COMPARATIVE SURFACE TOPOGRAPHIC ANALYSIS OF POLISHED NANOCOMPOSITE RESINS AND THE ADHERENCE OF STREPTOCOCCUS MUTANS BIOFILM ON THE POLISHED SURFACES USING TWO DIFFERENT COMMERCIAL POLISHING KITS AND AN INDIGENOUS POROUS NANOSILICA ABRASIVE - AN IN VITRO 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 2012 – 2015

DECLARATION BY THE CANDIDATE



I hereby declare that this dissertation titled "COMPARATIVE SURFACE TOPOGRAPHIC ANALYSIS OF POLISHED NANOCOMPOSITE RESINS AND THE ADHERENCE OF STREPTOCOCCUS MUTANS BIOFILM ON THE POLISHED SURFACES USING TWO DIFFERENT COMMERCIAL POLISHING KITS AND AN INDIGENOUS POROUS NANOSILICA ABRASIVE - AN IN VITRO STUDY" is a bonafide and genuine research work carried out by me under the guidance of Dr.B.Ramaprabha, Professor, Department Of Conservative Dentistry and Endodontics, Tamil Nadu Government Dental College and Hospital, Chennai- 600003.

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ENDORSEMENT BY HEAD OF THE DEPARTMENT / HEAD OF THE INSTITUTION



This is to certify that the dissertation titled "COMPARATIVE SURFACE TOPOGRAPHIC ANALYSIS OF POLISHED NANOCOMPOSITE **RESINS AND THE ADHERENCE OF STREPTOCOCCUS MUTANS BIOFILM** ON THE POLISHED SURFACES USING TWO DIFFERENT COMMERCIAL POLISHING KITS AND AN INDIGENOUS POROUS NANOSILICA ABRASIVE - AN IN VITRO STUDY" is a bonafide research work done by **Dr. VIDYA. K. G**, Post Graduate student (2012-2015) in the Department of Conservative Dentistry & Endodontics under the guidance of Dr. B. RAMAPRABHA, M.D.S, Professor and Guide, Department Of Conservative Dentistry & Endodontics, Tamil Nadu Government Dental College and Hospital, Chennai-600003.

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ACKNOWLEDGEMENT

I wish to place on record my deep sense of gratitude to my mentor **DR. M. KAVITHA, M.D.S.,** for her keen interest, inspiration, immense help and expert guidance throughout the course of this study as **Professor and HOD** of the Dept. of Conservative Dentistry and Endodontics, Tamil Nadu Govt. Dental College and Hospital, Chennai.

I take immense pleasure to extend my heart felt gratitude and sincere thanks to **DR. B. RAMAPRABHA M.D.S., Professor and Guide**, Department of Conservative Dentistry and Endodontics, Tamil Nadu Govt. Dental College and Hospital for her timely guidance, motivation, suggestions and inspiration.

I take this opportunity to convey my sincere thanks and gratitude to **DR. S. PREMKUMAR MDS., Principal i/c**, Tamil Nadu Govt. Dental College for permitting me to utilize the available facilities in this institution.

I am extremely grateful to **Mr. Hari**, PhD Research Scholar, Crystal Growth Centre, Anna University, **Dr. Parthiban**, **Dr. Senthil Kumar** and **Mrs. Usha**, Dept of Biotechnology, Madras Veterinary College, for their tireless help, patience and without whom this study would not have become a reality.

I express my heartfelt gratitude to **Dr. Junaid Mohammed MDS**., Assistant Professor, **Mr. Srinivasan**, Anna University, **Mr. Siva Kumar**, Dept of Pharmaceutics, College of Pharmacy, Madras Medical College for their meticulous help and guidance throughout my study.

I sincerely thank **Dr. K.AMUDHALAKSHMI M.D.S., and Dr. ARUNA RAJ M.D.S.,** Associate professors, for all the support and encouragement throughout this study.

My extended thanks to Dr. G. VINODH M.D.S., Dr. A. NANDHINI M.D.S., Dr. P. SHAKUNTHALA M.D.S., Dr. M. S. SHARMILA M.D.S., Dr. SUDHARSHANA RANJINI M.D.S., Dr. SMITHA.N M.D.S., Dr. JOTHI LATHA M.D.S., and Dr. VENKATESH M.D.S., Assistant professors, for all the help, suggestions, encouragement and guidance throughout this study.

None of my aims in life would have been fulfilled, had it not been for the constant support and encouragement of **MY LOVING PARENTS**, my dear sister **DR. DIVYA K.G.** and my **IN-LAWS**, who stood by me in all the good and bad times of my life.

Words fail to express when it comes to my beloved husband **Dr. SARATH. S. S**, for his unconditional love and support throughout my life, without whom my dreams would not have come true.

I also thank my dear co-PGs, seniors and juniors for their timely help and friendship.

Above all I pray and thank **THE ALMIGHTY GOD** for HIS continuous blessings in my every endeavour.

DECLARATION

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And

Mrs. Dr. B. Ramaprabha aged 45 years working as **Professor** in Department of Conservative Dentistry & Endodontics at the college, having residence address at 191/5, Green Fields Apts. R-30A, Ambattur, Thirumangalam High Road, Mugappair, Chennai-3 (herein after referred to as the Principal Investigator')

And

Mrs. Dr. VIDYA. K. G aged 27 years currently studying as **Post Graduate student** in Department of Conservtive Dentistry & Endodontics, Tamil Nadu Government Dental College and Hospital, Chennai 3 (herein after referred to as the PG student and coinvestigator⁶).

Whereas the PG student as part of her curriculum undertakes to research on "COMPARATIVE AFM SURFACE TOPOGRAPHIC ANALYSIS OF POLISHED NANOCOMPOSITE RESINS AND THE ADHERENCE OF STREPTOCOCCUS MUTANS BIOFILM ON THE POLISHED SURFACES USING TWO DIFFERENT COMMERCIAL POLISHING KITS AND AN INDIGENOUS POROUS NANOSILICA ABRASIVE - AN IN VITRO 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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1.

2.

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ABSTRACT

<u>AIM</u>: To evaluate the surface topography of nanocomposite resin discs using Atomic Force Microscope (AFM) and the adherence of Streptococcus mutans biofilm on the surfaces after polishing using two different commercial polishing kits and indigenously prepared porous nanosilica abrasive.

MATERIALS AND METHODS: 60 nanocomposite resin discs were prepared and were standardized using a surface Profilometer. Samples were randomly divided into 4 groups. Group1- unpolished, Group2- polished with Sof-Lex system, Group3- polished with Super-Snap and Group4- polished with the indigenously prepared porous nanosilica abrasive slurry. Average surface roughness values (Ra) were measured using an Atomic Force Microscopy (AFM). Streptococcus mutans biofilm was allowed to form over the resin discs and the corresponding Optical Density (OD) values were measured using a UV-Spectrophotometer. The surfaces were cleaned off the biofilm and the surface topography changes were measured again using an AFM.

<u>RESULTS</u>: When analyzing the surface roughness values after polishing and Streptococcus mutans biofilm formation and the Optical Density of all the 4 groups, group 1 (unpolished) showed the highest values followed by group 3 (Super-snap) and group 2 (Sof-lex). Group 4 (porous nanosilica) showed the smoothest surface in AFM after polishing. Statistical analysis was done using one- way ANOVA and Tukey's post hoc tests which demonstrated a highly significant difference (p<.001) between the mean values of all the 4 groups.

<u>**CONCLUSION</u>**: Within the limitations of this in vitro study, it was concluded that the smoothest surface with least bacterial adherence was produced by porous nanosilica abrasive slurry when compared with the commercially available micropolishing systems-Sof-lex and Super-Snap. Biofilm produces the roughest surface on the unpolished group and porous nanosilica group showed the least changes in surface topography.</u>

Keywords: Nanocomposite resin, Sof-Lex, Super-Snap, Porous nanosilica, Surface topography, Atomic Force Microscopy, Streptococcus mutans, Optical Density, UV-Spectrophotometer.

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ABBREVIATIONS

AFM	ATOMIC FORCE MICROSCOPE
SEM	SCANNING ELECTRON MICROSCOPE
OD	OPTICAL DENSITY
СМР	CHEMICAL MECHANICAL PLANARIZATION
MRR	MATERIAL REMOVAL RATE
Ra	AVERAGE SURFACE ROUGHNESS
SBA	SANTA BARBARA ACIDS
PBS	PHOSPHATE BUFFERED SALINE
TEOS	TETRA ETHYL ORTHO SILICATE
TEGDMA	TRI- ETHYLENE GLYCOL DIMETHACRYLATE
BisGMA	BISPHENOL-A GLYCIDYL METHACRYLATE



INTRODUCTION

Patients' increasing demand for esthetic restorations have led to a rapid development in the field of resin- based composite materials. Ever since the introduction of these materials in the early 1960s, they have been constantly evolving. In the last 15 years, a wide array of different types of composite materials has been marketed, from the earlier microhybrid and hybrid types to the recent nanohybrid ones. Nanohybrid composites are nanofilled with a particle size of $0.005 - 0.1\mu$ range. Their physical properties are equivalent to those of hybrid composite resins and they have good handling properties, greater polishing ability, high stain resistance, good colour stability and a low wear rate.⁶⁸ The final esthetic appearance for any composite restoration depends on: (1) the artistic ability of the clinician, (2) the contouring and shaping of the restoration and (3) the finishing and polishing of the restoration.

Effective finishing and polishing of composite restorations not only provides optimal aesthetics but also acceptable oral health of soft tissues and the marginal integrity of the restorative periodontal interface.⁶⁸ Finishing refers to the process of gross contouring in order to obtain a desired anatomy; whereas, polishing is the process of reducing the scratches that has been created by the use of finishing instruments. Finishing and polishing encompasses a sequential progression of steps starting from gross reduction and contouring to the final polishing. Composite restoration should be highly polished to maintain a plaque-free environment. Surface roughness determines the degree of initial bacterial adhesion to the restoration.¹⁰ Composite cured under a Mylar polyester strip produces the smoothest surface. But, in the clinical environment restorations have to be

finished and polished. The efficacy of polishing depends on how well the abrasive polish without damaging the surface of the composite or the adjacent enamel-dentin.

Secondary caries is one of the primary reasons for the replacement of any composite resin restorations.²² This is due to the formation of biofilm and excessive bacterial accumulation on the surface of composite materials. The streptococci bacteria, especially *Streptococcus mutans* (*S. mutans*), play an important role in the initiation and pathogenesis of secondary caries since these are the pioneer colonizers in the biofilm.²² They have got capability of adhesion, high acidogenicity and aciduric properties. These characteristics, especially the high affinity for adhesion, could be responsible for surface damage and biodegradation of resin restorations. Surface roughness of the restoration is an important factor in assessing the amount of plaque accumulation. A poorly finished and polished restoration can initiate biofilm adherence on its surface and the adjoining areas of the oral cavity.²²

Different polishing kits are commercially available to eliminate the scratches and grooves created in the restoration and hence to achieve a smooth plaque- free surface. Chairside polishing of the composite restoration is important for an esthetic appearance and a smooth well polished surface with less plaque accumulation.²² The various finishing and polishing instruments include: carbide and diamond burs, abrasive discs, abrasive strips, abrasive-impregnated rubber cubs and points & finishing and polishing pastes. However, disadvantage is that it is difficult to obtain a smooth surface on composite materials at the end of polishing procedure due to the shape and size of the filler particle and the proportion of the filler to the overall composition.⁹⁴ Traditionally,

the micron-sized silica and aluminium-oxide particle coated discs were used for polishing composite restorations.

According to the concept of chemical-mechanical planarization, nano abrasives are able to produce a smoother and finer surface.⁷¹ Various types of nanosilica abrasive slurries have been used in chemical-mechanical planarization (CMP), which have been traditionally used for polishing the semiconductors, computer hard discs etc.to a nano level. Colloidal silica nanoparticle has been used for polishing the tooth in order to reduce the bacterial adhesion for preventing dental caries.²⁵ These nanoabrasives are very stable, have good biocompatibility, easy method of preparation and a very low cost. It has been found that the traditional solid nanosilica abrasive which is been used in CMP slurries may cause surface defects owing to higher hardness. Therefore, more recently, porous nanosilica abrasives have been tried in CMP which produces fewer scratches and lower surface topographical variations with efficient Material Removal Rate (MRR).

Hence, this study is conducted to assess the efficiency of porous nanosilica abrasive in polishing nanocomposite compared with conventional micron- sized aluminium oxide polishing discs.

3



AIM AND OBJECTIVES

<u>AIM:-</u>

To evaluate the surface topography of nanocomposite resin discs using Atomic Force Microscope (AFM) and the adherence of Streptococcus mutans biofilm on the surfaces after polishing using two different commercial polishing kits and indigenously prepared porous nanosilica abrasive.

OBJECTIVES:-

- To evaluate the surface topography of nanohybrid composite after polishing with Sof-lex, Super-Snap and indigenously prepared porous nanosilica abrasive slurry using AFM.
- To evaluate the concentration of Streptococcus mutans adherence on the surface of nanohybrid composite after polishing with the three different polishing agents using UV-Spectrophotometer.
- To evaluate the surface topography of nanohybrid composite after the Streptococcus mutans biofilm formation using AFM.



REVIEW OF LITERATURE

FINISHING/POLISHING OF COMPOSITES & SURFACE ROUGHNESS:

Health JR and Wilson HJ (1976)³² conducted an invitro study investigating the effects on the time of placement and removal of the matrix strip on the quality of the restoration. They also studied the effect of tooth brush/ dentifrice on the surface roughness of the restorations.

From the study, the authors recommended application of the matrix strip before curing the resin. They also recommended the use of bonded abrasives such as discs and rubber wheels. Also, they have concluded that a good surface can be restored in composites by using an effective glazing agent. Then only the restorations may have an acceptable life when subjected to tooth brush & dentifrice.

R.Terrell Weitman et al (1975)⁷⁰ have studied the effectiveness of several composite finishing techniques in producing a plaque-resistant surface (both clinically & laboratory). 11 groups of class V composites were finished with 4 different finishing techniques (carbide finishing burs, white stones, brown & green finishing stones with aluminium oxide & brown & green stones with zirconium silicate). Laboratory measurements revealed that aluminium oxide slurry produced the smoothest surface, however, composite surfaces were covered with plaque after 24 hrs regardless of the finishing technique used. Aluminium oxide slurry was thought to produce a smoother surface than zirconium silicate due to the 1 μ m particle size and the incorporation of quartz with a Mohr hardness greater than 7. **Savoca D.E and Felkner L.L (1980)**⁷⁷ conducted an in vitro study to evaluate the effect of finishing composite resin surfaces at different time intervals such as 5, 7, 10, 15, 20 & 30 minutes and 1, 24, 48 hrs.

The study showed no difference in surface roughness of composite resins finished at different time intervals. The results also confirmed that the smoothest surface occurs after the removal of the matrix. The authors concluded that the smoothness of composite resin after finishing is not a function of the time at which it is finished.

Hachiya Y et al (**1984**)²⁹ conducted an in vivo and an in vitro study to evaluate the retention of various finishing and polishing agent techniques and the discoloration of two composite resins. The results for the studies were obtained in the laboratory for the specimens and clinically for the in vivo cases.

The authors concluded that oily foods are a major cause of discoloration of composite resin restorations. Hence, immersing composite specimens in a solution of orange oil, olive oil offers a satisfactory laboratory test for clinical discoloration. The glossy surface of composite resin created under a matrix discoloured more than a polished surface, which was rougher. Silicon cup blue produced the surface least susceptible to discoloration. Polishing immediately after insertion increases the discoloration. Polishing at the next appointment is recommended.

Stanford W.B et al (1985)⁸⁷ studied the effect of finishing & polishing on color and gloss of composites with different fillers. A conventional composite (Concise), small particle composite (Prisma-fit) and three macro filled composites (Silor, Durofil and Zeon) were used in this study. Finishing and polishing was done with 600 grit silicon

carbide paper on a polishing wheel at 3500 rpm. Composite cured with mylar strips were used as controls. A spectrophotometric colorimeter and glossometer were used to record the tristimulus color values and gloss respectively. A surface analyzer was used to record the surface profiles. Filler particle size and shape were also examined by SEM and optical microscopy.

All composites polished with silicon carbide showed statistically significant higher tristimulus values than mylar finished composite surface indicating strong reflection from a specific part of visible spectrum and hence appear lighter (whiter). Polishing also resulted in significant decrease in gloss (less for macro filled composite due to filler particle size). Surface profile tracing showed that gloss was related to surface roughness of polished surface. In general, polished composites tend to appear lighter and less glossy and this change is relevant when using composite in restorative dentistry.

Alan Boghasian et al. (1987)³ studied micro-filled and small particle hybrid composite resins's rotary finishing of coarse, five tungsten carbide and diamond burs. 2 light cured composites –Silux (with prepolymerised particles) and Herculite XR were used in this study. Finishing was done using 12 fluted and 30 fluted tungsten carbide and 25, 15 micron diamond burs. The specimens were cut using the above on a specially designed platform with the specimen advancing at a constant rate. 48 cut surfaces were examined randomly under SEM.

The results indicated that carbide burs caused a disrupted surface on the micro filled resin due to higher concentration of BISGMA causing occlusion of the carbide bur. Carbide burs (12, 30 flute) however produced smoother surfaces on hybrid composite. The result of this study was suggesting that carbide finishing burs for hybrid composite and diamond burs for micro filled composites.

Protten DH and Johnson GH (1988)⁶⁹ conducted an invitro study to evaluate the surface finish produced by 18 finishing instruments used on both a highly filled posterior composite and a blended anterior composite.

The authors summarized the results of the study that same finishing instruments and techniques revealed no significant difference in surface roughness of the anterior and posterior composite samples. A series of abrasive discs produced the smoothest surface. A five diamond bur with 25μ particles produced the roughest surface. The diamond burs in general produce less roughness at a low speed than at a high speed, probably from accentuated bur chatter and excessive heat build up in a high speed hand piece . Hence , an extra fine diamond with 15μ particles produced surface smoothnes superior to that produced with a white stone and similar to the smoothness produced with a carbide bur and rubber point. SEM analysis revealed qualitative difference in the surface texture produced by different finishing instruments, even though the average roughness was not shown to differ.

Herrgott AL, Zeimiecki TL and Dennison JB (1989)³⁴ conducted an invitro study to evaluate the different composite resin system finished with the currently available composite finishing instruments.

The authors have reviewed the previous studies and summarized that specially designed diamonds with very fine abrasive particles size (40 & 50μ) and white Arkansas stones have produced the smoothest surface. However, the use of diamond bur as

polishing agents is mostly limited to initial contouring because of their ability to remove equal amounts of adjacent enamel. They also conveyed that the use of conventional polishing agents increased the surface roughness of large particle composite resin.

According to this study, the surface roughness of the finished composite resin does not depend on the size of filler particles. There was significant difference in the surface produced by coarse, medium, fine and super fine discs. However, there was no statistical difference in the final finish produced by the commercially available finishing discs. It was concluded that the currently available polishing disc system could create a surface finish equivalent to that produced by Mylar strips.

W. W. Dodge et al (**1991**)¹⁰⁰ compared the wet and dry finishing and polishing of 4 composites (Herculite, Vision-dispers, Silux, Prisma-fil) and evaluated the surface smoothness, average hardness and color stability. The aluminium- oxide impregnated disks (Sof-lex) were used for finishing and polishing and profilometer, Knoop hardness tester and tristimulus colorimetry were used to evaluate the composites. The results indicated no difference in surface smoothness between wet and dry finish of Prisma-fil, Silux or Herculite while wet finished Vision-dispers was rougher than the dry finished (may be attributed to the presence of smear layer formed due to excess surface temperature exceeding the glass transition point as a result of being dry polished). There was no statistic difference between wet and dry finishing on the surface hardness. Dry finishing proved superior for Herculite, Prisma-fil with regard to color stability. Dry finishing of Silux produced color change. This study found that dry finishing was superior or equal to wet finishing (except for color change for Silux).

Stoddard JW and Johnson GH (1991)⁸⁸ conducted an invitro study to evaluate the best polishing agent for varios composite resins.

In this study, the surface of four anterior and posterior composite resins were compare d using Mylar strips, polishing with three rubber polishers and three different manufacturer's series of discs.

From this study the authors recommended that pairing a specific composite resin with a matching polishing system produced the smoothest surface and because of the in the size, shape, number of filler particles and type of resin, one system is incapable of creating the smoothest surface for all composite resins.

Jefferis SR and Borkmeier (1992)⁴⁰ conducted an invitro study to evaluate three composite finishing system. In this study the authors compared the effectiveness of three specific finishing and polishing systems when used to prepare the surfaces of composite restorative materials provided by the respective manufacturer and statistically significant differences in mean surface roughness were found between various finishing systems.

The authors concluded that a hybrid composite resin finished and polished with its respective finishing and polishing system gave a significantly smoother surface than a micro filled composite surface prepared with its corresponding sequentially coated abrasive disc system.

JL Ferracane et al (1992)⁴¹ studied the sub surface defects created during the finishing of composites. The materials used were a microfilled (Silux plus) and hybrid composites (P50, Herculite) . the composite specimens were finished with a 12 fluted carbide bur or a fine diamond within 3 minutes of light curing and subsequently stained with silver

nitrate. Microscopic evaluation revealed that significant penetration of stain occurred in the unfinished as well as in the finished surfaces. The extent of dye penetration area was less than 10μ being greatest for microfilled composites. This was attributed to the high invitro wear rate of posterior composites. The results showed that only a very limited subsurface damage may be created in certain composites during the initial contouring of a restoration and may be a function of difference in degree of cure, quality of adhesion, size and volume of fillers.

William G, Lambrechts and Braem (1993)⁹⁹ conducted an in vitro study using human enamel as the physiologic standard to compare the composite resins. According to their study, the intrinsic surface roughness of the composite resins must be equal to or lower than the surface roughness of human enamel, on enamel to enamel contact areas. Differential wear between enamel and composite surface on the same tooth is a new criterion for visually qualifying the wear resistance of composite resin in a biologic way. It was concluded that the ultrafine compact filled composite resins may be the material of choice for restoring posterior cavities.

Kwok – Hung Chung (1994)⁴⁸ conducted an in vitro study to investigate the effects of finishing and polishing procedures on the surface texture and color of resin composites. One of the major problem during the finishing and polishing of composite material is the discrepancy in hardness between the resin matrix and the organic filler. Therefore, they do not abrade uniformly. Often, shade of the composite restoration does not match as expected after the finishing and polishing procedure. Thus, finishing and polishing procedures contribute to the appearance of the color and gloss of a composite restoration.

From this study the authors have concluded that a mylar strip can create a smoother surface than any other type of polishing procedure. Because of the greater color difference and surface roughness values, the tested composite samples were lighter in shade after the polishing procedures. In conclusion, the authors highly recommended a custom made shade guide produced with different composites covered with a mylar strip in order to obtain near perfect color match.

Barry A Kaplan et. al (**1996**)¹⁰ studied the effect of three polishing systems on the surface roughness of 4 hybrid composites. They have polished composite discs using various systems including Enhance, Kerr composite finishing kit, MES/MPS polishing kit. The surface roughness was evaluated using Profilometer and SEM. The results revealed that MFS/MPS gave a superior surface polish for the three of the four composites tested. Further more, the Enhance system gave the poorest polish with all the four composites tested. MFS/MPS was thought to give a better polish due to the presence of diamond abrasives than carbide systems.

Fruits TJ and Miranda (1996)²³ have conducted an in vitro study to investigate the effects of equivalent abrasive grit sizes utilizing different polishing motions on selected restorative materials. The different motions investigated were rotary, planar and reciprocal. Fine, medium and coarse abrasives were used with each motion. The average surface roughness were used to compare the effects of the type of motion. The authors concluded that among all the combinations of the motion and abrasive grits, the planar motion produced a significantly lower surface roughness value.

S O Hondrum et. al (**1997**)⁸⁶ studied the effects of finishing and polishing of three materials used for class V restorations- composite resin, GIC and RMGIC. Seven methods of polishing including only the Matrix, polishing with Enhance system, polishing with two strips of MPS system and contouring burs were used. Quantification of surface roughness and gross reflectance were done. The results have indicated that the GIC surface was roughest followed by RMGIC and composite resin. Furthermore, the original matrix smoothness and gloss could not be produced with any contouring, finishing and polishing techniques used.

A V J Yap et. al (**1998**)⁸ studied the effects of immediate and delayed finishing and polishing procedures on the surface characteristics of tooth colored restorations including a microfilled, Compomer, RMGIC with 84 samples divided into two groups. Group 1 was immediately polished while group 2 was stored for 1 week and polished using the Enhance system, white stones and Super-snap. The results concluded that finishing and polishing generally was not influenced by polishing time with regard to surface roughness while delayed finishing/polishing resulted in a surface of similar or greater hardness compared to immediate finishing/polishing or control group.

Setcos J C and Torim B (1999)⁸¹ conducted an in vitro study to evaluate the effect of new polishing systems on the surface of resin composites. From the study, the authors have concluded that the Super-snap rainbow kit produced the smoothest surfaces, followed by Sof-lex pop- on discs and Enhance system.

Ceciliad P Turssi et. al $(2000)^{13}$ evaluated the effects of finishing and polishing techniques on the surface roughness of resin based composites. 40 cured composite

specimen discs were finished and polished using Sof-lex discs, Sof-lex followed by Prisma-gloss, Enhance points, Enhance followed by Prisma-gloss. Profilometric analysis showed that Sof-lex discs with subsequent use of Prisma-gloss provided superior finishing and polishing of composites, while Enhance points used alone showed the least favourable results. The use of polishing paste seems to have reduce the surface roughness and may be attributed to the surface temperature exceeding the glass transition point as a result of dry polishing.

Joniat S B and Gregoire (2000)⁴² conducted an in vitro study to evaluate the best finishing method on three composites. The study determined the finishing sequence best suited for available composites from a clinical view point. According to the authors, finishing burs left a rough surface. The tungsten carbide burs left irregularities harder to eliminate than those created by diamond burs. Therefore, intermediate polishing with silicon points appear to be necessary. Both aluminium oxide discs and polishing paste impregnated discs provided a good finish. Concerning the materials, the presence of microfine particles composed of microfillers strongly bound to the organic matrix produced an excellent polished surface.

Yap A V and Tan S (2000)¹⁰³ conducted an in vitro study to investigate the effect of polishing systems on the microleakage of conventional and RMGIC. The restored teeth were finished with various finishing systems. They have concluded that the microleakage at the dentinal margins of conventional GIC and enamel margins of RMGIC are significantly affected by different polishing systems.

Guilherme C et.al (2002)²⁸ investigated the influence of finishing/polishing procedures under wet/ dry conditions on the marginal integrity of microfilled and hybrid resin composite restorations immediately and after 24 hours of polymerization. Class V restorations were made using a hybrid or a microfilled composite. Finishing and polishing was done using Sof-lex aluminium oxide discs or fine and extra fine diamond burs under wet or dry conditions, immediately or after 24 hours of storing in water. After thermocycling, teeth were immersed in 2% methylene blue solution for 24 hours, sectioned and observed under stereomicroscope (20x). Results showed that delayed wet finishing produced best results in all groups of teeth restored with microfilled composite.

It was concluded that the microfilled composite restorations in dentin margins finished with diamond burs under wet condition after 24 hours exhibited significantly lower microleakage. Hybrid composite restorations had equivalent levels of microleakege regardless of the finishing protocol.

Halim Nagem Filho et. al (2003)³¹ evaluated the surface roughness of composite resin after using mylar strip, diamond bur, diamond bur and aluminium oxide disc in various composites. The results showed no statistical difference in average surface roughness between mylar strips and aluminium oxide discs. Finishing with diamond showed the highest roughness for all of the composites.

G Ozgumaltay et. al (**2003**)²⁴ investigated the effects of various finishing and polishing procedures on the 3 new tooth colored restorative materials (hybrid composite, packable composite and Ormocer). The finishing and polishing was carried out using diamond bur/

silicon polishers, diamond bur/ Sof-lex discs, carbide bur/ silicon polisher and carbide bur/ Sof-lex discs. Surface roughness tester and SEM were ysed to assess the surface roughness and topography. The results showed that the use of carbide burs with Sof-lex produced the smoothest surface. This was attributed to the ability of the aluminium oxide discs to cut the filler particles and matrix equally. The planar motion of the discs may also contribute to the smoother surface. Furthermore, the finishing diamond burs were more effective in removing material but tended to leave a more irregular surface due to their high cutting efficiency and this should be used for gross removal and contouring.

André F Reis et.al (2003)⁵ investigated the influence of various finishing systems on the surface roughness and staining of three packable resin composites Solitaire, ALERT and a conventional microhybrid one (Z250—3M-ESPE). Polishing was done with Poli I and Poli II pastes, Ultralap diamond paste, the Enhance system, Politip rubber tips, sequential fine, extra fine diamond burs and then 30-blade tungsten carbide burs used according to the manufacturers' instructions. After polishing, the surfaces were evaluated with a profilometer, and then immersed in 2% methylene blue for 24 h. Afterwards, the specimens were prepared for the spectrophotometric analysis.

No correlation was found between surface roughness and staining susceptibility. Z250 presented the smoothest surfaces and the dye uptake was found to be the minimum. The roughest surface was that of ALERT, and Solitaire showed the highest dye concentration. Stain resistance was not correlated with the smoothness of the surfaces, but was found to be influenced by each composite monomer and filler composition. M B Uctasli et. al $(2004)^{58}$ conducted a study to compare the surface roughness of flowable and packable composite material finishing with Sof-lex disc by means of average surface roughness measurement using a surface profilometer and SEM. The results of this study showed that after the finishing procedures, similar surface textures were observed for both packable and flowable composites with roughness of 0.23 to 0.38µm range.

Megeratt Baseran et. al (**2004**)⁵⁹ evaluated the effect of several finishing and polishing procedures on the surface roughness of nanofill and nanohybrid composites and Ormocer based dental restorative materials. Forty specimens of each material were polished with diamond and tungsten carbide burs along with Super- snap and Astropol. The average roughness was measured with Mahr Perthometer 54P. The results of this study showed that the Super-snap abrasive discs produced a smoother surface than Astropol for composite resins.

AVJ Yap et al (2004)⁷ investigated the texture of composite and compomer restoratives after treatment with different one-step finishing/polishing systems, which include One-Gloss, Shofu, PoGo, and Sof-lex. The surface roughness was compared with a matrix-strip, a two-step rubber abrasive and a graded abrasive disc (Super-snap). The results concluded that the effectiveness of finishing/polishing systems was material dependent. The surface finish produced by PoGo and Sof-lex was superior to that of the others.

Ahmet Umut Guler et. al (2005)¹ conducted an in vitro study to investigate the effect of different polishing methods- diamond polishing paste, pumice and polishing discs on
color stability of 2-3 component auto polymerized BISGMA light polymerized composite and a methyl methacrylate based material upon exposure to a staining agent.

The methyl methacrylate based PR material was found to be more color stable than the tested composites. The use of diamond polishing paste after polishing with pumice significantly reduced the staining of methyl methacrylate and bisacryl composites.

C S Jones et. al $(2005)^{16}$ investigated the load, speed and time required to achieve the smoothest surface on samples of amalgam, composite resin and GIC using 4 grades of a disc type of polishing system. These tests were conducted on a specially fabricated jig.

The load, speed and the time to produce a smoother surface is specific for each material. For amalgam and composite, the surface roughness values decressed as the discs became finer. There was a reduction in the roughness value for GIC using the 2 roughest discs.

Rustu Gedik et. al (2005)⁷⁵ evaluated the influence of various finishing and polishing techniques on the surface roughness of 4 microhybrid resin composites. The use of Astrobrush technique caused the greatest roughness of all composites. The Sof-lex technique produced the smoothest surface than Enhance and Astropol systems.

Tamayo Watanbe et. al (2005)⁹¹ investigated the influence of polishing duration on surface roughness of 2 light cured resin composites using 4 different polishing systems. In the profilometric analysis, the surface finish produced by multiple- step polishing systems was superior to that obtained with single step system.

Duygu Sarac et. al (2006)¹⁹ evaluated the surface roughness and color change of a hybrid, a microhybrid, and a nanohybrid composite resin polished with the use of polishing discs, wheels, and a glaze material. Surface characteristics may affect the color change and surface roughness of composite resins.

The composite resins tested were nanohybrid, Grandio; microhybrid, Filtek Z250; hybrid, Quadrant Universal and were polished using Sof-Lex, Astropol and glaze application. Color was assessed using a small area colorimeter and the surface roughness was evaluated using a profilometer.

The results showed that the polishing technique and type of composite resin significantly affected the surface roughness and color change. The polishing wheels produced the highest surface roughness values when compared to the other polishing techniques and the nanohybrid composite resin showed the lowest roughness values compared to the other compared to the other composite resins.

The highest roughness values with hybrid composite resins may be due to the size of the filler particles that got exposed after the polishing procedure. The resulte showed that the smoothest surfaces were obtained with polyester strips. After polishing discs or polishing wheels, glaze usage resulted in significantly lower roughness and color change values. The glaze appears to fill the structural microdefects and hence can provide a more uniform surface.

Heintze S.D. et al $(2006)^{33}$ analyzed the influence of polishing time and press on force on the surface gloss and roughness of dental materials by using a 3 component rubber – based polishing system and to compare results with those obtained in conjunction with an optimal polishing procedure & application of metal matrix.

The results showed that both surface gloss and surface roughness were material dependent and influenced by the polishing time and applied force.

M Jung et al (2007)⁵⁴ assessed the surface topography of 4 nano composites and 1 hybrid composite after polishing with 3 different systems. Nano composites used were Premise, Tetric EvoCeram, Filtek Supreme and Ceram X Duo and the hybrid Herculite XRV. Polishing was done using Sof-lex and a sequence of diamond polishing followed by tungsten carbide finishing bur, which was later polished with Astropol, OptiShine and Enhance/PoGo systems. Surface roughness was analysed using optical laser stylus profilometer. The results concluded that polishing was significantly influenced by 3 factors: composite material, finishing protocol and polishing method. Astropol achieved the lowest roughness on all composites.

Z Ergucu et al (2007)¹⁰⁵ analyzed the surface roughness of five novel resin composites that contain nanoparticles after polishing with 3 different one-step systems. The resin composites used were Ceram X, Filtek Supreme XT resin composite, Grandio, Premise and Tetric EvoCeram and polished using PoGo, OptraPol, and OneGloss. Surface roughness was evaluated using surface roughness tester and SEM. The results concluded that the effectiveness of the polishers seems to be material dependent.

Ana Coralina Valinoti et al (2008)⁴ demonstrated the effect of acidic medicines (Claritin & Dimetapp) under pH cycling conditions, on surface degradation of composite

resins. It was observed that acidic medicines degrade the surface of composites than pH cycling.

Richard Koh et al (2008)⁷³ evaluated the differences in surface roughness of a microhybrid and a Nanofilled composite using four polishing systems.

It was concluded that the Nanocomposite were smoother than the microhybrid and the Sof-lex provide the smoothest surface when used with either composite.

Zeynep D Yesil et al (2008)¹⁰⁶ evaluated the relative wear characteristics of 2 nano filled composite, microhybrid and microfilled materials.

The incorporation of nanofillers in composite did not significantly improve their wear resistance or the amount of opposing tooth cusp wear when compared to the conventional resin materials.

Ahmet Umut Güler et. al (2010)² investigated the effects of different air polishing powders on the surface roughness of different types of composite resin restorative materials. Polishing was done with a series of aluminum oxide polishing discs (Sof-Lex) and two different air-powder applications (Cavitron Prophy-Jet; and Sirona ProSmile prophylaxis powder). A standard air polishing unit (ProSmileHandly) was used. Surface roughness measurements were performed using a profilometer. Results concluded that air polishing applications increased the surface roughness of all composite resin restorative materials that have been used in this testing. So, it was concluded that composite restorations may require re-polishing after air polishing. **J. Janus et. al** (**2010**)³⁸ assessed the surface roughness and morphology of three nanocomposites polished with two different polishing systems. The nanocomposites Filtek Supreme, Grandio and Synergy D6 were polished with CompoSystem or Sof-Lex polishing discs. The average surface roughness (Ra) before and after polishing was measured using optical profilometry. AFM and SEM scanning were additionally used to analyze the surface morphology after polishing with the aim of relating the surface morphology and the surface roughness. Within the same polishing system, Filtek Supreme exhibited the smoothest surface, followed by Synergy D6 and Grandio. Sof-Lex polishing discs produced the smoothest surface compared to CompoSystem. AFM and SEM observations confirmed that the surface roughness was related to the surface morphology and to the average filler size.

Positive correlation between the average filler size and the surface roughness suggest that using nanoparticles in the formulation does not necessary improve the surface texture. The nanofilled composite Filtek Supreme, which contains only nanosized fillers, showed the best results when it was been associated with the Sof-Lex polishing discs.

D Atabek et. al (2010)¹⁷ evaluated the efficiency of a new nanotechnology polishing system on the surface roughness of 2 nano resin based composites. The polishing systems used were Enhance, PoGo, and nanotechnology liquid polish system (Lasting Touch). Surface roughness of the samples were analysed using optical profilometer. The results concluded that with the combination of finishing and polishing procedures, the nanotechnology liquid polish can provide a more glossy surface.

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B Zimmerli et. al (2011)⁹ evaluated the operator variability of different finishing and polishing techniques. 12 operators with different experience levels polished composite specimens using different finishing/polishing methods, which includes method 1- 40 μ m diamond(40D), 15 μ m diamond(15D), 42 μ m silicon carbide polisher(42S), 6 μ m silicon carbide polisher(6S) and Occlusobrush; method 2- 40 μ m diamond, 42 μ m Silicon carbide, 6 μ m Silicon carbide and O; method 3- 40 μ m Diamond, 42 μ m Silicon carbide, 6 μ m Silicon carbide and PoGo; method 4- 40 μ D, 42 μ S and PoGo; and method 5-40D, 42S and O. The mean surface roughness was measured with a profilometer and were qualitatively assessed using Scanning Electron Microscopy. The methods 3 and 4 showed the best polishing results and method 5 demonstrated the poorest and the most dependent on the skills of the operator. It was concluded that polishing procedures can be simplified without increasing variability between operators and without jeopardizing polishing results.

J B da Costa et. al (**2011**)³⁷ evaluated the surface finish and gloss of a two- step composite finishing/polishing disc system compared with two multistep systems on five composites. The systems used were two-step Enhance, and four-step Sof-lex, Super-snap. Surface gloss was measured with a glossmeter and the surface roughness was measured with a profilometer. Results concluded that Enhance was capable of providing similar gloss and surface roughness to Sof-lex on the 4 composites evaluated but was not able to produce as glossy as Super-snap for 3 of the 5 composites.

Sibel A Antonson $(2011)^{84}$ compared four finishing/polishing systems on surface roughness and gloss of different resin composites. Nanofill – Filtek Supreme Plus (FS) and a micro-hybrid resin composite – Esthet-X (EX) were used. Following 24h storage in $37 \ ^{0}$ C water, the top surfaces of each sample were roughened using 120-grit sandpaper. Surface roughness (Ra, μ m) and gloss were recorded.

Finishing & Polishing was done using Astropol system, Enhance/PoGo, Sof-Lex system[SL], and an experimental disk system, EXL-695[EXL]. SEM evaluation done. The Sof-Lex F/P system provided the smoothest surface. In gloss, FS composite with the EXL-695 system provided a significantly higher gloss. EX treated by Soflex revealed the least gloss. SEM images revealed comparable results for F/P systems but EX surfaces included more air pockets.

Nihan Gönülol et. al (**2012**)⁶³ has conducted an in vitro study to evaluate the effects of different finishing and polishing techniques on the surface roughness and color stability of nanocomposites.

Two nanohybrid (Grandio, Aelite Aesthetic Enamel), two nanofill (Filtek Supreme XT Dentin and Translucent), and a microhybrid (Filtek Z250) composites were used. A profilometer was used for assessing the surface roughness. The colour ΔE was calculated with a colorimeter at baseline and 48 h after storage in a coffee solution. The results showed no significant difference in roughness values between mylar strips and Sof-Lex polishing discs. The Enhance system showed the lowest color differences among all the finishing systems.

It was concluded that the composites with smaller filler size did not necessarily show low surface roughness and discoloration. The degree of staining of the composite resins was dependent on the chemical structure of the monomer, as well as the surface irregularities. **Ugur Erdemir et. al** (**2012**)⁹⁴ evaluated the surface roughness of various tooth-colored restorative materials after polishing them with three different polishing systems. The materials tested were glass ionomer cement, compomer restorative, microhybrid, and nanofil composite and were randomly polished with Sof-Lex disks, Poli-pro disks, and the Hiluster Plus systems. The mean surface roughness of each polished specimen was determined with a profilometer and examined using scanning electron microscopy. The results showed that the type of tooth-colored materials, their polishing technique, and the interactions they have were statistically significant. Mylar strip showed the smoothest surfaces of all the materials. Glass ionomer cement showed statistically significantly higher Ra values than the others. Compomer produced the smoothest surface. No significant difference was observed between the microhybrid and nanofil composites.

Barakah et. al (**2014**)³⁰ compared the Effect of 3 polishing systems on stain susceptibility and surface roughness of 2 nanocomposite resins and a microhybrid composite resin. The polishing systems used were PoGo, Astropol, or Hi-Shine. Using a profilometer, the average roughness was measured, and with a spectrophotometer, the baseline color was recorded. All specimens were incubated for 3 weeks after soaking in a staining solution of either coffee, green tea, and berry juice.

All polishing systems improved the staining resistance of Filtek Supreme XT and Z250 but did not affect that of Tetric EvoCeram. Filtek Supreme XT showed the most significant surface colour change and was the smoothest after polishing with PoGo, whereas the roughest surface was produced by Hi-Shine but with the lowest color change.

The study concluded that staining susceptibility and surface roughness depend mainly on material composition and on the polishing procedures. Nanocomposite resins did not exhibit better staining resistance or surface roughness than microhybrid composite resin.

Tijana Lainović et. al (2014)⁹² investigated the influence of diamond paste finishing on surface topography and roughness of two dental resin-based nanohybrid composites. The nanocomposites tested were Evetric and IPS Empress Direct. They were polished by two dental polishing protocols: multi-step dental polishing protocol with SuperSnap and the same multi-step polishing protocol followed by DirectDia diamond paste applied by SuperBuff polishers (Shofu, Inc. Kyoto, Japan). The surface topography was assessed using AFM. Results of this study showed that diamond paste polishing was useful processing method which significantly reduced surface roughness and created favorable topography of tested nanohybrid composite materials.

SILICA AS AN ABRASIVE

Kailiang Zhang et. al (2007)⁴⁶ has synthesized colloidal nano-abrasives with different particle sizes by an ion-exchange and hydrothermal processes, and their particle size and stability were characterized by using Transmission Electron Microscope (TEM) and a Zeta potential instrument. The results show that the colloidal nano-abrasives obtained were having the diameters of 10-20 nm, 50-70 nm, 80-90 nm, and the zeta potential (less than -45 mV) also illustrates that the colloidal nano-abrasives was of high stability. The colloidal nano-abrasives with average diameter of 80-90 nm was made into a slurry and were used to polish silicon wafers. The root mean square (RMS) of surface roughness for polished silicon wafers was less than 0.4 nm, which shows that this slurry made of colloidal abrasives gives a higher polishing rate along with less surface roughness.

Kazuaki and Yoshida $(2007)^{47}$ found out that the nanoabrasive colloidal silica fabricated by sol-gel method has high polishing potential rate when there is 1.0 wt% particle concentration, and increase in pH (13.4) leads to high polish rate.

Mohammed Q Al-Rifaiy (2010)⁶¹ evaluated the effect of mechanical polishing (MP) and chemical polishing (CP) on the surface roughness of heat cured (HC) and auto cured (AC) denture base acrylic resins. Surface roughness was measured using surface analyzing instrument in microns. There was no significant difference between MP and CP of HC and AC acrylic resin groups. It was concluded that mechanical polishing produces lower surface roughness compared with chemical polishing. The mean surface roughness values of mechanical polishing are not influenced by acrylic resin type. Chemical polishing effect on the surface roughness value depends on the acrylic resin type. Mechanical polishing is the most effective polishing technique.

Hong Lei et. al (2012)³⁶ reviewed that abrasives play an important role in chemical mechanical polishing (CMP). The most widely used abrasives in CMP slurries were compact solid silica particles which has the main disadvantage of causing surface defects owing to their high hardness. While it has been found out that porous silica abrasive exhibits a better surface planarization and fewer scratches than traditional solid silica abrasive when used for the polishing of hard disk substrates. But, there was not much significant improvement in the material removal rate (MRR).

Therefore, porous Fe2O3/SiO2 nanocomposite abrasives were prepared and their CMP performances on hard disk substrates were tested. The results indicates that the material removal rate of this new slurry containing porous Fe2O3/SiO2 nanocomposite abrasives is much higher than that of the slurry containing pure porous silica abrasive

under the same testing conditions. It was also seen that the surfaces polished by slurries containing the porous Fe2O3/SiO2 nanocomposite abrasives exhibits a lower degree of surface roughness, having fewer scratches and lower topographical variations than that by pure porous silica abrasive.

M Sivanandini et. al (2013)⁵⁵ reviewed the use of colloidal silica in chemical mechanical polishing (CMP). CMP is an indispensable process step in semiconductor device fabrication, common technique used in wafer polishing for dynamic memory, microprocessor applications and glass mechanical polishing.

An abrasive in the slurry provides both mechanical action with nanometer-sized abrasive particles and chemical action from the solution additives with a synergistic effect that causes material removal. The slurry designed for optimal performance should produce reasonable material removal rates, should have an acceptable polishing selectivity, lower surface topographic defects after polishing and good stability in slurry. Choosing slurry which provides good removal rates without causing defects is of utmost importance in CMP. Colloidal sized SiO2, CeO2 and Al2O3 particles are used in the manufacturing of CMP slurries. They are applied in different fields, but silica abrasives are promising.

Colloidal silica is effective as an additive for the intermediate diamond polishing of metals and is also the best polishing abrasive for eliminating subsurface and surface because of its polishing action on CMP. These polishes can be chemically stabilized to produce a nearly "perfect suspension"; can also have additives that minimize the effect of particle aggregation of crystallization. The silica based compositions are applied successfully for the finish polishing of Si, Ge, GaAs, InP, variety of materials, metals, dielectrics and other semiconductors in industries.

STREPTOCOCCUS ADHESION ON RESTORATIONS

Shintani H et. al (**1985**)⁸² conducted an in vitro study to evaluate the effect of various polishing methods on staining and accumulation of S. mutans on composite resins.

The author reviewed the previous studies and summarized that micro filled composite resins capable of obtaining a high glossy surfaces were resistant to plaque accumulation. However, the inorganic filler particles of the microfilled resin (30-50%) gave the resin some inferior characteristics like high water sorption. This high water sorption characteristic of the microfilled composite resin may cause the staining of the resin. The authors concluded that the bacterial accumulation on the polished surface was higher than that on the smooth surface. However, there was no appreciable difference in the bacterial accumulation between the surfaces finished by different finishing and polishing methods.

Yamamato K, Ohashis and Takit (**1996**)¹⁰¹ evaluated the adherence of oral streptococci to composite resin of varying surface roughness. The adherence of oral streptococci to composite resin plays an important role in the development of secondary caries. The bacterial adhesion test was carried out under a sucrose independent condition. The surface roughness values of each specimen ranged between $0.2\mu m$ and $3.0\mu m$. The authors have concluded that there is no relationship observed between the surface

roughness values and bacterial adhesion because Streptococcus oralis adhered firmly to the filler particles of all composite resin surfaces.

P Kldalichi et. al (2004)⁶⁶ reviewed and have summarized that the bishydroxy propoxyphenyl propane (bis HPPP), ethoxylated bisphenol A (E- bis PA), methacrylic acid (MA) and triethylene glycol (TEG) are the hydrolytic degradation byproducts of composite resins and are generated from interaction of human salivary enzymes and composite.

They conducted a study to investigate the influence of TEGDMA derived degradation products MA and TEG on the growth of oral bacteria S.mutans and S.salivarius at 37^oC and pH 5.5. The results showed that at neutral pH, the growth of oral bacteria was significantly reduced by MA and TEG and that these modulate the growth of bacteria.

Nurit Beyth et. al (2008)⁶⁴ reported that the polymerized resin composites and polymerized monomers accelerated the bacterial growth. They conducted a test to hypothesis that bacteria composite surface interaction causes changes in surface topography.

The results showed that S. mutans growth on resin composite increases surface roughness without affecting the microhardness. Because of this change in surface integrity, it may further accelerate bio film accumulation.

Ralf Burgers et. al (2009)⁷² compared the Streptococcus mutans adherence on the novel silorane based composite and 4 different conventional composites and related the surface roughness with that of hydrophobicity and also the type of matrix.

The results showed that there was a lower quantity of adhering Streptococci on the novel silorane composite than the other. This condition might result from its increased hydrophobicity. This may increase the longevity of the restoration and reduces the recurrent caries.

Filiz Aykent et. al (**2010**)²² examined the effect of different surface finishing and polishing methods on surface roughness and the adhesion of *S. mutans* bacteria to indirect, direct composite resin, and 1 ceramic material. The materials used were indirect composite resins (SR Adoro, Estenia), direct composite resin (Tetric), and a ceramic material (VITABLOCS Mark II). The following 4 surface finishing techniques were used: diamond rotary cutting instrument, sandpaper discs (Sof-Lex), silicone-carbide rubber points (Shofu), or a felt wheel with diamond paste. Surface roughness was then measured using the profilometer.

Artificial saliva and mucin were added to the specimens to produce pellicle. Bacterial suspension (109 CFU/ml) was then added to the specimens, and bacterial adhesion was determined using a confocal laser microscope. The highest surface roughness values were recorded in SR Adoro and diamond rotary cutting groups and the lowest in the ceramic group and in SR Adoro indirect composite resin. Bacterial adhesion to indirect composite resin materials differed from that to ceramic material after the surface treatments. It was concluded that the surface roughness and the vital *S. mutans* adhesion are positively related.

Li Mei et. al (2011)⁵² determined the streptococcal adhesion forces with composite resins with different surface roughness. Polishing and grinding were applied to obtain smooth (roughness 20 nm), moderately rough (150 nm) and rough (350 nm) surfaces of two light-cured composites for orthodontic use. The forces of adhesion between *Streptococcus sanguinis* and *Streptococcus mutans* and the composite surfaces were measured using atomic force microscopy in absence or presence of a salivary conditioning film. Initial forces as well adhesion after 120 s were measured, since longer contact times will result in stronger adhesion forces ("bond strengthening"). The results showed that streptococcal adhesion forces after bond-strengthening were significantly stronger than upon initial contact, for all of the composite types used. The use of salivary conditioning films significantly decreased the surface roughness of the composite and also the streptococcal adhesion forces.

It was concluded that streptococcal adhesion forces to orthodontic composite resins increase with increasing roughness of the composite surfaces. The adhesion forces with *S. mutans* than with *S. sanguinis* were less affected by the roughness of the composites.



ARMAMENTARIUM

- 1. 10ml syringe
- 2. Aluminium mold (10 mm× 2mm)
- 3. Analytical balance
- 4. Atomic Force Microscopy (Park Systems Corporation, Suwon, Korea)
- 5. Autoclave
- 6. Centrifuge (Eppendorf Centrifuge 5810 R, Eppendorf AG, Hamburg, Germany)
- 7. Conical rubber cups
- 8. Contra angle micro motor hand piece (NSK, Japan)
- 9. Cover slips
- 10. Diamond burs (TF- 12EF)
- 11. Glass slabs
- 12. Incubator
- 13. Magnetic stirrer
- 14. Mandrel
- 15. Measuring jar
- 16. Micropipette
- 17. Planetary ball milling machine
- 18. QTH Light curing unit (CU100A, Rolence Enterprise Inc. Chung Li, Taiwan)
- 19. Quick fix adhesive (FeviKwik)
- 20. Scanning electron microscope (HTAC-1, S-3400N)
- 21. Surface profilometer (Surtronic 3+, Taylor Hobson Ltd, Leicester, England)
- 22. Surgical gloves

- 23. Teflon coated instrument
- 24. Test tubes, beakers, petri dishes and non- absorbant cotton plugs
- 25. Ultrasonic bath cleaner
- 26. UV Spectrophotometer (BioPhotometer Plus, Eppendorf AG, Hamburg, Germany)
- 27. UV- light sterilization chamber

LIST OF MATERIALS USED

MATERIALS FOR POLISHING NANOCOMPOSITE RESIN DISCS

Sl No.	Product	Manufacturer
1.	Filtek ZT 250	3M ESPE Dental Products St. Paul, MN, USA
2.	Sof-Lex	3M ESPE Dental Products St. Paul, MN, USA
3.	Super- Snap	Shofu Inc., Kyoto, Japan
4.	Porous nanosilica abrasive	Indigenously prepared

MATERIALS FOR PREPARATION OF POROUS NANOSILICA

- 1. Ethanol, Sigma Aldrich Corporation, USA.
- 2. Tetraethoxysilane, Sigma Aldrich Corporation, USA.
- 3. Tri-Block Polymer Pluronic P123 (Poly(ethylene glycol)-block-poly (propylene glycol)-block-poly (ethylene glycol), Sigma Aldrich Corporation, USA.

MATERIALS FOR BACTERIAL CULTURE AND BIOFILM FORMATION

- 1. 0.9% Sodium Chloride solution
- 2. Distilled water
- 3. Freeze- dried Streptococcus mutans (MTCC 890), IMTECH, Chandigarh
- 4. Phosphate Buffered Saline (pH 7.4), Himedia
- 5. Tryptic Soy Broth, Himedia

SPECIMEN PREPARATION

A circular aluminium mold with a diameter of 10 mm and a depth of 2 mm was custom made for preparing the nanocomposite resin disc specimens. The light cured composite used in this study is: Filtek Z250 XT (3M ESPE Dental Products, St. Paul, MN, USA) (Fig:1).

60 such discs were prepared using the aluminium mold. The molds were filled with the nanocomposite material and placed between 2 transparent glass slabs and then light cured for 40 seconds by the QTH light curing polymerization unit (CU100A, Rolence Enterprise Inc. Chung Li, Taiwan). The prepared samples were then fixed to a cover slip using water insoluble glue (Fevi Kwik) (Fig:2).

The samples were standardized by measuring the average surface roughness (Ra) at three different positions in each sample initially before polishing using a surface profilometer (Surtronic 3+, Taylor Hobson Ltd, Leicester, England) (Fig:4). The surface roughness was kept at a cut- off value of 0.8mm and the traversing distance of stylus was 6mm. The radius of the tracing diamond tip was 5µm and the measuring force and speed were 1mm/sec.

The samples were then randomly divided into four groups of 15 each, (n=15).

Group 1 \longrightarrow **Unpolished** nanocomposite resin discs.

Group 2 — Polishing with **Sof- Lex** discs

(3M ESPE Dental Products, St.Paul MN, USA).

Group 3 → Polishing with Super- Snap (Shofu Inc., Kyoto, Japan).

Group 4 — Polishing with porous nanosilica abrasive slurry.

SYNTHESIS OF POROUS NANOSILICA ABRASIVE

Porous nanosilica abrasives were synthesized by sol– gel method.⁵⁵ For the synthesis of nanosilica with a typical P6mm pore arrangement and a mesoporous structure, 2.3 g of Tri-Block Polymer Pluronic P123 (Poly(ethylene glycol)-block-poly (propylene glycol)-block-poly (ethylene glycol), Mav=5800, EO20PO70EO20, Sigma Aldrich Corporation, USA were used (fig:5). These amphiphilic co- polymers acts as templating agents or structure directing agents to synthesize large- pore mesoporous nanosilica materials. The pluronics were dissolved in 15 ml of ethanol and stirred for 2 h (fig:6). Then 4.16 g of Tetraethoxysilane, Sigma Aldrich Corporation, USA was added to the above mixture and stirred for 1 hr. Tetraethoxysilane OR Tetraethyl orthosilicate (TEOS) is the source for silica.

The resulting homogeneous solution was transferred to Petri dishes and underwent solvent evaporation at room temperature for 2 days to get a rigid gel. This gel was then dried at 80 °C for 12 h to remove the residual ethanol. Finally, the as-made bulk samples were calcined at 550 °C in air for 5 h with a heating rate of 1 °C/min to remove the

surfactant. During the process of silica hydrolysis and condensation, the shape of the spherical micelles changes to rod-like. After silica condensation, the organic template is removed by calcination, thus creating the large mesopores (5-30 nm) characteristic for the SBA-family (which is an acronym of Santa Barbara Acids, which refers to the university where this material was first discovered). This material also typically exhibits microporosity originating from the corona micellar chains, which are burned upon calcination. The crystalline powder hence obtained is ball milled for 30 hours to synthesize the nanoparticle (fig:7,8). The average particle size of the hence synthesized porous silica nanocomposite abrasive was measured using Scanning Electron Microscopy. The size obtained was 70 nm (fig:9,10).

A schematic representation of the synthesis is given below:



POLISHING PROCEDURE

GROUP 2 (Sof-lex discs) :

Each of the 15 nanocomposite disc samples were polished sequentially with medium Sof- lex discs which contains 40 μ m size coated aluminium oxide particles, fine discs of 24 μ m particle size and ultrafine discs with 7 μ m size (fig:3). The Sof-lex discs were fixed on to its mandrel attached to a slow speed contra angle hand piece (NSK, Japan) rotating at 20,000 rpm. The whole polishing procedure was carried out by a single operator as per manufacturer's instruction using light pressure and brushing strokes for 20 seconds per disc. For each of the sample, a new set of Sof-lex discs were used. After completion of polishing, the nanocomposite discs were rinsed in running water in order to remove the debris.

<u>GROUP 3 (Super Snap)</u> :

Each of the 15 nanocomposite samples were polished with medium aluminium oxide discs (35 μ m paricle size), fine discs (20 μ m size) and superfine (8 μ m size) Super Snap discs (Shofu Inc., Kyoto, Japan) (fig:3). The discs attached to its mandrel were mounted on to the slow speed contra- angle hand piece (NSK, Japan) rotating at a speed of 20,000 rpm and used with a light pressure and brushing strokes for 20 seconds per disc. A new series of Super Snap discs were used for each specimen as per manufacturer's instruction. The samples were rinsed in running water to remove the debris.

GROUP 4 (Porous Nanosilica abrasive) :

Each of the 15 nanocomposite samples were first smoothened with finishing diamond burs (TF- 12EF) using a light pressure. The porous nanosilica abrasive slurry was applied on to each of the 15 samples and simultaneously polished with the conical rubber cup for 20 seconds with light pressure in a circular motion. After polishing the discs were rinsed in running water to remove the debris.

ATOMIC FORCE MICROCSOPY (AFM)

AFM is a very high resolution type of scanning probe microscopy with resolution fraction of a nanometer (fig:25). AFM has a technique based on the detection of deflective forces between the silicon cantilever with a sharp tip and sample surface. In this study, AFM (Park Systems Corporation, Suwon, Korea) operating in non-contact mode was used (fig:26). The other modes of an AFM are contact mode and tapping mode.

After polishing the specimens, a $10\mu m \times 10\mu m$ area for imaging was randomly selected with the 'V' shaped silicon cantilever in 1 Hz with 256×256 pixel resolution. The mean surface roughness (Ra) value calculations were done with the AFM in built- Park XEI 100 Version- 1.8.3 software (fig:27).

BACTERIA CULTURE

Streptococcus mutans (**MTCC 890**) was received as freeze-dried from IMTECH, Chandigarh (fig:11). It was regenerated by dissolving in 10 ml of Tryptic Soy Broth (Himedia) (fig:12). The solution was then kept in incubator for 24 hrs at 37^oC (fig:13). The resultant bacterial solution (bacteria + culture medium) was centrifuged for about 5 minutes at 10,000 rpm (Eppendorf Centrifuge 5810 R) (fig:14). The supernatant obtained was discarded to retain the pellet of bacteria at the bottom of the tube (fig:15). The pellet was resuspended in 2 ml of Phosphate Buffered Saline (PBS) (Himedia) to wash away the broth and to maintain the neutral pH. Then it was centrifuged twice for 5 minutes at 10,000 rpm. After discarding the supernatant, again the pellet of bacteria was resuspended in 5 ml of PBS. The optical density of the suspension was adjusted to 0.33 at 550 nm using the UV-Spectrophotometer (BioPhotometer Plus, Eppendorf AG, Hamburg, Germany) (fig:16).

BACTERIAL BIOFILM FORMATION

A 100 μ l (1×10⁸ bacterial cells) of the bacterial suspension was added to each 60 test tubes containing 10 ml of fresh Tryptic Soy Broth (fig:18). After sterilizing the specimens under UV- radiation, they were kept in 60 test tubes (fig:19,20) and were incubated at 37^o C for 1 day for the Streptococcus mutans biofilm formation on the surface of the composite specimens (fig:21).

After incubation, the test materials were washed three times with 5 ml of sterile 0.9% NaCl solution in order to remove the non-adhering cells. Each disc was then placed in a beaker containing 5 ml of sterile saline solution. The beakers were placed in an ultrasonic bath cleaner and sonicated for 5minutes in order to detach bacteria adhered to the surfaces of the specimen (fig:22). The discs were removed and the suspension is added to 5 ml of fresh broth in test tubes (fig:23). The tubes were incubated at 37^{0} C for 24 hrs.

After incubation, the concentration of bacteria in the broth was finally measured with UV-Spectrophotometer (BioPhotometer Plus, Eppendorf AG, Hamburg, Germany) (fig:24). The specimens were then air dried at room temperature and the surface topography was analyzed under AFM (fig:26).

PROCEDURAL FLOW CHART:

A circular aluminium mold of dimensions 10mm $\times 2$ mm was custom made for preparing the nanocomposite discs.





A 100 μ l of bacterial suspension is added to each 60 test tubes containing 10 ml of fresh Tryptic Soy broth. The specimens were sterilized under UV radiation and were placed in the test tubes, and incubated for 1 day at 37^oC



A 100 μ l of bacterial suspension is added to each 60 test tubes containing 10 ml of fresh Tryptic Soy broth. The specimens were sterilized under UV radiation and were placed in the test tubes, and incubated for 1 day at 37^oC



Wash the specimens for 3 times in 5 ml of 0.9% NaCl

to remove the non-adhered cells



Specimens were washed with 5 ml of sterile saline in ultrasonic bath for 5 minutes



SPECIMEN PREPARATION, POLISHING ARMAMENTARIUM

AND STANDARDIZATION



Fig 1: Nanocomposite resin disc preparation



Fig 2: Grouping of samples 4 groups (n=15)



Fig 3: Sof-Lex, Super-Snap polishing kits & porous nanosilica with conical rubber cup



Fig 4: Standardization of specimens using Surface **PROFILOMETER**

SYNTHESIS OF POROUS NANOSILICA



<u>Fig 5</u>: TriBlock polymer- templating agent & TetraEthyl OrthoSilicate- silica source



Fig 6: Magnetic stirrer for homogenizing the mixture



<u>Fig 7</u>: Ball milling in a planetary ball mill to obtain the porous nanosilica



Fig 8: Synthesized porous nanosilica along with the tungsten carbide balls

SCANNING ELECTRON MICROSCOPY FOR ASSESSING

THE PARTICLE SIZE



Fig 9: Scanning electron microscope



Fig 10: SEM image of porous nanosilica particles

STREPTOCOCCUS MUTANS CULTURE, BIOFILM FORMATION & OPTICAL DENSITY ASSESSMENT USING UV-SPECTROPHOTOMETER



Fig 11: freeze dried Streptococcus mutans (MTCC 890)



Fig 12: Tryptic Soy Broth and Phosphate Buffered Saline for regenerating bacteria



Fig 13: incubating the bacteria at 37^oc for 24 hrs in an incubator



Fig 14: Centrifuging the bacteria after incubation

Colour plates



Fig 15: Pellet of bacteria obtained after centrifugation



Fig 17: Subculturing the bacteria using Trvotic Sov Broth as the culture media



Fig 16: Measuring the initial Optical Density in a UV-Spectrophotometer



Fig 18: Bacteria added to test tubes



Fig 19: UV light sterilization chamber with laminar flow



Fig 20: Specimens sterilized using UV-light sterilization chamber


Fig 21: Specimens placed in test tubes with bacteria for biofilm formation



Fig 22: Adhered bacteria removed from the discs using sonication in an ultrasonic cleaner



Fig 23: Sonicated bacterial suspension transferred to test tubes containing the culture media



Fig 24: Optical Density measured using UV-Spectrophotometer

ATOMIC FORCE MICROSCOPY WITH IN-BUILT SOFTWARE



Fig 25: Atomic Force Microscope



Fig 26: Scanning the specimen



Fig 27:AFM in-built software

ATOMIC FORCE MICROSCOPY IMAGES

Ra VALUES IN INCREASING ORDER







Fig31a: Group1:unpolished: after polishing

Fig31b: Group1: unpolished: after biofilm formation



RESULTS

The surface roughness (Ra) values in nm obtained after AFM analysis for the 4 groups after polishing are presented in Table 1:

TABLE - 1

SPECIMEN	GROUP 1	GROUP 2	GROUP 3	GROUP 4
No.	UNPOLISHED	SOFLEX	SUPERSNAP	NANOSILICA
1	38.569	17.956	23.458	7.953
2	40.933	20.475	22.490	9.903
3	38.964	17.978	24.921	7.361
4	40.536	20.743	24.435	6.335
5	42.000	21.978	23.131	6.842
6	42.469	20.590	24.176	7.355
7	38.134	16.549	23.864	7.913
8	38.676	16.433	22.908	6.033
9	39.649	17.546	23.822	6.527
10	39.479	16.435	24.347	7.414
11	41.361	19.231	23.154	7.193
12	40.897	20.683	24.186	6.200
13	40.009	16.567	24.178	7.413
14	42.546	17.654	23.190	7.001
15	39.698	18.134	24.267	7.054

The mean and standard deviation of surface roughness values of all the 4 groups after polishing are presented in Table 2:

TABLE-2

Group	Mean	Std. Deviation	Std. Error
Group 1	40.262000	1.4202273	0.3667011
Group 2	18.597333	1.8696733	0.4827476
Group 3	23.769333	0.6806244	0.1757365
Group 4	7.233133	0.9357351	0.2416058
Group 3 Group 4	23.769333 7.233133	0.6806244 0.9357351	0.1757365 0.2416058

The surface roughness (Ra) values in nm obtained after AFM analysis for the 4 groups after the removal of Streptococcus mutans biofilm are presented in Table 3:

TABLE-3

SPECIMEN	GROUP 1	GROUP 2	GROUP 3	GROUP 4
No.	UNPOLISHED	SOFLEX	SUPERSNAP	NANOSILICA
1	60.000	23.397	31.572	14.034
2	58.790	22.119	31.438	13.739
3	58.114	24.488	33.043	14.489
4	62.805	22.108	30.186	13.784
5	63.503	23.756	30.591	14.264
6	54.463	24.127	30.084	14.764
7	62.510	24.670	32.114	13.965
8	59.661	23.232	30.780	12.894
9	59.387	24.135	31.256	13.223
10	58.534	24.628	33.964	14.776
11	63.133	22.179	32.175	13.980
12	59.678	24.486	30.889	13.452
13	64.445	20.765	32.854	14.441
14	63.891	23.591	33.156	14.745
15	59.675	22.906	33.348	13.845

The mean and standard deviation of the surface roughness values after biofilm formation for all the 4 groups are presented in Table 4:

TABLE- 4

Group	Mean	Std. Deviation	Std. Error
Group 1	60.572667	2.7365214	0.7065668
Group 2	23.374667	1.1562060	0.2985311
Group 3	31.830000	1.2288787	0.3172951
Group 4	14.026000	0.5684163	0.1467645

OPTICAL DENSITY (OD) MEASUREMENT

Streptococcus mutans adherence on the polished surface of the specimens were measured as the optical density using UV- Spectrophotometer is presented in Table 5:

SPECIMEN	GROUP 1	GROUP 2	GROUP 3	GROUP 4
No.	UNPOLISHED	SOFLEX	SUPERSNAP	NANOSILICA
1	0.834	0.419	0.692	0.321
2	0.898	0.428	0.634	0.335
3	0.964	0.510	0.599	0.360
4	0.890	0.487	0.657	0.411
5	0.778	0.429	0.634	0.372
6	0.831	0.425	0.612	0.407
7	0.823	0.493	0.628	0.323
8	0.901	0.483	0.701	0.334
9	0.971	0.550	0.676	0.400
10	0.767	0.441	0.634	0.312
11	0.853	0.497	0.621	0.410
12	0.876	0.453	0.502	0.333
13	0.790	0.448	0.639	0.413
14	0.881	0.454	0.608	0.390
15	0.859	0.464	0.614	0.331

TABLE- 5

The mean and standard deviation of OD values of all the 4 groups are presented in Table 6:

TABLE- 6

Group	Mean	Std. Deviation	Std. Error
	0.0.000	0.0.601001	0.0155016
Group I	0.863000	0.0621821	0.0155916
Group 2	0.468714	0.0362352	0.0096054
Group 3	0.625643	0.0449300	0.0120223
Group 4	0.366500	0.0379428	0.0099157

STATISTICAL ANALYSIS

In analyzing the results of all the samples, the following statistical techniques were employed after estimation of arithmetic mean and standard deviation:

- ✤ Analysis of variance (ANOVA)
- Post hoc test Tukey HSD
- ✤ Independent sample t- test



STATISTICAL ANALYSIS WITHIN THE GROUPS

STATISTICAL ANALYSIS OF SURFACE ROUGHNESS AFTER POLISHING:

TABLE 7: ANOVA analysis after polishing

AFM values	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8481.047	3	2827.016	1650.433	.000
Within Groups	95.922	56	1.713		
Total	8576.969	59			

TABLE 8: Post Hoc Tests after polishing- Multiple Comparisons

Tukey HSD

I GROUPS	J GROUPS	Mean	Std. Error	Sig.	95% Confidence Interval	
		Difference (I-J)			Lower Bound	Upper Bound
	2	21.6646667*	.4778973	.000	20.399249	22.930085
GROUP1	3	16.4926667*	.4778973	.000	15.227249	17.758085
	4	33.0288667*	.4778973	.000	31.763449	34.294285
	1	-21.6646667*	.4778973	.000	-22.930085	-20.399249
GROUP2	3	-5.1720000^{*}	.4778973	.000	-6.437418	-3.906582
	4	11.3642000^{*}	.4778973	.000	10.098782	12.629618
	1	-16.4926667*	.4778973	.000	-17.758085	-15.227249
GROUP3	2	5.1720000^{*}	.4778973	.000	3.906582	6.437418
	4	16.5362000^{*}	.4778973	.000	15.270782	17.801618
	1	-33.0288667*	.4778973	.000	-34.294285	-31.763449
GROUP4	2	-11.3642000*	.4778973	.000	-12.629618	-10.098782
	3	-16.5362000*	.4778973	.000	-17.801618	-15.270782

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

STATISTICAL ANALYSIS OF Ra AFTER THE REMOVAL OF BIOFILM:

TABLE 9: ANOVA analysis after the removal of biofilm

AFM values	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	18196.113	3	6065.371	2276.235	.000
Within Groups	149.220	56	2.665		
Total	18345.334	59			

TABLE 10: Post Hoc Tests after the removal of biofilm

Multiple Comparisons

Tukey HSD

I GROUPS	J GROUPS	Mean Difference	Std. Error	Sig.	95% Confidence	Interval
		(I-J)			Lower Bound	Upper Bound
	soflex	37.1980000 [*]	.5960593	.000	35.619702	38.776298
control	supersnap	28.7426667*	.5960593	.000	27.164369	30.320965
	nanosilica	46.5466667*	.5960593	.000	44.968369	48.124965
	control	-37.1980000*	.5960593	.000	-38.776298	-35.619702
soflex	supersnap	-8.4553333*	.5960593	.000	-10.033631	-6.877035
	nanosilica	9.3486667*	.5960593	.000	7.770369	10.926965
	control	-28.7426667*	.5960593	.000	-30.320965	-27.164369
supersnap	soflex	8.4553333 [*]	.5960593	.000	6.877035	10.033631
	nanosilica	17.8040000^{*}	.5960593	.000	16.225702	19.382298
	control	-46.5466667*	.5960593	.000	-48.124965	-44.968369
nanosilica	soflex	-9.3486667*	.5960593	.000	-10.926965	-7.770369
	supersnap	-17.8040000*	.5960593	.000	-19.382298	-16.225702

 $\ensuremath{^*}.$ The mean difference is significant at the 0.05 level.

STATISTICAL ANALYSIS OF OD VALUES:

TABLE 11: ANOVA analysis for OD value

OD values

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.103	3	.701	324.306	.000
Within Groups	.121	56	.002		
Total	2.224	59			

TABLE 12: Post Hoc Tests for OD value

Multiple Comparisons

Dependent Variable: OD values

Tukey HSD

I GROUPS	J GROUPS	Mean	Std. Error	Sig.	95% Confidence	Interval
		Difference (I-J)			Lower Bound	Upper Bound
	soflex	.3956667*	.0169751	.000	.350718	.440615
unpolished	supersnap	.2310000*	.0169751	.000	.186052	.275948
	nanosilica	.4944667*	.0169751	.000	.449518	.539415
	unpolished	3956667*	.0169751	.000	440615	350718
soflex	supersnap	1646667*	.0169751	.000	209615	119718
	nanosilica	$.0988000^{*}$.0169751	.000	.053852	.143748
	unpolished	2310000*	.0169751	.000	275948	186052
supersnap	soflex	.1646667*	.0169751	.000	.119718	.209615
	nanosilica	.2634667*	.0169751	.000	.218518	.308415
	unpolished	4944667*	.0169751	.000	539415	449518
nanosilica	soflex	0988000*	.0169751	.000	143748	053852
	supersnap	2634667*	.0169751	.000	308415	218518

 $\ast.$ The mean difference is significant at the 0.05 level.

STATISTICAL ANALYSIS BETWEEN THE GROUPS

TABLE 13: INDEPENDENT SAMPLE T TEST FOR UNPOLISHED GROUP:

Comparing the AFM values after polishing and after biofilm formation

Group Statistics

	GROUPS CODED 1 –	N	Mean	Std. Deviation	Std. Error
	CODED 2 - AFTER BIOFILM				Mean
AFM VALUES	Before biofilm	15	40.262000	1.4202273	.3667011
	After biofilm	15	60.572667	2.7365214	.7065668

AFM VALUES	Levene's for Equa	s Test ality of	t-test for H	-test for Equality of Means							
	Variance	es									
	F	Sig.	t	df	Sig. (2-	Mean	Std. Error	95% Confidenc	e Interval of the		
			tailed) Difference Difference Difference								
								Lower	Upper		
Equal variances assumed	6.511	.016	-25.514	28	.000	-20.3106667	.7960567	-21.9413150	-18.6800183		
Equal variances not assumed			-25.514	21.032	.000	-20.3106667	.7960567	-21.9660055	-18.6553278		

TABLE 14: INDEPENDENT SAMPLE T TEST FOR SOFLEX GROUP:

Comparing the AFM values before and after biofilm

Group Statistics

	GROUPS CODED 1 - BEFORE	N	Mean	Std. Deviation	Std. Error
	BIOFILM				Mean
	CODED 2 - AFTER BIOFILM				
AFMVALUES	1	15	18.597333	1.8696733	.4827476
	2	15	23.374667	1.1562060	.2985311

AFMVALUES	Levene's	Test	t-test for	t-test for Equality of Means							
	for Equa	ality of									
	variance	es		r			r	r			
	F	Sig.	t	df	Sig. (2-	Mean	Std. Error	95% Confide	nce Interval of		
				tailed) Difference Difference the Difference				<u> </u>			
								Lower	Upper		
Equal variances assumed	6.396	.017	-8.417	28	.000	-4.7773333	.5675967	-5.9400025	-3.6146642		
Equal variances not assumed			-8.417	23.342	.000	-4.7773333	.5675967	-5.9505466	-3.6041201		

TABLE 15: INDEPENDENT SAMPLE T TEST FOR SUPERSNAP GROUP:

Comparing the AFM values before and after biofilm

Group Statistics

	GROUPS CODED 1 - BEFORE BIOFILM CODED 2 - AFTER BIOFILM	Ν	Mean	Std. Deviation	Std. Error Mean
AFM VALUES	1 2	15 15	23.769333 31.830000	.6806244 1.2288787	.1757365 .3172951

AFM VALUES	Levene's for Equa	ality of	t-test for Equality of Means						
	F	Sig.	t df Sig. (2- Mean Std. Error 95 tailed) Difference Difference the				95% Confidence Interval of the Difference		
								Lower	Upper
Equal variances assumed	7.440	.011	-22.223	28	.000	-8.0606667	.3627113	-8.8036471	-7.3176862
Equal variances not assumed			-22.223	21.851	.000	-8.0606667	.3627113	-8.8131824	-7.3081510

TABLE 16: INDEPENDENT SAMPLE T TEST FOR NANOSILICA GROUP:

Comparing the AFM values before and after biofilm

Group Statistics

	GROUPS CODED 1 - BEFORE BIOFILM	N	Mean	Std. Deviation	Std. Error Mean
	BIOFILM				
AFM VALUES	1	15	7.233133	.9357351	.2416058
All MI VALUED	2	15	14.026000	.5684163	.1467645

AFM VALUES	Levene	s Test	t-test for Equality of Means								
	for Equality of										
	v arran	ces									
	F	Sig.	t	df	Sig. (2-	Mean	Std. Error	95% Confide	nce Interval of		
					tailed)	Difference	Difference	the Difference			
								Lower	Upper		
Equal variances assumed	.854	.363	-24.029	28	.000	-6.7928667	.2826892	-7.3719292	-6.2138042		
Equal variances not assumed			-24.029	23.094	.000	-6.7928667	.2826892	-7.3775224	-6.2082110		

BAR DIAGRAM OF MEAN SURFACE ROUGHNESS (Ra) VALUES IN nm



<u>GRAPH 1:</u> After polishing of the nanocomposite resin discs

<u>GRAPH 2:</u> After the removal of biofilm from the nanocomposite resin discs



GRAPH 3: BAR DIAGRAM COMPARING THE MEAN OF OD VALUES



INTERPRETATION OF THE RESULTS

SURFACE ROUGHNESS VALUES AFTER POLISHING:

GROUP 1 (UNPOLISHED)

In this group, the nanocomposite resin disc specimens were not polished and the mean surface roughness (Ra) measured using AFM was 40.262000nm, which was the highest among the 4 groups (fig:31a).

GROUP 2 (SOF-LEX)

The specimens in this group which were polished using Sof-lex polishing system had a mean surface roughness (Ra) of 18.597333nm (fig:29a).

GROUP 3 (SUPER-SNAP)

The specimens were polished with Super-Snap discs and the mean surface roughness value was 23.769333nm. This value is higher than that for the Sof-lex system (fig:30a).

GROUP 4 (POROUS NANOSILICA)

The mean surface roughness value for this group was 7.233133nm, which was the lowest among the others (fig:28a).

When analyzing the surface roughness values of all the 4 groups, group 1 (unpolished) showed the highest surface roughness followed by group 3 (Super-snap) and group 2 (Sof-lex). Group 4 (porous nanosilica) showed the smoothest surface in AFM after polishing.

GROUP 1 > GROUP 3 > GROUP 2 > GROUP 4

Within group analysis of the surface roughness values obtained after AFM analysis for the 4 groups was done using one- way ANOVA with Tukey's post hoc tests. The tests demonstrated a highly significant difference (p<.001) between the mean surface roughness of all the 4 groups.

SURFACE ROUGHNESS VALUES AFTER BIOFILM FORMATION:

The mean surface roughness (Ra) after biofilm formation for groups 1, 2, 3 and 4 were 60.572667, 23.374667, 31.830000 & 14.026000 nm respectively (fig:31b,29b,30b,28b).

The adherence of Streptococcus mutans biofilm on the polished nanocomposite surface increased the surface roughness in all the 4 groups. Analysis of surface roughness after biofilm formation of all the 4 groups showed values in the following order:

GROUP 1 > GROUP 3 > GROUP 2 > GROUP 4

Within group comparison was made using one- way ANOVA with Tukey's post hoc tests which revealed a highly significant difference between the mean Ra values of all the 4 groups (p<.001).

OD VALUES SHOWING BACTERIAL ADHERENCE:

The mean of the OD values for the groups 1, 2, 3 and 4 were 0.863000, 0.468714, 0.625643 and 0.366500 respectively. The results showed that the OD value for the group 4

(porous nanosilica) was lower than the other groups, which was statistically significant. Group 1 showed the highest concentration of bacterial adherence followed by group 3, group 2 and group 4 which had the least amount of adhered bacteria.

GROUP 1 > GROUP 3 > GROUP 2 > GROUP 4

One- way ANOVA with Tukey's post hoc tests revealed a highly significant difference between the mean OD values of all the 4 groups.

BETWEEN GROUPS COMPARISON :

The mean AFM values after polishing and after biofilm formation were compared for each group using independent sample t- test which demonstrated that the AFM surface roughness values of each group after biofilm formation was significantly higher than the values after polishing (p<.001).



DISCUSSION

Finishing and polishing are significant procedures after the placement of a resin composite restoration. Proper finishing and polishing have been related to less plaque retention, consequently decreased secondary caries rate and marginal discoloration, thus enhancing the longevity and esthetics of the restoration.⁹⁸

Surface roughness can be expressed as a function of the microrelief of the surface created during the finishing and polishing procedure.²⁰ During these processes, abrasion of resin matrix and filler particles can be accompanied: (i) by the softening of resin matrix due to the production of highly localized heat¹⁴; (ii) by the creation of residual defects and surface flaws caused by dislodgement or debonding of the glass fillers^{14,39,79} and (iii) by scratch lines left by abrasives of greater size.^{26,79} The microrelief of the surface especially voids, cracks and pits is of critical clinical relevance as it has been reported to create protected sites for bacteria.⁶⁵

Polishing is complicated by the heterogeneous nature of dental composite resins with both hard filler particles and soft resin matrix.^{11,45} Resin removal rather than glass filler abrasion during the polishing procedure contributes to the exposure of filler particles and increases the surface roughness.²⁰ In order to effectively polish a resin composite, an abrasive should remove the resin matrix as well as cut the relatively harder filler particles. It has been suggested that the filler particle size, shape, hardness and load have the potential to influence the surface characteristics of a resin composite.^{15,67}

According to Sen et. al $(2002)^{78}$, the polishing of methacrylate resin matrix produced the smoothest surface than the bisacryl resin matrix due to the presence of a homogenous composition.

Pallav et. al (1989)⁶⁷ reported that the filler particles should be situated as close as possible in order to protect the resin matrix from abrasives. Reduced interparticle distance in resin composite is achieved by decreasing the size and increasing the volume fraction of filler particles.

Rough surface of the composite restoration may have influence in the development of discoloration. Coffee, red wine, edible oils may stain the tooth colored restorations by both adsorption and absorption of the colorants into the organic phase of the composite resin.^{19,83,96} To achieve less color change, the smoothest surface finish is mandatory.

Various studies^{44,80,85,102} have reported recently that the nanocomposite have better physical and handling properties than the micro hybrid composites. The nanocomposite resin have nil/ less amount of TEGDMA and increased filler load (82 wt%). The size of the filler particle (40-300nm) is also smaller than the filler particle size in micro hybrid.

In the present study, we have selected Filtek Supreme Z250 XT as the nanocomposite material, which has a homogenous filler structure and is close to that of microfilled composite .Hence it can be classified in the nanofilled composite subclass and the other nanocomposite subclasses being nanofilled hybrid and complex (or blended) nanofilled hybrid composites. It has been used clinically as a universal restoration for both anterior and posterior surfaces. The filler structure includes: surface- modified zirconia/ silica with a mean particle size of approximately 3μ m/less; non-agglomerated/ non-aggregated 20nm surface- modified silica particles and the filler loading is 82% (by wt.) or 68% (by vol.).

Previous studies^{6,80,85} have shown that Filtek SupremeXT has produced smoother surface among all the 3 subclasses of nanocomposites. This result could be related to the specific composition of Filtek Supreme, which contains only nanofillers, which is in the same size range as the microfillers. The nanofillers are discretely dispersed or organized in clusters. These purely inorganic clusters are formed by individual primary nanoparticles bonded between them by weak intermolecular forces. Hence, these nanoparticles may break away from the clusters during wear or polishing.^{60,62,93}

Studies^{24,89} stated that curing composites against a Mylar polyester strip (Du Pont Co., Wilmington, Del.) produced the smoothest surface and the surface had a high glossy finish. But, the surface was rich in unpolymerized resin and resin matrix alone. This surface when exposed to oral environment may undergo degradation and the filler particles were exposed. There was an increase in the rate of plaque accumulation and degradation of the restoration. Therefore, finishing and polishing of the surface of a resin composite restoration is critical in the clinical success.

Many studies^{12,24,74} reported that aluminium oxide discs gave smoother finish than diamond and silicon carbide polishing systems. In accordance with those results, Sof-lex and Super-Snap produced smoother surface than other polishing systems such as Compomaster, Po-Go. This may be due to the size and hardness of the aluminium oxide particles incorporated in the polishing system to cut the filler particles and the resin matrix simultaneously.⁹⁵

Tamoyo Watanbe et. al (2005)⁹¹ reported that Super-snap produced a smooth surface than Compomaster and Enhance system as its ability depended on the cutting property of filler particle and matrix resin equally.

According to a previous study, the load of the finishing device to the surface influences the polishing result.³³ However, it was also reported that the pressure applied by the disk seemed to be less critical for flexible discs like Sof-Lex.³³ In the present study, a single operator performed finishing procedures in order to better simulate clinical conditions.⁴⁵ For the same purpose, immediate polishing was preferred as compared to delayed polishing⁸⁵ as no negative effect on surface roughness was noted.⁹⁷

A surface profilometer was used to measure the average surface roughness of the resin discs for the purpose of standardization of the specimens. The main disadvantage of a profilometer is that it provides only two-dimensional data of the threedimensional surface. For analyzing the surface topography after polishing, AFM was used as it has got a higher resolution (in the level of nanometers) and capability to distinguish surface roughness than profilometer and SEM.^{27,33,43,56} AFM images represents the surface morphology of the specimens caused by the exposed fillers. The high-resolution capacity of AFM permits accurate views of the surface topography, with 3D imaging of individual glass particles. The AFM calculated roughness comes as a complementary and local result to characterize the surfaces. AFM gives a higher lateral resolution (<30 nm) compared to optical profilometry (2µm) and a smaller surface size for investigation (10µm×10µm for AFM and 1000µm×1000µm for profilometry). Hence, AFM roughness is representative of a local order rather than a global roughness provided by the profilometry.³³ Filler size distribution might not be homogeneous and AFM views of the observed area could not be a representative of the entire surface, which is one of the limitation of AFM.^{86,101}

Chemical mechanical planarization (CMP) introduced by Monsanto in 1965 is used to produce mirror- like surfaces with no measurable subsurface damage.⁷⁶ CMP has been traditionally used in the field of engineering for procedures like semiconductor polishing, optical lithography, producing reflecting surfaces for mirrors, lenses and the planarization of computer chips. Colloidal silica with different particle sizes are predominantly used in the different CMP slurries. Colloidal silica are also been used in fields as diverse as catalysis, metallurgy, electronics, glass, ceramics, paper and pulp technology, optics, elastomers, food, health care and industrial chromatography, polishing sophisticated microcircuit parts to outer space and play vital role in the safe reentry of space vehicles. Various modifications have been done in the traditional colloidal silica slurry for improvements in CMP, like the reduction in particle size to produce nanosilica abrasive.

Rajiv et. al (2002)⁷¹ stated that the nanosilica particle abrasive slurry have the smoothest finishing and polishing in chemical mechanical planarization. The nanosilica abrasives with average diameter of 80-90 nm were used to prepare polishing slurry for silicon wafers. The polishing rate was more than 600 rpm and the root mean square (RMS) of surface roughness for polished silicon wafers was less than 0.4 nm. Gaikwad et. al (2008)²⁵ reported that the silica nanoparticle with a diameter of 64 nm produced smoother surface on the tooth, which decreased the caries rate and Streptococcus mutans adherence. The colloidal nano-abrasive particles not only provides high polishing rate, but also achieves a very smooth surface.

The colloidal nanosilica particles tried in previous studies^{49,50,41} were of the compact solid type which is said to cause surface defects owing to high hardness. In our study, we have synthesized and used **porous nanosilica** abrasive which according to recent studies^{35,36,53} are said to exhibit better surface planarization and fewer scratches than traditional solid silica abrasive during the polishing. The porous nanosilica abrasive that we have synthesized in our study through a sol- gel process has a typical hexagonal mesoporous structure with a p6mm pore arrangement belonging to the SBA-15 family of porous structures. The ball milled porous nanosilica particle that we finally obtained had an average particle size of 70 nm in diameter which was characterized using SEM.

Hence in this study we have assessed the polishing ability of the synthesized porous nanosilica abrasive slurry, Sof-lex and Super-snap polishing agents on nanocomposite resin discs, testing the adherence of Streptococcus mutans bacteria on these polished discs and to check whether the bacterial interaction has caused any changes in the surface topography of the polished nanocomposite resin discs.

The results showed that Group 4 (porous nanosilica) produced the smoothest surface among the 4 groups in this study. According to Rajiv et. al $(2002)^{71}$ and Gaikwad et. al $(2008)^{25}$, when the particle size of the abrasive slurry was decreased (to the level of nanometers), the material removal from the particle may also be reduced due to lower stresses (in nanoscales). The degree of surface scratching may be decreased due to the reduced indentation as the abrasive particle size was smaller.

In this study the results showed that the Group 2 (Sof-lex) produced smoother surface than the Group 3 (Super-Snap) with statistical significance (p<0.05) which is in accordance with the results of previous studies conducted.⁶² Increased smoothness of Sof-lex polished surface may be due to the fact that the abrasive particle size in Super-Snap (ultrafine disc has particle size of 8µm) is larger than that of Sof-lex (ultrafine is 7μ m).

The mechanical properties of a restoration can also be judged by its biological properties such as anti- plaque effect. In general, the adherence of microorganism is considered to be of utmost importance for the longevity of a restoration. It may lead to recurrent caries, microleakage etc. The adhesion of microorganism seems to be strongly dependent on the surface roughness. The other factors include the type of resin matrix, hydrophobicity of the surface and the unpolymerized monomer on the outer surface of the restoration.^{72,82,90} Therefore, the bacterial adherence study provides another parameter to describe the surface roughness.

Biofilm formation coincided with surface roughness and increased exposure to inorganic, positively charged elements in the surface. Thus, in composite resin, the exposure of fillers like Si⁺⁺, Al⁺⁺⁺ and Ba⁺⁺ were increased which in turn led to a considerable decrease in the ratio between the organic and inorganic compounds. The most of the bacteria- binding salivary pellicle constituents are acidic in nature and are positively charged resulting in increased formation of biofilm.

Some authors stated that bacteria on the rough surface of the restoration decreased the pH of the restoration. Hence, degradation of the surface occurs by

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disintegration of the resin matrix which exposes the filler particles. This may lead to increase in the surface roughness.^{57,64,90}

In this study, the Streptococcus mutans biofilm was allowed to form over the polished surfaces of the specimens in each group by culturing it in an incubator at 37°C for 24hrs. The bacteria adhered on to the polished surfaces were removed by sonication in an ultrasonic bath. The bacterial suspension obtained was cultured in Tryptic Soy Broth for one day and the optical density (OD) of the bacterial solution was taken using a UV- Spectrophotometer.

The least bacterial adherence is showed by group 4, the nanosilica group which has produced the smoothest surface after polishing, corroborating the finding that bacterial adherence over the composite restoration can be effectively minimized by effective finishing and polishing techniques. The roughest surface of unpolished group has attracted the maximum amount of bacteria.

Results of the studies conducted by Ralf Buerger et. al $(2009)^{72}$ indicated that Streptococcus mutans bacterial adherence seems to be strongly dependent upon the type of matrix used, filler size and the chemical composition of the resin composite used. They found that the silorane- based composition have a lower susceptibility to adhere streptococci. Eugenio Brambilla et. al $(2009)^{21}$ reported that the curing time is also one of the crucial factors in determining the biological behavior of composite resins. But in our study we have not assessed the variations in resin chemistry and curing time which was kept at uniform.

According to the reports of Nurit et. al $(2008)^{64}$, Suzana et. al $(2008)^{90}$, the Streptococcus mutans biofilm changes the surface topography of the nanocomposite and micro hybrid composite resins.

In the present study, the average surface roughness (Ra) was increased in all the 4 groups tested after the biofilm formation as the bacterial adherence degraded the nanocomposite resin surface. This was marked in case of group 1 as there was increased bacterial adherence, which was statistically significant (p<0.001). In this study, the changes in surface topography obtained after the Streptococcus mutans biofilm formation were in the following order: **GROUP 4** (**POROUS NANOSILICA**) < **GROUP 2** (**SOF-LEX**) < **GROUP 3** (**SUPER-SNAP**) < **GROUP 1** (**UNPOLISHED**) (p<0.05). The porous nanosilica (group 4) showed the least increase in surface roughness after biofilm formation because it had accumulated the least amount of Streptococcus mutans which was confirmed by our OD values. So, in this study it was proved that efficient polishing can decrease the bacterial adherence and surface degradation which is the main factor that causes secondary caries formation and ultimate failure of a composite restoration.

The biodegradation resistance of composite resin materials may also be contributed in part to the presence of Bis-EMA in its matrix composition. The Filtek Z250 ZT used in this study has a resin chemistry of Bis-GMA, UDMA, Bis-EMA, PEGDMA and TEGDMA. Nanocomposite resins containing the ethoxylated version of Bis-GMA, the Bis-EMA showed a lower amount of release of by- products and was highly stable. Yap et. al (2005)¹⁰⁴ also showed that the hardness, surface roughness and shear strength of a Bis-EMA- based composite was not affected by food, liquids, including lactic acid. This is due to decrease flexibility and elimination of the hydroxyl groups from the Bis-GMA monomer to Bis-EMA, thus decreasing the hydrophobicity of the monomer. Hence, the reduction in water uptake may be partially responsible for the biochemical stability of the composites that are composed of this monomer.

Meth acrylic Acid (MA) and Tri-ethylene glycol (TEG) are the hydrolyzed by-products of TEGDMA, the primary diluent co-monomer in many composite resins. In varying concentration range, TEG was effective in bacterial growth stimulation and MA was shown to inhibit bacterial growth. TEG and MA could interfere with various cellular activities such as nutrient uptake, signal transduction and gene expression when it comes in direct contact with oral bacteria. Thus, the presence of excess amount of TEGDMA in cured composite resin is also one of the factors that increase the growth of oral bacteria.

Within the limitations of the present study, it was concluded that polishing of nanocomposite resins with porous nanosilica abrasive slurry gave a smoother surface topography than the commercially available Sof-lex and Super-snap polishing systems. The smoother nanocomposite resin surface reduces the adherence of bacteria, thereby the longevity and the aesthetics of the restoration.



SUMMARY

The purpose of this in vitro study was to evaluate the surface topography of nanocomposite resin discs using Atomic Force Microscope (AFM) and the adherence of Streptococcus mutans biofilm on the surfaces after polishing using two different commercial polishing kits and indigenously prepared porous nanosilica abrasive.

Sixty nanocomposite resin discs were prepared from Filtek Z250 XT (3M ESPE Dental Products, St. Paul, MN, USA) using a custom made aluminium mold of dimensions 10mm×2mm. The specimen surfaces were standardized using a profilometer and were divided into 4 groups of 15 samples:

GROUP 1-UNPOLISHED (n=15)

GROUP 2-POLISHED USING SOF-LEX (n=15)

GROUP 3-POLISHED USING SUPER SNAP (n=15)

GROUP 4-POLISHED USING POROUS NANOSILICA ABRASIVE (n=15)

Porous nanosilica was synthesized by a sol- gel method and was ball milled for 30 hrs to produce nanoparticles of 70 nm size, which was characterized using SEM. After polishing using the respective methods, AFM analysis was done.

Freeze dried Streptococcus mutans were regenerated and were allowed to form a biofilm over the polished samples and the concentration of bacterial adherence was quantitatively measured as OD values using UV-Spectrophotometer. The changes in surface topography after biofilm formation was again assessed using AFM. Statistical analysis was carried out and the results were found to be highly significant (p<.0.001). The AFM surface roughness values after polishing were of the following order:

GROUP 1 > GROUP 3 > GROUP 2 > GROUP 4

The OD values of the amount of bacterial adherence were of the following order:

GROUP 1 > GROUP 3 > GROUP 2 > GROUP 4

The AFM surface roughness values after biofilm formation were of the following order:

GROUP 1 > GROUP 3 > GROUP 2 > GROUP 4

So in this study, the indigenously prepared porous nanosilica abrasive slurry produced the smoothest surface topography after polishing, adhered the least amount of Streptococcus mutans bacteria and the least surface degradation after the biofilm formation.


CONCLUSION

Within the limitations of this in vitro study, the following conclusions were drawn:

- Composite restoration should be polished to produce a smooth surface.
- The smoothest surface was produced by porous nanosilica abrasive slurry than the commercially available polishing systems- Sof-lex and Super Snap.
- The Streptococcus mutans bacterial adherence was lowest in the porous nanosilica group, which produced the smoothest surface after polishing.
- After the biofilm formation, the roughest surface was produced by the unpolished surface due to surface degradation and porous nanosilica group showed the least increase of surface roughness and degradation.

This study emphasized the concept that a highly polished restoration surface will adhere fewer bacteria and degrades less. The notorious factor behind the clinical failure of composite restoration, the secondary caries can be effectively reduced by this nanopolishing technique using porous nanosilica abrasive, which has been shown to be highly successful when compared to the traditional micropolishing methods for composites.



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