		1045000	
		Std. Deviation		1022.252	
		Minimum		12500	
		Maximum		15000	
		Range		2500	
		Interquartile Range		1900	
		Skewness		-0.309	0.913
		Kurtosis		-1.787	2
	G2	Mean		27040	1562.562
		95% Confidence Interval for Lower Bound		22701.63	
		Mean	Upper Bound	31378.37	
		5% Trimmed Mean		26933.33	
		Median		26000	
		Variance		12208000	
		Std. Deviation		3493.995	
		Minimum		24000	
		Maximum		32000	
		Range		8000	
		Interquartile Range		6600	
		Skewness		0.731	0.913
		Kurtosis		-1.299	2

STRAIN	GROUPS	DESCRIPTION		STATISTIC	STD. ERROR
A 6 Hrs (Clinical)	**G3**	Mean		10800	1291.511
		95% Confidence Interval for Lower Bound		7214.19	
		Mean	Upper Bound	14385.81	
		5% Trimmed Mean		10722.22	
		Median		10400	
		Variance		8340000	
		Std. Deviation		2887.906	
		Minimum		8000	
		Maximum		15000	
		Range		7000	
		Interquartile Range		5400	
		Skewness		0.709	0.913
		Kurtosis		-0.638	2
	G4	Mean		15000	1080.74
		95% Confidence Interval for Lower Bound		11999.38	
		Mean	Upper Bound	18000.62	
		5% Trimmed Mean		15011.11	
		Median		15200	
		Variance		5840000	
		Std. Deviation		2416.609	
		Minimum		12000	
		Maximum		17800	
		Range		5800	
		Interquartile Range		4700	
		Skewness		-0.149	0.913
		Kurtosis		-2.009	2

STRAIN	GROUPS	DESCRIPTION		STATISTIC	STD. ERROR
A 6 Hrs (Clinical)	**G5**	Mean		34240	2211.244
		95% Confidence Interval for Lower Bound		28100.6	
		Mean	Upper Bound	40379.4	
		5% Trimmed Mean		34088.89	
		Median		33200	
		Variance		24448000	
		Std. Deviation		4944.492	
		Minimum		29200	
		Maximum		42000	
		Range		12800	
		Interquartile Range		8600	
		Skewness		1.077	.913
		Kurtosis		1.121	2.000

TABLE 5 A TEST OF NORMALITY AND TEST OF VARIANCE

	Kolmogorov-Smirnov[a]				Shapiro-Wilk		
	groups	Statistic	df	Sig.	Statistic	df	Sig.
A 6 Hrs (Clinical)	**G1**	.252	5	.200*	.934	5	.620
	G2	.217	5	.200*	.885	5	.330
	G3	.197	5	.200*	.930	5	.596
	G4	.172	5	.200*	.955	5	.774
	G5	.192	5	.200*	.936	5	.641
A 18 Hrs (Clinical)	**G1**	.331	5	.078	.824	5	.125
	G2	.225	5	.200*	.911	5	.475
	G3	.293	5	.184	.867	5	.253
	G4	.321	5	.101	.793	5	.071
	G5	.307	5	.138	.853	5	.203
B 6 Hrs (MTCC)	**G1**	.209	5	.200*	.938	5	.654
	G2	.237	5	.200*	.958	5	.795
	G3	.202	5	.200*	.948	5	.726
	G4	.218	5	.200*	.908	5	.457
	G5	.332	5	.074	.818	5	.113
B 18 Hrs (MTCC)	**G1**	.385	5	.015	.722	5	.016
	G2	.279	5	.200*	.781	5	.056
	G3	.270	5	.200*	.931	5	.602
	G4	.214	5	.200*	.919	5	.525
	G5	.278	5	.200*	.844	5	.175

5 b TEST OF HOMOGENITY

Test of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
A 6 Hrs (Clinical)	Based on Mean	2.059	4	20	.124
	Based on Median	1.187	4	20	.347
	Based on Median and with adjusted df	1.187	4	10.946	.369
	Based on trimmed mean	1.930	4	20	.145
A 18 Hrs (Clinical)	Based on Mean	13.594	4	20	.000
	Based on Median and with adjusted df	3.675	4	7.547	.059
	Based on trimmed mean	13.138	4	20	.000
	Based on Mean	8.610	4	20	.000
	Based on Median	2.930	4	20	.047
	Based on Median and with adjusted df	2.930	4	5.467	.125
	Based on trimmed mean	8.460	4	20	.000
	Based on Mean	6.972	4	20	.001
	Based on Median	1.703	4	20	.189
	Based on Median and with adjusted df	1.703	4	9.848	.226
	Based on trimmed mean	6.377	4	20	.002

TABLE 6 A - ANOVA

Sum of Squares			df	Mean Square	F	Sig.
A 6 Hrs (Clinical)	Between Groups	2001321600	4	500330400.0	48.219	.000
	Within Groups	207524000.0	20	10376200.00		
	Total	2208845600	24			
A 18 Hrs (Clinical)	Between Groups	2581156400	4	645289100.0	12.234	.000
	Within Groups	1054872000	20	52743600.00		
	Total	3636028400	24			
B 6 Hrs (MTCC)	Between Groups	8969908400	4	2242477100	38.386	.000
	Within Groups	1168382000	20	58419100.00		
	Total	1.014E+10	24			
B 18 Hrs (MTCC)	Between Groups	2126288000	4	531572000.0	9.269	.000
	Within Groups	1146992000	20	57349600.00		
	Total	3273280000	24			

TABLE 6B Post Hoc Tests

Games-Howell

Multiple Comparisons

Mean Dependent Variable (I) groups (J) groups Difference (I-J)				Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
A 6 Hrs (Clinical)	G1	G2	-13240.000*	1628.066	.003	-19955.59	-6524.41
		G3	3000.000	1370.036	.314	-2501.77	8501.77
		G4	-1200.000	1173.456	.836	-5772.45	3372.45
		G5	-20440.000*	2258.008	.003	-30077.97	-10802.03
	G2	G1	13240.000*	1628.066	.003	6524.41	19955.59
		G3	16240.000*	2027.215	.000	9175.53	23304.47
		G4	12040.000*	1899.895	.002	5272.84	18807.16
		G5	-7200.000	2707.619	.157	-16813.36	2413.36
	G3	G1	-3000.000	1370.036	.314	-8501.77	2501.77
		G2	-16240.000*	2027.215	.000	-23304.47	-9175.53
		G4	-4200.000	1684.043	.187	-10062.33	1662.33
		G5	-23440.000*	2560.781	.000	-32828.45	-14051.55
	G4	G1	1200.000	1173.456	.836	-3372.45	5772.45
		G2	-12040.000*	1899.895	.002	-18807.16	-5272.84
		G3	4200.000	1684.043	.187	-1662.33	10062.33
		G5	-19240.000*	2461.219	.002	-28574.69	-9905.31
	G5	G1	20440.000*	2258.008	.003	10802.03	30077.97
		G2	7200.000	2707.619	.157	-2413.36	16813.36
		G3	23440.000*	2560.781	.000	14051.55	32828.45
		G4	19240.000*	2461.219	.002	9905.31	28574.69

Mean Dependent Variable (I) groups (J) groups Difference (I-J)				Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
A 18 Hrs (Clinical)	G1	G2	-19930.000	6877.500	.160	-48466.06	8606.06
		G3	7050.000	2348.446	.097	-1175.05	15275.05
		G4	3910.000	2103.331	.422	-3901.18	11721.18
		G5	-11870.000*	2394.410	.008	-20214.99	-3525.01
	G2	G1	19930.000	6877.500	.160	-8606.06	48466.06
		G3	26980.000	6782.035	.063	-1838.12	55798.12
		G4	23840.000	6701.104	.095	-5276.00	52956.00
		G5	8060.000	6798.088	.761	-20705.70	36825.70
	G3	G1	-7050.000	2348.446	.097	-15275.05	1175.05
		G2	-26980.000	6782.035	.063	-55798.12	1838.12
		G4	-3140.000	1766.352	.451	-9428.13	3148.13
		G5	-18920.000*	2104.566	.000	-26195.05	-11644.95
	G4	G1	-3910.000	2103.331	.422	-11721.18	3901.18
		G2	-23840.000	6701.104	.095	-52956.00	5276.00
		G3	3140.000	1766.352	.451	-3148.13	9428.13
		G5	-15780.000*	1827.019	.000	-22334.81	-9225.19
	G5	G1	11870.000*	2394.410	.008	3525.01	20214.99
		G2	-8060.000	6798.088	.761	-36825.70	20705.70

GAMES-HOWELL

Mean Dependent Variable (I) groups			Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
		G3	18920.000*	2104.566	.000	11644.95	26195.05
		G4	15780.000*	1827.019	.000	9225.19	22334.81
B 6 Hrs (MTCC)	G1	G2	-43150.000*	7244.136	.015	-74283.49	-12016.51
		G3	2050.000	1635.084	.724	-3748.56	7848.56
		G4	3490.000	1953.228	.441	-3266.06	10246.06
		G5	-27750.000*	2184.834	.000	-35421.03	-20078.97
	G2	G1	43150.000*	7244.136	.015	12016.51	74283.49
		G3	45200.000*	7183.592	.014	13827.50	76572.50
		G4	46640.000*	7262.617	.011	15573.35	77706.65
		G5	15400.000	7328.301	.354	-15450.68	46250.68
	G3	G1	-2050.000	1635.084	.724	-7848.56	3748.56
		G2	-45200.000*	7183.592	.014	-76572.50	-13827.50
		G4	1440.000	1715.109	.910	-4708.43	7588.43
		G5	-29800.000*	1974.842	.000	-37128.20	-22471.80
	G4	G1	-3490.000	1953.228	.441	-10246.06	3266.06
		G2	-46640.000*	7262.617	.011	-77706.65	-15573.35
		G3	-1440.000	1715.109	.910	-7588.43	4708.43
		G5	-31240.000*	2245.351	.000	-39065.98	-23414.02
	G5	G1	27750.000*	2184.834	.000	20078.97	35421.03
		G2	-15400.000	7328.301	.354	-46250.68	15450.68
		G3	29800.000*	1974.842	.000	22471.80	37128.20
		G4	31240.000*	2245.351	.000	23414.02	39065.98

Mean Dependent Variable (I) groups				Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
B 18 Hrs (MTCC)	G1	G2	-17000.000	5477.518	.113	-38256.71	4256.71
		G3	80.000	2383.107	1.000	-9108.25	9268.25
		G4	3560.000	2273.324	.572	-5769.44	12889.44
		G5	-17840.000	5539.675	.100	-39390.41	3710.41
	G2	G1	17000.000	5477.518	.113	-4256.71	38256.71
		G3	17080.000	5120.859	.112	-4877.22	39037.22
		G4	20560.000	5070.700	.064	-1593.62	42713.62
		G5	-840.000	7157.653	1.000	-25568.98	23888.98
	G3	G1	-80.000	2383.107	1.000	-9268.25	9108.25
		G2	-17080.000	5120.859	.112	-39037.22	4877.22
		G4	3480.000	1178.134	.109	-727.89	7687.89
		G5	-17920.000	5187.292	.101	-40181.70	4341.70
	G4	G1	-3560.000	2273.324	.572	-12889.44	5769.44
		G2	-20560.000	5070.700	.064	-42713.62	1593.62
		G3	-3480.000	1178.134	.109	-7687.89	727.89
		G5	-21400.000	5137.782	.059	-43856.64	1056.64
	G5	G1	17840.000	5539.675	.100	-3710.41	39390.41
		G2	840.000	7157.653	1.000	-23888.98	25568.98
		G3	17920.000	5187.292	.101	-4341.70	40181.70
		G4	21400.000	5137.782	.059	-1056.64	43856.64

*. The mean difference is significant at the 0.05 level.

TABLE 7 MEAN SURFACE VALUE Ra

GROUPS	MEAN ROUGHNESS VALUE Ra IN μm
G1	0.0073
G2	0.3785
G3	0.0161
G4	0.0717
G5	0.1491

PROFILOMETERIC MEASUREMENTS

Fig 21 - GROUP 1 - MYLAR

Ra	0.0073	μm	Rq	0.0093	μm			
Rsk	-0.2896							
Rp	0.0162	μm						
			Rz	0.0361	μm			
RS	2.04	μm						

Fig 22 - GROUP 2 - CAULK

Ra	0.3785	μm	Rq	0.5107	μm			
Rsk	-0.9839							
Rp	0.7635	μm						
			Rz	1.9266	μm			
RS	13.78	μm						

Fig 23 - GROUP 3 - PERMASEAL

Ra	0.0161	μm	Rq	0.0396	μm				
Rsk	8.0745								
Rp	0.0673	μm							
			Rz	0.1162	μm				
RS	11.28	μm							

Fig 24 - GROUP 4 OPTIGUARD

Ra	0.0717	μm	Rq	0.1459	μm				
Rsk	2.4645								
Rp	0.1813	μm							
			Rz	0.3249	μm				
RS	21.21	μm							
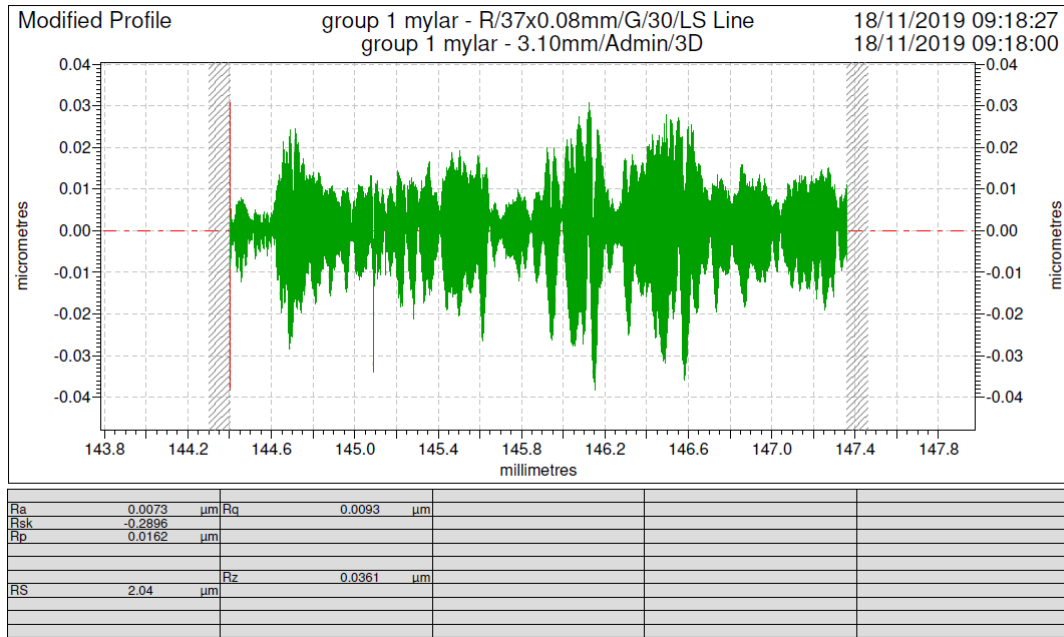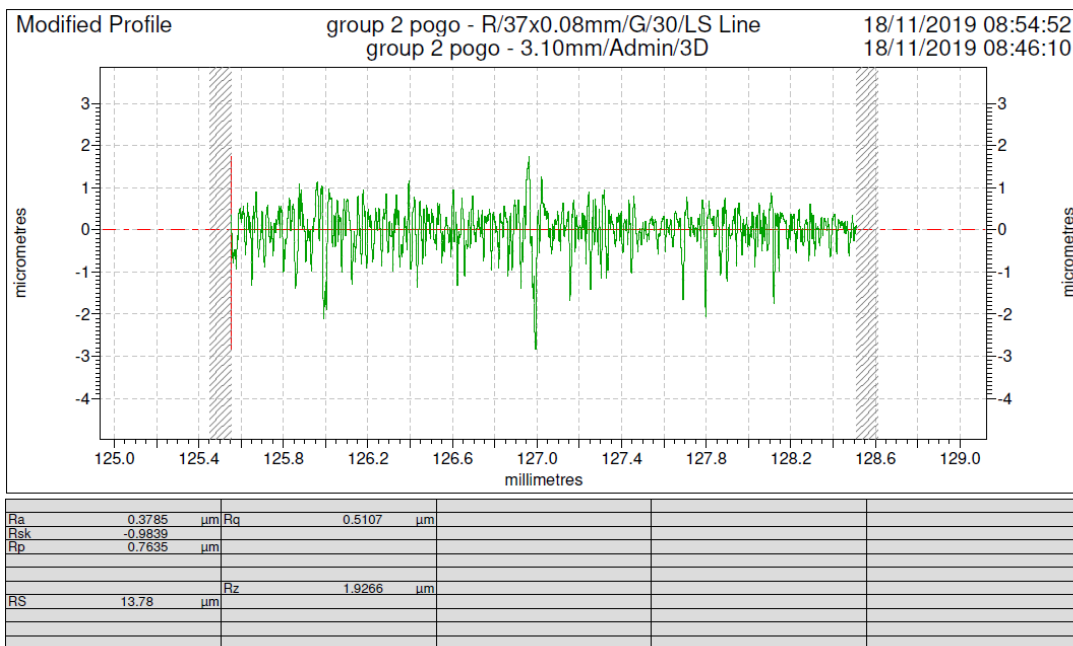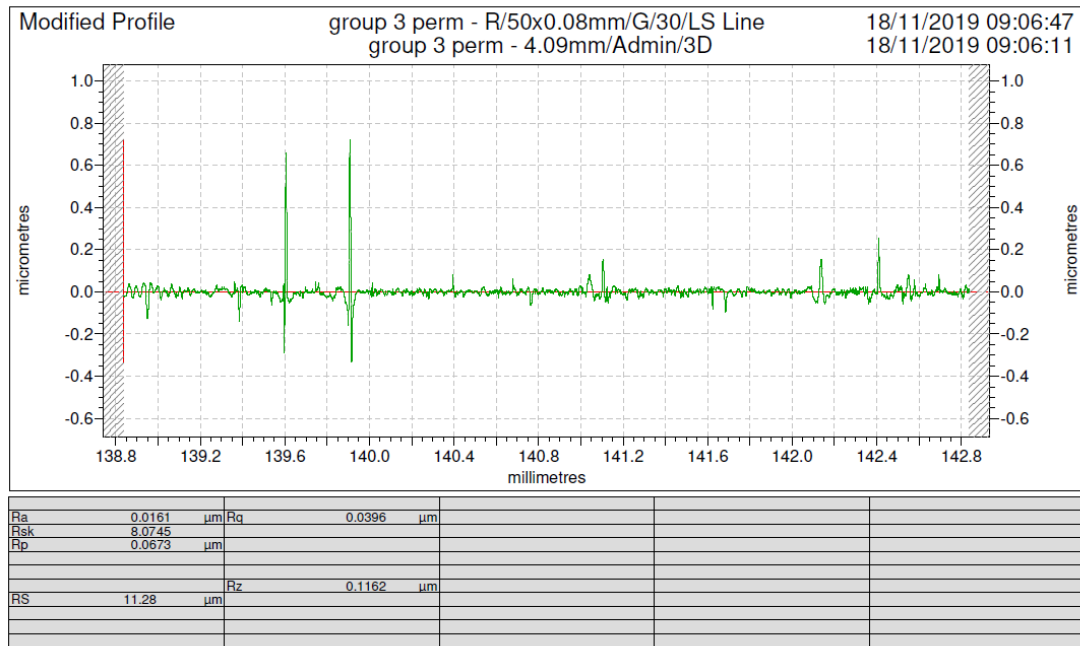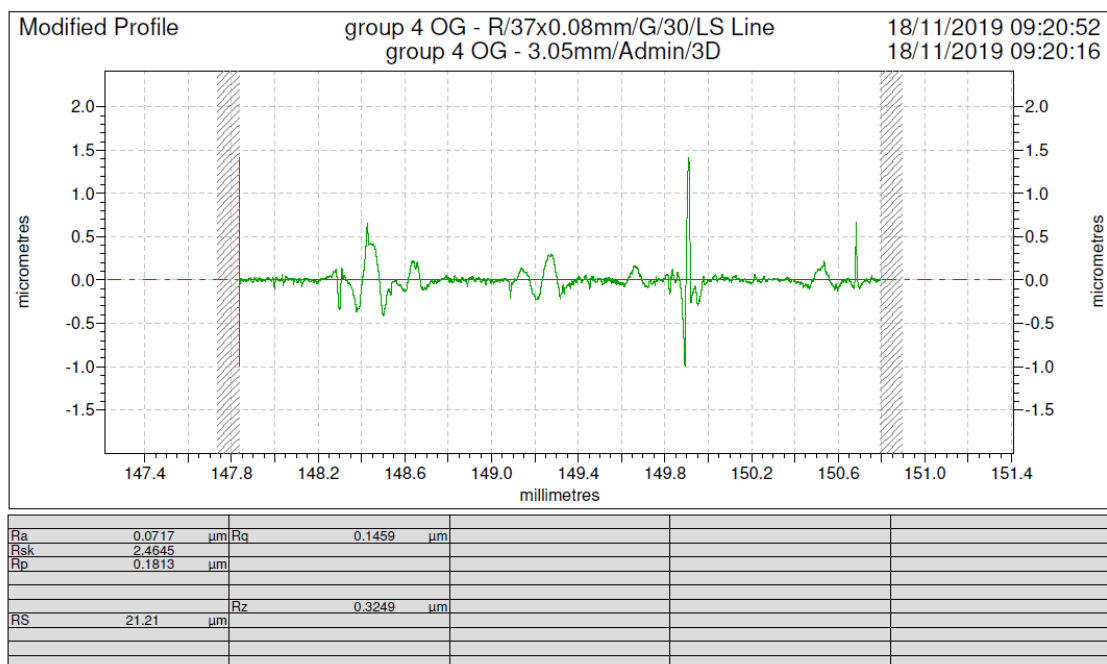
Fig 25 GROUP 5 – G COAT PLUS
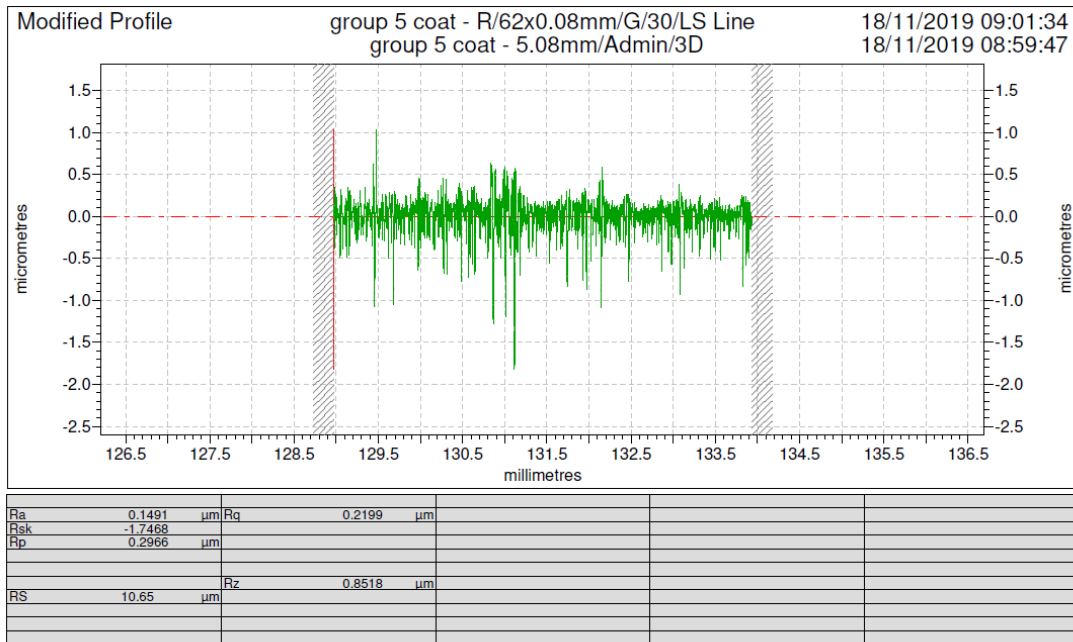
Fig 26 (i-v) SEM IMAGES AT 500X
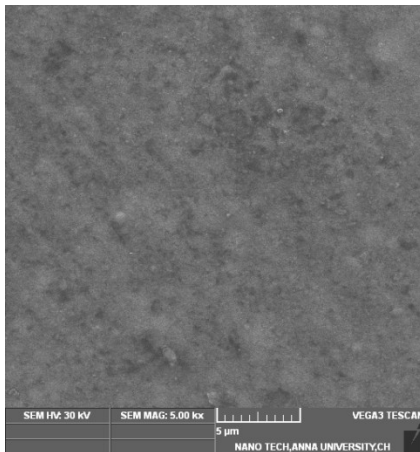
i)G1(MYLAR)   **ii) G2(CHAULK)**
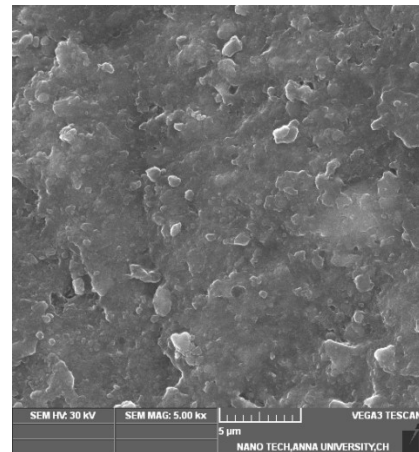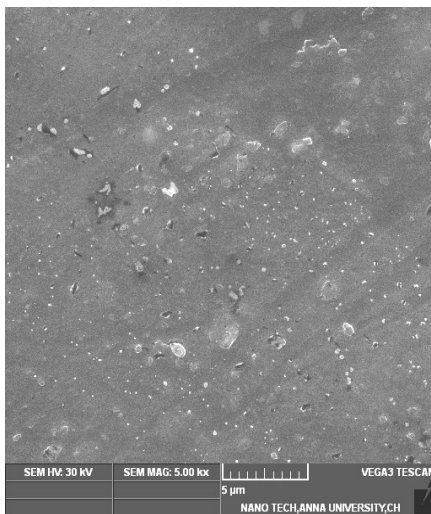
iii) G3 (PS)   **iv) G4 (OG)**
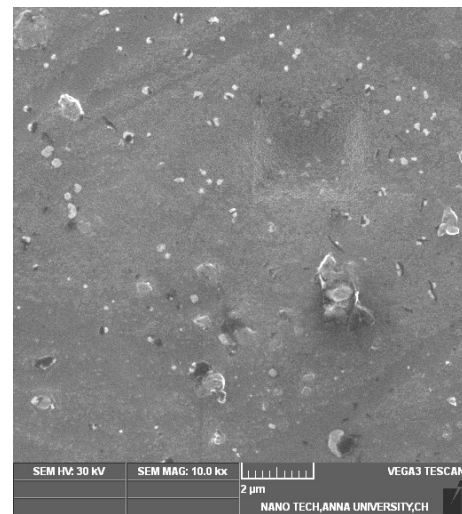
v) G5(GC COAT PLUS)
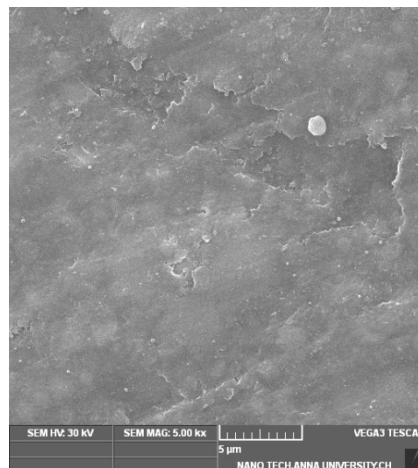
COLONY FORMING UNITS OF STREPTOCOCCUS MUTANS
Fig 27 (i - v) GROUPS 1-5 A(CLINICAL) 6 HRS

i)G 1 A(CLINICAL) 6 HRS

ii) G 2 A(CLINICAL) 6 HRS
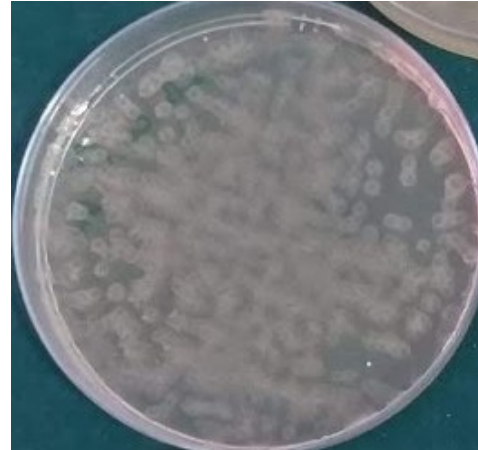
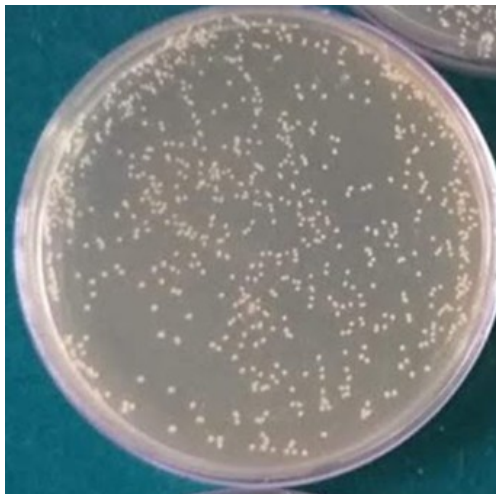
iii) G 3 A(CLINICAL) 6 HRS
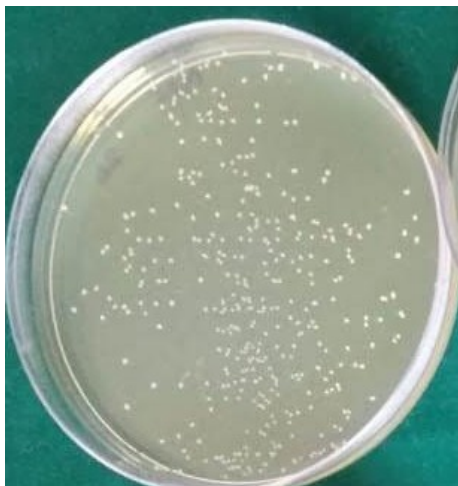
iv) G 4 A(CLINICAL) 6 HRS

v) G 5 A (CLINICAL) 6 HRS

Fig 28(i - v) GROUPS (1 – 5) A (CLINICAL) 18 HRS

i) G1 A (CLINICAL) 18 HRS
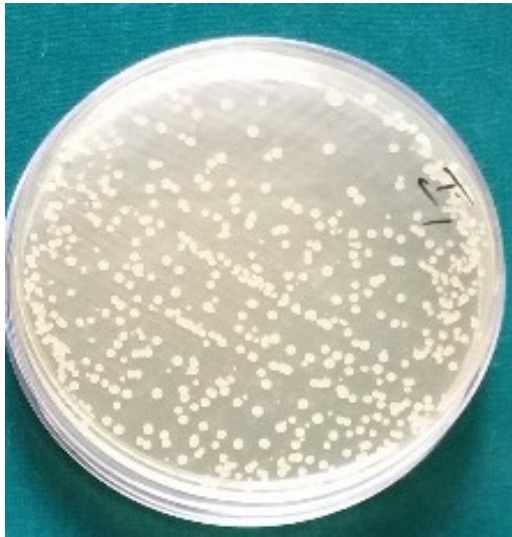
ii) G2 A (CLINICAL) 18 HRS

iii) G3 A (CLINICAL) 18 HRS

iv) G 4 A (CLINICAL) 18 HRS
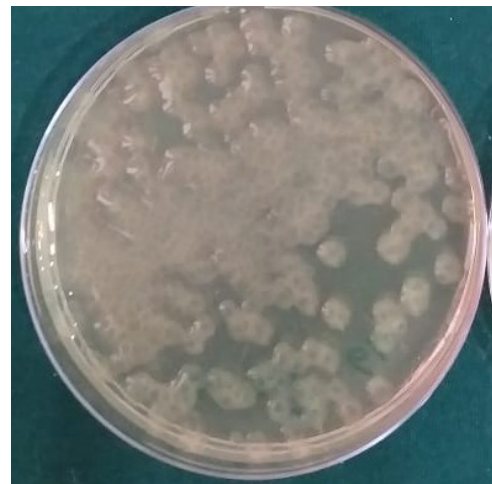
v) G 5 B (CLINICAL) 18 HRS

Fig 29 (i -v)GROUPS (1 -5) – (B MTCC) 6HRS
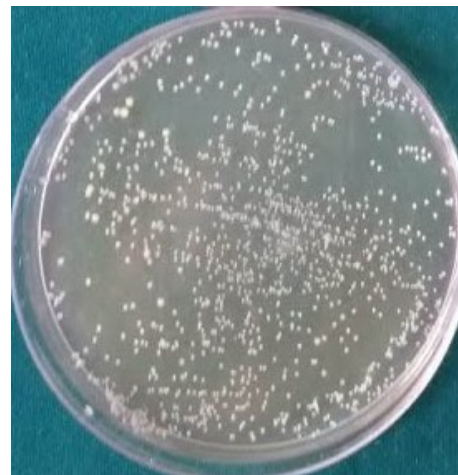
i)G 1 B (MTCC) 6 HRS

ii) G 2 B (MTCC) 6 HRS
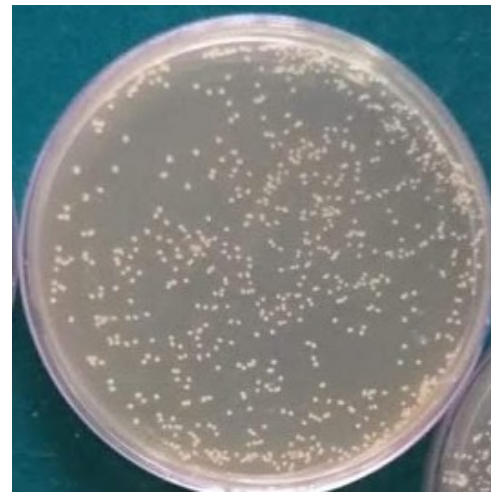
iii) G 3 B (MTCC) 6 HRS
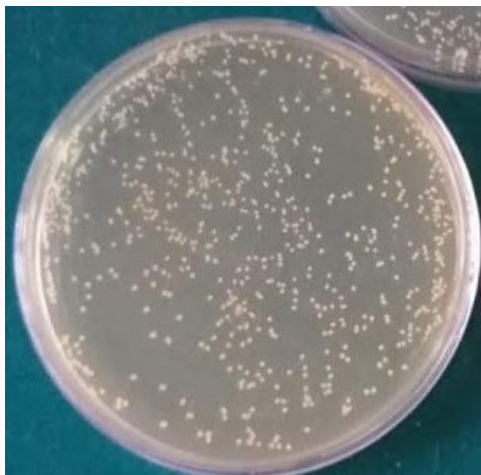
iv) G 4 B (MTCC) 6 HRS

v) G5 B (MTCC) 6 HRS

Fig 30 (i -v) GROUPS (1 -5) – B (MTCC) 18 HRS
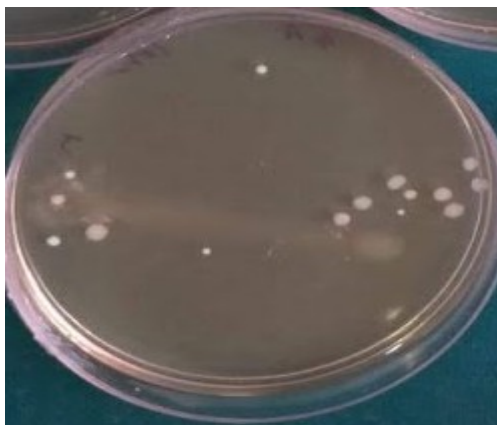
i) G1 B (MTCC) 18 HRS

ii) G2 B (MTCC) 18 HRS

iii) G3 B (MTCC) 18 HRS

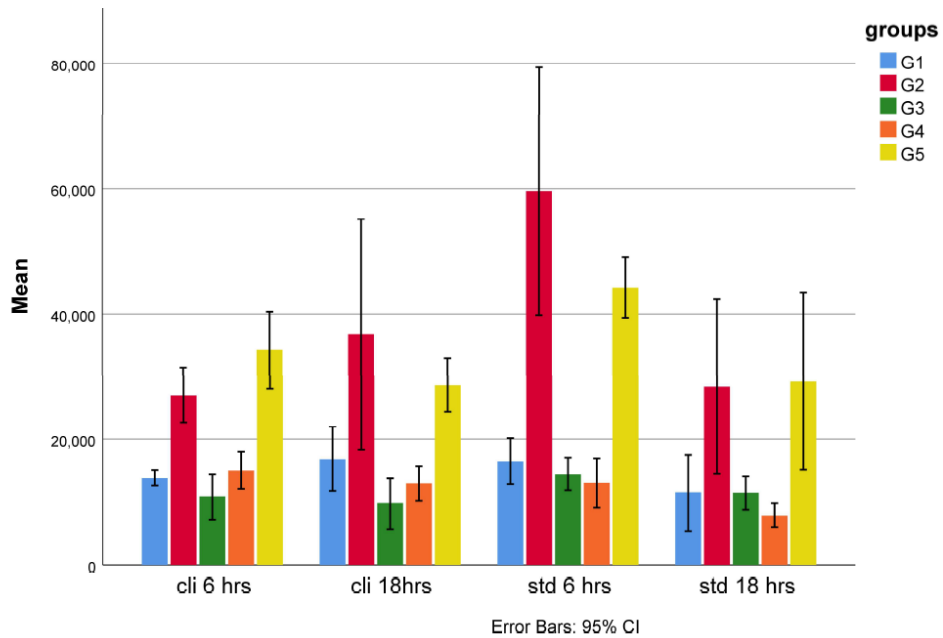**iv) G4 B (MTCC) 18 HRS**

v) G5 B (MTCC) 18 HRS

Graph 1

CLUSTERED BAR MEAN OF CLINICAL 6 HRS, MEAN OF CLINICAL 18HRS, MEAN OF STANDARD 6 HRS, MEAN OF STANDARD 18 HRS BY INDEX BY GROUPS

INFERENCE

Table 4 illustrated that the mean of number of colony forming units were least in G3 (PS), followed by G4 (OG) and G1 (Mylar). The highest mean value of the number of colony forming units was observed in G5 (G coat plus) followed by G2 (Caulk). This signified that the surface sealants G3 (PS) and G4 (OG) had lesser mean colony counts similar to the control group G1(Mylar).

With Clinical Strains, 6 hour incubation, G1(Mylar) (p =0.03), G3(PS) (p = 0.000), G4(OG) (p = 0.002) was statistically significant than G2(Caulk) and , G1(p =0.02), G3(PS) (p = 0.000), G4(OG) (p = 0.003) was statistically significant than G5 (G coat plus), (G1,G3 G4 <G2, G5). There was no significant difference between G1(Mylar), G3 (PS), G4(OG) (p > 0.05). There was no significant difference between G2(Caulk) and G5 (G coat plus) (p > 0.05),(G1=G3 G4<G2=G5).

With Clinical Strains, 18 hour incubation, G1 (Mylar) (p = 0.008), G3 (PS) (p=0.000), G4 (OG) (p=0.000) was statistically significant than G5, (G1=G3=G4 < G5).

With Standard Strains, 6 hour incubation, G1(Mylar) (p = 0.15), G3 (PS) (p =0.14), G4(OG) (p =0.11) was statistically significant than G2(Caulk) (p= 0.015) (G1<G2) and G1(Mylar) (p = 0.000), G3(PS) (p = 0.000), G4(p = 0.000) was statistically significant than G5,(G1=G3=G4<G5).

With Standard strains, 18 hour incubation, there was no statistically significant between the groups.

In all the composite groups with both Clinical and Standard strains at 6 hours, G1(Mylar), G3 (PS) and G4 (OG) performed better than G2 (Caulk)and G5(G Coat

plus). At 18 hours, in Clinical strains, G3 (PS) and G4 (OG) performed better than G2 (Caulk)and G5(G Coat plus).

Table 7 represented Mean Roughness Value (Ra) for the five groups. The increase in the number of mean colony forming units was associated with increase in the Mean Roughness Values. Group 1 (Mylar) achieved the smoothest mean surface value. Followed by G3 (PS), G4 (OG) , G5 (G Coat Plus). G2 (Caulk), the surface polished with chaulk micropolisher had the highest Mean Roughness Value. The surface sealants group had smoother finish than the surface finished with Caulk micropolisher alone. G1<G3<G4<G5<G2.

Fig 26 represents scanning electron microscopy (SEM) surface images for the groups at 500x. G1 (Mylar) had the smoothest surface among the groups, G3 (PS) and G4 (OG) had similar surfaces. Few microcracks and craters were observed in G5. G2(Caulk) showed the roughest surface among the groups.

DISCUSSION

Streptococcus mutans are the early colonizers for both the primary and secondary caries. Surface roughness greater than 0.2 μm has been a risk factor for biofilm formation on restorations which increases the chances for secondary caries[14]. The early phase of colonisation occurs by the interaction of the intrinsic physico-chemical superficial properties of the restorative materials and by the passive and active mechanisms of bacterial adhesion to underlying substrata [55]. From material aspect, the biofilm formation depends on the surface roughness and the physicochemical property. Bacterial adhesion and retention to a dental substrate surface occur through the following steps: transport of the bacterium towards the surface, initial adhesion, attachment by specific interactions, and colonization to form a biofilm. Van der Waal's attractive forces and electrostatic repulsive forces combine to aid the initial bacterial adhesion and retention to the surface of substrate [11].

Blocking of the caries development can be brought by sequestering biofilm formation that is due to microbial colonization[8]. A rough surface of the restoration means increased surface area where the bacteria has more points to attach to the substrate because of the favourable environment, the bacteria are protected from salivary flow and masticatory forces. Several methods were introduced to maintain a smooth surface of the tooth colored restorations such as improvements in composite resin material – from macrofilled to nanofilled composites, improvements in finishing and polishing and so on. Finishing and polishing procedures are necessary to achieve esthetics and longevity of tooth-colored restorations[37].

Surface sealants are one such development in adhesive dentistry to improve the finishing and polishing, introduced in the 1970's to preserve surface quality of

traditional quartz containing resin composites. These first generation Surface Sealants consisted of unfilled, light-cured liquid resins were placed on cured resin composite surfaces. These surface sealants enhanced surface gloss, color stability, wear resistance, and decrease the marginal leakage [22, 68]. Disadvantages such as formation of a non-uniform layer, film thinning, cracking, and debonding, thus creating a rough texture, vulnerable to staining and discoloration were noted [31,40]. In 90's, the second generation surface sealants were again advocated as a coating material for GIC, which sets by Acid- Base reaction. The third generation of Surface Sealants has come out to improve marginal and surface defects of composite resins. Newer Surface Sealants are unfilled or low-filled resin composites containing cross-linkable monomers, which has oxygen inhibition to a minimal extent. It was recommended for use in tooth colored direct and indirect restoration and reapplied in successive visits [2]. Various studies on surface sealants focussed mainly on microleakage, marginal deterioration, wear properties.The adhesion of Streptococcus mutans to composite resins coated with surface sealants was less studied. Mutans streptococci were the commonest species among bacteria causing initial colonizing and proliferating in the dental biofilm[13]. Streptococcus mutans MTCC 497, an ATCC analogue of the UA159 strain of S. mutans (purchased from IMTECH, Chandigarh, India) was used in this study. The clinical isolates of S. mutans used in this study were isolated and characterized earlier for study purpose. CLSI guidelines were followed for the isolation and characteization of S. mutans from samples. Standard and Clinical isolates were used to study the adhesion properties of Streptococcus mutans. Two different incubation time (6 hour and 18 hour) for intragroup comparison were assessed in the study. Bacterial adhesion to a biomaterials surface involved two-phases: an initial, rapid and reversible physical phase (phase one) which is pursued by time-depended and irreversible molecular and cellular phase (phase two) [36].

Filtek Z250 was used in the study, they are microhybrid composites consist of glass fillers (average particle size 0.4-0.7 μm) with 0.04 μm silica fillers[43]. They have high strength and wear resistance and has all-purpose application [21]. It primarily consist of hydrophobic resin monomers, such as bisphenol-A glycidyl dimethacrylate (Bis-GMA), triethylene glycol dimethacrylate (TEGDMA), and urethane dimethacrylate (UDMA) similar to the main components of the surface sealants used. In a previous study, nanofilled composites had good polishability which does not necessitate the use of surface sealant to improve surface smoothness[63]. Therefore, Microhybrid composite Filtek Z250 was used in the study. The study was designed with 5 groups, G1(Mylar), G2(Caulk), G3(Permaseal), G4(Optiguard) and G5(G Coat Plus) where G1(Mylar) was the control group. Caulk Dentsply polishing system was used in this study because studies suggest that one step polishing system performed better than the multi-step system by enhancing the surface smoothness[67]. Permaseal, Optiguard and G Coat Plus were the three sealants used. PermaSeal and Optiguard composite surface sealants consisting mainly BisGMA (60%) and TEGMA (35 – 40%). They are unfilled, low viscosity, and has better surface moisture capability. G Coat Plus was a nanofilled surface sealants primarily recommended as coating agent for composite resins, GIC and RMGIC and. It consist of mainly Methyl methacrylate, silanated colloidal SiO2, the nanofillers upto 20% by weight. **Arthilakshmi et al**[5] states that the G Coat Plus forms a "microlamination effect'" with uniform flow and complete wetting of the GIC surface due to the presence of single-phase dispersed nanofillers (30 nm). The thickness of the protective coating is about 35–40 μm. This toughened laminate layer provides a smooth and glossy surface.

Specimens were cured under the Mylar strip to have less bubble inclusion and to obtain an uniform surface. **Pereira et al.** estimated the S.mutans adhesion on the surface of

three composites after different finishing and polishing methods and concluded that the composite strips finished with Mylar strip alone with no additional finishing and polishing had the least bacterial adhesion in both microhybrid and nanofilled composites in the absence of saliva[52].

Yuehuei et al suggested that Spread plate method was a reliable method for viable plate counts in the estimation of adhesion of bacteria. In Spread plate method, the total number of colony forming units can be accounted on a single plate at once. The concentration of cells in the tube from which the sample was plated can be enumerated[69]. In Spread plating method, enrichment and selection media can be used[30]. The profilometer was used in the study as it determines the profile along 3 lines on the surface with a tracing device, and it expresses roughness by the undulations of the profile relative to some base line.

Miranda[48] et al concluded in her study that with the Profilometer, the surface topography can be characterized over a length scale from 0.01 μm to 4 mm and, the results were more reliable and precise. SEM was preferred over TEM which required ultrathin sectioning of the samples[28]. Though AFM was more specific and sensitive for surface characterization, SEM was preferred over AFM in the study[66].Initial Streptococcus mutans adhesion was the key parameter, Surface topography with SEM was satisfactory for the study.

The findings of the present study suggest that the initial Streptococcus mutans adhesion decreased significantly in two surface sealants groups. Thus, the null hypothesis that the surface sealants does not play a role in initial Streptococcus mutans adhesion on resin composites polished with Caulk Micropolisher was partially rejected.

Quirynen M and Bollen[55] advocated that the physiochemical properties of the adherent surfaces are responsible for the adhesion of the bacteria. Adhesins present in the surface appendages which enhance the bacteria to form highly specific, stereochemical bonds with corresponding receptor molecules on the pellicle layer. Studies on early plaque formation suggest that the bacterial colonization occurs at 4 hours of exposure to the surface. Further studies on initial bacterial adhesion on plastic films infer that the increase in the number of bacteria within 24 hr due to multiplication and proliferation of the adherent micro- organism [36]. In the present study, initial adhesion of Streptococcus mutans adhesion at two incubation 6 hr and 18 hr was done to evaluate the difference in the number of colony forming units, the results inferred that the mean colony forming units increased between 6 hr and 18 hr significantly increased in G2 (Caulk micropolisher) . Surfaces polished by Caulk micropolisher alone had greater number of colony forming units indicating that the microdefects provided the niches for the adherent biofilm and their number increased significantly at 18 hours irrespective of the groups. **Hannig et al** illustrated in his TEM analysis that there was substrata-related morphological changes in adherent biofilm as a result of distinct differences in bacterial detachment[28]. According to Hannig, there may be morphological changes in adherent biofilm in the Caulk group due to increased surface area, surface energy which may require further analysis.

The role of surface sealants were appreciable in Microhybrid composite, **Khalaj et al** performed similar study on nanohybrids and concluded that the application of surface sealants improves the surface roughness[37]. **Lopes et al** tested surface sealants on nanofilled composite resins and inferred that the surface sealants did not improve the surface roughness of nanofilled composite resins[42]. According to **Lopes** , the surface sealants was not necessary for improving the surface quality of nanofilled resin

composites. When critically analyzing the surface roughness of the surface sealants applied over the composite overtime, **Biazuz et al** studied the water sorption, solubility and surface roughness of surface sealants concluded that the surface sealants undergo hydrolytic degradation overtime[10]. However , **Dickinson[24] et al** in his clinical trial recommended that the surface penetrating sealant's effectiveness could be enhanced if the material was reapplied twice a year based on the probability of wear of the surface sealant. **Cilli** elucidated the effect of surface sealants after simulated tooth brushing and found that the sealed counterparts behaved well when compared to the unsealed resin composites[14].

In this study, G1(Mylar) showed the smoothest mean surface value. Followed by G3 (Permaseal), G4 (Optiguard), G5 (G Coat Plus). G2 (Caulk) had the highest Mean Roughness Value. The surface sealants groups PS, OG had smoother finish than the surface finished with Caulk micropolisher alone. Many studies suggest that the Ra value of less than 0.2 μm has lowest threshold for bacterial adhesion [15]. In the present study, G1(Mylar),G3 (Permaseal) and G4(Optiguard) showed lesser than 0.2 μm and G2(Caulk), G5 (G Coat Plus) showed higher than 0.2 μm. This finding suggested that on application of surface sealants PS, OG in respective groups had reduced the average roughness (Ra) to half the initial roughness value obtained with Caulk micropolisher. **Bagheri et al** concluded that the surface sealant improved the surface texture with a thickness range of 0 to 70 micrometers [6]. A high Ra value indicated that the rough surface provide a safe haven for the bacteria against the shear forces, creating a necessary time and a vicious anaerobic environment for the bacteria to adhere to surface [8].

Scanning microscopic images at 500x magnification demonstrated the Mylar surface had smoother finish. The Permaseal and Optiguard groups had finish comparable to the

Mylar group. The microcracks and microdefects created during polishing with Caulk Micropolisher were sealed by the surface sealants. G coat plus showed a rougher finish similar to Caulk micropolisher. This might have been due to the uneven coating of the G5 (G Coat plus), tears on the surface of G5 (G Coat plus) were also noted[2]. Roughness value Ra measured in profilometer was least in G1 (Mylar), followed by G3 (PS) and G4 (OG) which showed comparable results.

For Clinical Strains at 6 hours and 18 hours, and for Standard Strains at 6 hour incubation, G3 (Permaseal) followed by G4 (Optiguard) had the least Streptococcus mutans counts followed by G1(Mylar). G Coat plus had the highest Streptococcus mutans counts. For Standard Strains at 18 hours, there was no statistical difference between the groups.

The adhesion and clumping of Streptococcus mutans is facilitated by the secretion of exopolysaccharide (glucans) which was very well noted in the clinical isolate groups which had slimy layer over the colonies. The G3 (PS) and G4 (OG) showed lesser bacterial counts due to smoother surface and physiochemical properties of these surface sealants. The results of Standard strains at 6 hour incubation were similar to the clinical strains, yet, the voluminous secretion of slime layer was not noted with Standard strains. At 18 hour incubation, the clinical strain G5 (G Coat plus) showed significantly higher colony counts. At 18 hours, standard strains had no significant difference between the groups suggesting that the adhesion of Streptococcus mutans to the surface sealants may be time dependent.

Cortopassi (2019) stated that the surface of resin composite coated with surface sealants has less structural defects since its viscosity allows it to flow freely in voids and porosities[15].

Microorganisms have a higher affinity towards resins composites than the enamel, ceramic and metal surfaces[22]. Filtek Z250 composite resin and the surface sealants had similar hydrophobic resin monomers (BisGMA, TEGDMA) and UDMA. The monomers are hydrophobic, yet they consist of hydrophilic moiety such as hydroxyl, ethylene oxide, Urethrane groups might be responsible for hydrophilic properties. BisGMA and TEGDMA are the major constituents of surface sealants PS, OG.

BisGMA has superior mechanical properties such as high stiffness. TEGDMA is smaller molecule, more mobile. For its enhanced polymerisation, it has been incorporated with Bis GMA. Hydroxyl groups are responsible for attracting water [10]. **Katsikogianni, M., Missirlis** explained the fact that materials with different functional group change bacterial adhesion in a manner depending on its hydrophobicity and charge. These functional groups provide template for water nucleation and forms a stable interfacial water layer so it prevents direct contact between bacteria and surface [36]. **Hannig** suggested that the presence of the pellicle layer, which apparently masks any difference among materials, with regard to surface properties and biocompatibility, bacterial interaction bound to occur overtime[28]. This explains that presence of hydrophilic functional groups initially attracts water layer which may have prevented initial Streptococcus mutans adhesion in G3 and G4. Permaseal consist of DMAEMA which is a zwitterion polymer which is an antifouling material due to their ability to form tightly bound water layers with strong ionic interaction which prevent protein deposition and further biofilm formation[48]. The results of the present study was in accordance to **Ruschel et al**[57] (2018) where the surface roughness of composites coated with three surface sealants, Permaseal which has smoothest surface,Fortify and Biscover where the other two sealants. **Kim et al** concluded that the Optiguard had lesser initial colonies of Streptococcus mutans due to its low Roughness value Ra

among three surface sealants (Optiguard, Permaseal, Fortify Plus) and it had the more hydrophilic surface[38].

Group 5 G Coat Plus showed higher no. of CFU statistically significant which may be attributed to the higher Surface Roughness appreciated in both Profilometer and SEM images[16]. Studies pertaining to Streptococcus mutans adhesion on various polymers suggest that Streptococcus mutans adhesion was higher in Methylmethacrylate [17]. Methacrylate being the main constituent of G Coat plus may be an additional factor for the poor performance of G Coat plus. The other explanation for poor performance of G Coat plus than its counterparts may be attributed to its filler content, high viscosity and properties of the individual components which has increased hydrophobicity.

Regardless of the individual properties of the surface sealants, the smooth surface which was obtained by coating PS and OG in respective groups was the main factor for less initial Streptococcus mutans adhesion in group PS, OG which was similar to control G1 (Mylar).

The study model was an in vitro model, pellicle formation and other factors present in invivo bacterial adhesion were not present in the study. The surface sealants were not coated with brush but uniformly cured under the Mylar Strip which was not the clinical scenario. The longevity of the surface sealants was not concerned in the study which may necessiate future studies. Further studies are needed to determine in detail physiochemical properties of surface sealants and the composite resins such as hydrophobicity, surface free energy and electrical charge.

However, the test conditions were suitable to assess the adhesive properties of composites and surface sealants in the protocol suitable for bacterial growth.

SUMMARY

The aim of the present study was to evaluate the initial adhesion of clinical and standard strains of Streptococcus mutans on the dental composite resins coated with three commercially available surface sealants. 110 resin composite discs made of Filtek Z 250 (8 mm x 1 mm) and were randomly divided into five groups.

GROUP 1 - Discs finished with Mylar Strip alone (n = 22).

GROUP 2 – Discs finished and polished with Caulk Micropolisher (n = 22).

GROUP 3 – Permaseal applied over finished and polished Discs (n = 22).

GROUP 4 - Optiguard applied over finished and polished Discs (n = 22).

GROUP 5 – G Coat plus applied over finished and polished Discs (n = 22).

Two representative discs from each group were taken for SEM and profilometric analysis. The Streptococcus mutans adhesion to the respective groups where done on Clinical strain and MTCC strains at 6 hour and 18 hour incubation by spread plate method. The results were tabulated. G3(PS) and G4(OG) reduced the surface roughness of composite resin discs finished and polished with Caulk Micropolisher. The reduction in the Streptococcus mutans adhesion to composite resins finished and polished with Caulk Micropolisher in G3 (PS) and G4 (OG) were comparable or even lesser than the control G1 (Mylar). The results of the present study encourages the use of surface sealants PS and OG after finishing and polishing procedure of composite resin to improve surface smoothness and to decrease the initial Streptococcus mutans adhesion.

CONCLUSION

The following conclusion can be brought out :

1)   PS and OG reduced the surface roughness of composite resin discs finished and polished with Caulk Micropolisher.

2)   The reduction in the Streptococcus mutans adhesion to composite resins finished and polished with Caulk Micropolisher in PS and OG were comparable or even lesser than the control Mylar Group.

3)   The adhesion of Streptococcus mutans increased with the increase in surface roughness of composites.

4)   The results of the present study encourages the use of surface sealants PS and OG after finishing and polishing procedure of composite resin to improve surface smoothness and to decrease the initial Streptococcus mutans adhesion.

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