

**COMPARATIVE EVALUATION OF PENETRATION OF TWO
DIFFERENT GENERATIONS OF BONDING AGENTS
IN DENTIN FOLLOWING REPLACEMENT OF CLASS I
AMALGAM RESTORATION OF PREMOLARS WITH
MORE ESTHETIC COMPOSITE RESIN:
AN IN VITRO CONFOCAL MICROSCOPIC ANALYSIS**

Dissertation submitted to

Maharashtra University of Health Sciences, Nashik

in the Partial Fulfillment of Regulations

for the award of the Degree of

MDS

IN

CONSERVATIVE DENTISTRY AND ENDODONTICS

BRANCH IV

2020

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LIST OF ABBREVIATIONS



Sr. No	Abbreviation	Full Form
1.	SEM	Scanning Electron Microscope
2.	TEM	Transmission Electron Microscope
3.	EDX	Energy Dispersive Xray
4.	CLSM	Confocal Laser Scanning Microscope
5.	OSHA	Occupational Safety and Health Administration
6.	CDC	Centre for Disease Control
7.	LED	Light Emitting Diode
8.	s	Seconds
9.	SPSS	Statistical Package for the Social Sciences
10.	μm	Micro meter
11.	S	Significant
12.	NS	Not Significant
13.	HS	Highly Significant
14.	N	Number of specimens
15.	n	Number of specimens in each group
16.	p-value	Probability of obtaining a test statistic at least as extreme as the one that was actually observed.
17.	max.	Maximum
18.	min.	Minimum
19.	no.	Number
20.	CI	Confidence Interval
21.	mm	Milli Meter
22.	CEJ	Cemento-Enamel Junction
23.	mW/cm^2	Milliwatt/square centimetre
24.	RDI	Resin-Dentin Interface
25.	UNEP	United Nations Environmental Programme
26.	Ag	Silver
27.	Hg	Mercury
28.	Sn	Tin
29.	Zn	Zinc

Sr. No	Abbreviation	Full Form
30.	F	Fluorine
31.	Al	Aluminium
32.	Cu	Copper
33.	RDIZ	Resin dentin interdiffusion zone
34.	nm	Nano meter
35.	°C	Degree Celcius
36.	ver	Version
37.	%	Percentage
38.	i.e.	That is

Introduction

“ Change is Inevitable. Change is Constant.”

-Benjamin Disraeli

Operative dentistry has always been regarded as a complete clinical practice of dentistry. Operative dentistry being a foundation of dentistry, has also provided a base over which other disciplines of dentistry evolved.¹

Evolving from “barber-dentists” sent from England, dentistry organised itself in the United States during 17th century. Back then dental practice was confined to tooth extractions. **G.V. Black (1896)** standardised cavity preparation and experimented with various mixtures of silver alloy with mercury, thus contributing immensely to the dental profession. Black’s research was further enhanced by his son, **Authur Black (1870–1937)**.²

Dental caries, a chronic disease known to human kind, is one of the most frequently occurring health problem in today’s world.³ The destruction of dental hard

acellular tissue by acidic by-products from the bacterial fermentation of dietary carbohydrates especially sucrose, cause caries. Dental caries being a major oral health disease, jeopardizes the achievement and maintenance of oral health in all age groups.⁴

The “dictum for oral health” in dentistry is to eliminate dental caries completely. However, caries remains to be omnipresent. A majority of the population needs or already has dental restorations. Once dental restorations are placed, their lifetimes are affected by multitude of factors which vary immensely. Decision of the choice material depends upon the knowledge of the properties of different materials available.⁵

Dental amalgam, probably introduced by **Monsieur Travaux** of Paris in the year 1826, was usually prepared by triturating silver coins, with mercury. The introduction of a dental amalgam alloy is attributed to **Dr. G.V. Black** of the USA in the year 1895, with a silver tin composition which is the forerunner of the modern day dental alloys. Since last 150 years the dental amalgam has been the most popular and effective filling material used from the perspectives of longevity, mechanical performance and economics.

Silver amalgam restorations comes with their own drawbacks which cannot be neglected in the present scenario:¹

- The presence of mercury limiting its usage to the posterior teeth.
- Require excessive tooth structure removal during cavity preparation and are unaesthetic.
- As amalgam does not bond to the tooth structure, micro-leakage immediately following placement of the restoration is unavoidable.

- Marginal deterioration: The “ditching” around amalgam restorations are considered to be a stress/corrosion dependent defect occurring in areas subjected to occlusal loading. It was observed that secondary caries is one of the most important factor leading to the replacement of amalgam restoration.⁶

In **1926**, **Alfred Stock**, Ph.D., a German chemist, published an article condemning amalgam restorations.⁷ **Dr. Stock** had been exposed to high mercury levels while working in his chemical laboratory.

In the **1970s**, **Dr. Hal Huggins** promoted the theory that amalgam restorations can cause a wide variety of diseases,⁷ including neurological, cardiovascular, immunological, collagen, emotional and allergic diseases.

A **1995 survey** reporting that 8.7 percent of dentists wanted to ban amalgam use and that 14.3 percent were undecided about its safety was published.⁷ The “60 Minutes “ segment broadcasted in 1990 in the media steered most of the opposition to amalgam.⁷

High oral levels of mercury vapour was revealed by **Berglund A (1990)** in people with amalgam restorations than people who do not have amalgam restorations.⁸

The Governing council of the United Nations Environmental Programme (UNEP) in **February 2009** unanimously agreed on the need to develop a global legally binding instrument, or treaty, on mercury, called the **Minamata Convention on Mercury** with effect from **16th August 2017**. Efforts have been taken to phase down and restrict the use of dental amalgam in accordance to the products

subject to article 4, paragraph 3. Convention also promotes research and development of quality dental restorations which are free from mercury.

As suggested in late nineteenth and early twentieth century, dentinal staining may be due to penetration of Ag and Hg ions from the overlying amalgam and deposits of metallic sulphides.⁹ **Scholtanuset et al.**¹⁰ revealed that dentin adjacent to an amalgam restoration was discoloured due to penetration of amalgam corrosion products deep into dentinal walls. It has been suggested that there is a relationship between ion penetration, discoloration, and demineralization.¹¹

The survival and modes of failure of amalgam restorations were retrospectively analyzed by **Letzel**. The study revealed that leading mode of failure was bulk fracture (4.6%), followed by tooth fracture (1.9%) and marginal ridge fracture (1.3%).¹²

Thus, as quoted by Heraclitus, Greek philosopher (540-480 BC),

“ Nothing endures but change.... ”

Hence, many amalgam restorations need to be replaced or repaired due to microleakage, recurrent caries, bulk amalgam fracture, or aesthetic demands of the patient.¹³

Buonocore in 1955, initiated use of composite resins, which unquestionably acquired a prominent place among the filling materials. As stated by **Terry DA et al. (2004)** “their considerable aesthetic possibilities give rise to a variety of therapeutic indications, which continue to grow as a result of great versatility of the presentations offered; also, these materials conserve tooth structure better because they are retained by adhesive methods rather than depending on cavity design.”¹⁴

In the current scenario, composite resins in conjunction with dental adhesives has evolved as the material of choice for replacement of existing amalgam restorations. However, after the removal of amalgam, dentin shows marked dark discolouration that is attributed to the penetration of metal ions into dentin which may affect the adhesive bond. **Carvalho RM et al. (2012)** has pointed out that reliable adhesion to remaining tooth tissue is paramount for durable composite restorations using adhesive techniques.¹⁵

Ghavannasiri M et al.¹⁶ in their study used SEM and Energy Dispersive X-ray (EDX) to analyze that the amount of Sn, Cu, Ag, and Hg at a 1000X magnification of the dentin. They found tin, copper, mercury in majority of the analysed samples.

Bonding to enamel is relatively simple process when compared to bonding to dentin, which presents a much greater challenge. Dentin thickness has a great impact on adhesion after tooth preparation. **Suzuki et al.** stated that bond strength is generally less in deep dentin than in superficial dentin.¹⁷ Dentinal adhesion depends on the penetration of adhesive monomers into the network of collagen fibrils which are exposed after acid etching. Different adhesive materials may interact with dentin in different ways-mechanically, chemically or both.¹⁸

Harnirattisai C, reported that there is no difference between self-etch and total-etch adhesives when bonding to discoloured dentin.¹⁹ In a different study it was found that the bond strength to darkened dentin to be lower than that of intact dentin.^{20,21} **Harnirattisai et al.** showed similar results, proving that bond strengths to dark dentine after amalgam removal are less than that to the normal dentin.¹⁹

Additionally, a study with scanning electron microscope (SEM) has showed that many dentinal tubules in discoloured dentin were open, but the conception of plasma proteins in dentinal fluid adjacent to corrosion products actually reduces the permeability of the dentin and intervenes with the infiltration of resin monomer.²² Therefore, discoloured dentin must be considered as a different substrate in clinical procedures when collate with sound, unaltered dentin.^{23,24}

Ghavamnasiri et al.²⁵ reported marked improvement in the dentinal marginal seal comparable to an initially placed composite, when the cavity wall was extended 0.5 mm into non-discoloured dentin after removal of an old amalgam restoration.

Methods used routinely to visualize bonded structures such as a hybrid layer and resin tags within dentin are transmission electron microscopy (TEM)^{26,27} and scanning electron microscopy (SEM).^{28,29} Confocal laser scanning microscopy (CLSM), in combination with fluorescent dyes, is a valuable new technique for the visualization of bonding structures such as a hybrid layer and resin tags in dentin. The superiority of confocal microscopy include non-destructive examination, as the layer envisioned can be up to 100 µm underneath the surface. Moreover, drying of samples, which is indispensable for conventional SEM or TEM, is not mandatory with CLSM. This decreases risk of shrinkage or other artefacts due to desiccation.^{30,31} **Watson and Boyde (1987)** were among the first to investigate the behaviour of adhesive resins on the dentin surface using the CLSM.³² CLSM serves many advantages, which includes, the ability to control depth of field, elimination or reduction of background data away from the focal plane, and the ability to collect sequential optical sections from thicker specimens.³³

Since it has been correctly said,

“ You arrive at precision....

When you become precise in your technique.”

The purpose of this study was to analyse the depth of penetration of adhesives by using Confocal Laser Scanning Microscope in premolars with Class I amalgam restorations replaced with composite restorations.

The nulls hypothesis proposed was, there is no significant difference in the penetration of two commercial bonding agents in dentinal tubules, when old silver fillings are replaced with composite resin restoration.

Aim and Objectives

Aim

To evaluate the effectiveness of penetration of two different adhesive bonding agents in case of replacement of existing amalgam restorations with restorative composite resin.

Objectives

1. To assess the penetration of Tetric N-bond and Tetric N-bond Universal in Class I cavity restored with composite resins.
2. To assess the penetration of Tetric N-bond and Tetric N-bond Universal in Class I cavity restored with silver amalgam and replaced with restorative composite resins.
3. To compare both the bonding agents (Tetric N-bond and Tetric N-bond Universal) under CLSM-imaging.

Review of Literature

The answers you get from literature depend on the questions you pose.

-Margaret Atwood

The current strategies for management of teeth requiring replacement of old silver amalgam fillings with composite resins, is based on the evolution of different adhesive materials and techniques combined with the ever-growing expertise and understanding of the clinician. Therefore, it is imperative to know these materials, techniques that various researchers have used and also, the difficulties faced over the years for the long-term survival of such composite resin restorations.

According to **Applebaum E (1929)**⁹ it is a very common observation that dentin underneath previous amalgam restorations shows extensive black discolouration. He suggested that dentin staining might be the result of the penetration of Ag and Hg ions from the overlying amalgam and deposition of metallic sulphides.

Massler M and Barber TK (1953)²⁴ analyzed the effects of amalgam fillings on the structure of the enamel and dentin. Three-hundred freshly extracted teeth, each

with an old amalgam restoration were hemisected and examined under a binocular dissecting microscope. A smaller number of tooth sections were examined under higher magnification by reflected light and in ground sections. The dentin underlying the amalgam restorations were found to be discoloured. A series of tests suggested that the discolouration might be the result of impregnation with metallic particles derived from the amalgam. They reported that mercury (Hg), silver (Ag), tin (Sn), zinc (Zn), and copper (Cu) were found in discoloured dentin by spectrographic analysis.

Sonemark R et al. (1968)³⁴ studied the efflux of metal ions from metal restorations and their penetration into dental tissues. The results showed significant concentration of metal ions in both enamel and dentin from amalgam, chromium-cobalt, and cast gold restorations after short periods in or on the teeth. They found considerable amount of Hg into dentinal tubules besides Zn and Sn, within short evaluation periods in artificially corroded amalgam restorations.

Mateer RS et al. (1970)³⁵ found that the dentin along the tooth-amalgam interface is exposed to corrosion products in several ways: the fluid in the marginal gap being a transport medium released by corrosion of amalgam at the interface can form complexes but might also diffuse into the fluid filled marginal gaps and subsequently into the underlying hydrophilic dentin.

According to **Kurosaki N et al. (1973)**¹¹ the superficial layer of softened dentin has deposits of Sn and Zn in high concentrations and with time it decreases in the superficial layer and increases in the deeper layer of softened dentin.

Application of cavity varnish under amalgam restorations has been a regular treatment over time. The rationale was to reduce the initial micro-leakage and to prevent discolouration of tooth structures by prevention of ion penetration.

Tveit and Hals³⁶ conducted a study with an aim to assess the effect of silicate cement of Copalite-covered cavity walls in extracted human teeth. Class V cavities were prepared in 24 premolars and filled with silicate cement (Bio-Tray). Four cavities were unlined, the rest of the cavities were lined with one or two layers of Copalite before insertion of the restorations. After 6 months, 70-100 microns thick longitudinal sections of the teeth were studied by polarized light microscopy, microradiography and electron probe microanalysis. When imbibed in water or quinoline, a subsurface zone of altered birefringence was noticed in nearly all cavity walls. By electron probe microanalysis F (0, 4-3% by weight), Zn (1-4%) and Al (0,2-6%) were measured in the outer 10-60 microns of the cavity walls. Thus, they demonstrated that copal varnish is no barrier for Zn ions.

Nakabayashi et al. (1982)³⁷ described the hybrid layer as the interdiffusion zone of demineralized intertubular and peritubular dentin and polymerized resin and proposed this principle of micromechanical interlocking as a prime mechanism of bonding.

In 1987, Watson and Boyde³⁸ for the first time described the use of fluorescent confocal microscopy for analysis of the interface of restorative materials and tooth structure. They advocated the use of fluorescent dyes, mixed into components of an adhesive system, to highlight the bonded interface.

Watson T (1989)³⁹ conducted a study to examine the interface between Scotchbond 2 and tooth tissue to determine its distribution within the tooth and

overlying restoration. The distribution of the Scotchbond 2 in the tooth was localized to the smear layer and dentin upto 50 microns deep into it. The sections showed excellent adaptation of the adhesive to the tooth surface, with considerable penetration into the etched enamel structure.

Van Meerbeek et al. (1992)⁴⁰ conducted a research to evaluate the morphological aspects of the resin dentin interdiffusion zone (RDIZ) with different dentin adhesive systems. They concluded that the application of these adhesive systems induced structural changes in the dentin surface morphology, creating a retentive interface, called the inter-diffusion zone, between the deep, untouched dentin layers and the composite filling material. This resin-dentin interdiffusion zone offers bonding sites for copolymerization with the resin composite and, concurrently, might have protective potential for pulp tissue as it blocks the normal passage of microorganisms and toxin.

Pashley et al. (1993)⁴¹ compared the substructure of fracture dentin with that of smear layer-covered dentin, before and after acid etching, by high-resolution SEM. They identified the surface porosities in dentin that permitted resin infiltration during dentinal bonding. The results indicated that the most ideal dentinal substrate for bonding resins to dentin, with systems designed to infiltrate resin into the dentinal matrix, would be the demineralized dentin just beneath the surface of dentin that was acid etched and never air dried. However, the act of acid etching, at least with a solution of 37% phosphoric acid for 30 seconds, seemed to reduce the potential porosity of dentin, as revealed by the difference between the arrangement of collagen fibres at the surface and that beneath the surface, by creating a very thin surface film of condensed collagen fibres. This was even more exaggerated in dentin that had been covered by a smear layer prior to acid etching.

Nakajima M et al. (1995)²⁰ in their study supported the hypothesis that bonding to caries-affected dentin with All Bond 2 and Clearfil Liner Bond II produces bond strengths lower than these systems achieve to normal dentin.

Pioch T et al. (1997)⁴² reported that CLSM offers a wealth of new information about bonding morphology and, therefore, should be used in addition to conventional methods.

According to **Mjor IA et al. (2002)**¹³ the clinical diagnosis of secondary caries is the main reason for replacement of all types of directly placed restorations. Small defects of secondary caries, stained and degraded margins may be removed by refurbishing/ refinishing procedures. These approaches can save tooth structure and be cost effective.

Wilson MA et al. (2002)⁴³ conducted a clinical trial to evaluate the performance of a low-shrinking resin composite compared with amalgam for restoration of class I and II cavities in posterior teeth in general practice setting and concluded that the resin composite evaluated, when used in conjunction with the recommended adhesive system, may be an appropriate alternative to amalgam restoration in posterior teeth over one year in clinical service.

Hashimoto M et al. (2003)⁴⁴ studied the degradation patterns of different adhesives and bonding procedures and stated that the bond degradation is associated with resin elution for the total-etch adhesive (GLUMA One Bond) of the wet bonding and the self-etching primer system (One-Up Bond F).

Calneto J et al. (2004)⁴⁵ compared the effect of a self-etching primer and a non-rinse conditioner with the effect of a conventional adhesive system on the

penetration depth in dentin of human teeth, using Scanning Electronic Microscopy (SEM). The authors concluded that the self-etching primer and the non-rinse conditioner provide a lower penetration depth in human tooth dentin than the conventional adhesive system.

Bitter K et al. (2004)⁴⁶ evaluated the resin-dentin interface of different adhesive systems and corresponding luting cements proposed for bonding fibre-posts to root canal dentin. The study stated that conditioning of the root canal dentin with phosphoric acid and the use of one- and two bottle-bonding systems give a thicker and more uniform hybrid layer with considerably more resin tags than observed with 'self-etching' adhesives. This might provide a more durable bond of the post to root canal dentin.

Luz MA et al. (2005)⁴⁷ investigated the dentin-resin interdiffusion zone using two different self-etching bonding systems, and compared them with a total-etch bonding system using SEM. The descriptive analysis of the resin-dentin unions in the different self-etching resin bonding systems showed that all of them had formed an interdiffusion zone. However, each one of them presented its own characteristics as to thickness of the hybrid layer, formation of resin tags, and degree of its penetration, besides adhesive layer aspects. They proposed that these differences probably occurred because of the proper characteristics of each bonding system that ultimately determine the degree of smear layer removal, underlying dentin demineralization, adhesive wettability and diffusion through dentin. The application technique for each system could also have an influence.

Kenshima S et al. (2006)⁴⁸ studied the conditioning effect on dentin, resin tags and hybrid layer of different acidity self-etch adhesives applied to thick and thin

smear layer and observed that the etch-&-rinse adhesive presented the thickest hybrid layer and was the only adhesive to produce resin tags in high density and uniform distribution along the whole dentin surface, independently of the smear layer thickness.

Harnirattisai C et al. (2007)¹⁹ concluded that the bond strengths of Single Bond and Clearfil SE Bond to normal dentin were greater than to discoloured dentin. However, no differences were found between the bond strengths to the discoloured dentin of both adhesives.

Baweja PS et al. (2007)⁴⁹ evaluated the hybrid layer and resin tag formation of a total-etch technique in comparison with self-etching primers with three different pH on Confocal Microscope. The Mean lengths of resin tag formation and thickness of hybrid layer formation in Group I (Blot dry) Prime & Bond NT (Dentsply) was maximum followed by Group IV [Mild self-etch systems having pH of about 2: Clearfil SE bond plus primer (Kuraray)]; Group II [Strong self-etch adhesives usually with a pH of 1 or below (Adper Prompt L-Pop (3M ESPE))] and Group III [Intermediary self-etch adhesives with a pH of about 1.5: AdheSE (Vivadent)]. Thus, they concluded that Prime & Bond NT (Group I) performed better than all the three self-etching primers in terms of resin tag formation & hybrid layer thickness.

Sauro S et al. (2008)⁵⁰ conducted a comparative study using a double-staining/confocal microscopy technique to evaluate the micro-permeability of several self-etching and etch-and-rinse adhesives and concluded that micro-permeability was detected in all the adhesives. DC-Bond, G-Bond, and Scotchbond 1XT showed voids along the resin-bonded interface. Silorane and Optibond FL showed an adhesive layer that was free of micro-permeability.

Scholtanus et al. (2009)¹⁰ reported that discoloured dentin underneath amalgam restorations containing amalgam corrosion products is demineralised and hence it must be considered a different substrate for clinical procedures than sound dentin.

Ghavamnasiri et al. (2012)²⁵ reported that after amalgam removal, a 0.5mm extension of the cavity walls could improve the dentinal marginal seal to replicate that of an initial composite restoration.

Marigo et al. (2012)⁵¹ conducted a study to evaluate the resin-root canal dentine interface of four “etch and rinse” adhesive systems and concluded that all the adhesives produced good results in terms of hybrid layer thickness and resin tag density.

Vashisth P et al. (2012)⁵² analysed and compared the changes in the ultra-morphology of dentin in primary teeth using different bonding agents and to study the resin/dentin interface produced by them on SEM. They reported that the three step bonding agent completely removed the smear layer. While the self-etch approach was not efficient in removing the smear layer and opening of the dentinal tubules. The longest resin tags with lateral branches were seen in 2 groups: Scotch Bond multipurpose and Optibond FL.

Kallepalli S et al. (2014)⁵³ conducted a study to evaluate the resin-dentin interface of three adhesive systems used for bonding the root canal under Scanning Electron Microscope (SEM). Statistical analysis showed that Group I (“Etch & rinse” adhesive system) showed highly statistical significant difference when compared with other groups. Thus, maximum thickness of hybrid layer and long resin tags were shown by etch & rinse adhesives followed by self-etch adhesives.

Ghavamnasiri et al (2015)¹⁶ reported that there is no need to extend the cavity walls of the preparation when replacing amalgam restorations with composite resin to obtain the same level of bond strength as the original composite restoration.

Ramya MK et al. (2015)⁵⁴ in their in-vitro study evaluated the resin-dentin interface using a self-etch and a total-etch adhesive system bonded to moist dentin using Confocal Laser Scanning Microscope. The occlusal surfaces of forty intact non-carious human premolars were grounded to expose the dentin. Group I (20): self-etch adhesive system [Filtek P90, 3M ESPE]. Group II (20): Total-etch adhesive system [Adper single bond plus, 3M ESPE]. A very high statistical significance ($P < 0.001$) was obtained among the groups. The resin dentin interface of conventional total-etch adhesives performed better than newer self-etch adhesive system in terms of resin tag length and hybrid layer thickness.

Awad M (2017)⁵⁵ in his in vitro study assessed the RDI morphology of two ethanol based universal adhesives using SEM. He found that both the universal adhesives could penetrate the dentin forming well-defined resin tags and lateral branches. He concluded that both the tested adhesives were one-step self-etch adhesives and the water-ethanol combination in both the adhesives may have helped to dilute the viscous monomers and facilitate its infiltration into dentin.

Ferreira JC et al. (2017)⁵⁶ carried out a research that aimed to analyse the morphology of RDI yielded by two step etch and rinse adhesive with different solvents. A total of 32 dentin disks were prepared and randomly assigned to four groups: Group I- Adper Scotchbond-IXT (ethanol/water): Group II-XP-Bond (tertiary butanol): Group III- Prime and Bond NT (acetone): and group IV- One Coat bond (5% water). SEM evaluation of the RDI revealed that adhesive systems with different

solvents led to significant differences in the interface morphology. The adhesives containing tertiary butanol seemed to originate a good quality hybrid layer, long and entangled tags and also appear to have a greater ability to originate microtags.

Rajaram N et al. (2018)⁵⁷ had done a study to analyse the morphology of dentinal tubules in carious lesions using confocal microscopy. Undecalcified ground sections of human teeth were stained with alizarin red and examined by confocal microscopy. The dentinal tubule morphology and the zones of dentinal caries were studied, and the histopathological changes of dentinal tubules showed areas of demineralised dentin, translucent dentin, and normal dentin. The study thus showed that confocal laser scanning microscope has the ability to focus through a carious lesion into the underlying sound dentin.

Materials and Method

Methodology is applied Ideology.

- *Mason Cooley*

Eighty freshly extracted human premolars were selected for the study. The teeth were cleaned, disinfected and stored as per the recommendations and guidelines laid down by OSHA and CDC (2003 report 17).⁵⁸ The selected teeth were stored in phosphate buffer saline solution (Severn, Biotech).⁵⁹

Approval from the Institutional ethical committee was taken for the study.

Selection criteria:

Inclusion criteria:

1. Sound premolars with intact marginal ridges.
2. Teeth extracted either for orthodontic or periodontal purposes were selected.

Exclusion criteria:

1. Teeth with caries, trauma, fractures or other defects.
2. Teeth with developmental anomalies.

Armamentarium:

Instruments and equipments:

- Excavator (GDC) (PLATE-I)
- Explorer (GDC) (PLATE-I)
- Straight probe (GDC) (PLATE-I)
- Pair of tweezers (GDC) (PLATE-I)
- Hand Scaler (Satelec P5 Newtron Worktop Scaler, Satelec Acteon)
- Cotton holder (GDC) (PLATE-I)
- Waste receiver (GDC) (PLATE-I)
- Diamond points (Mani) (PLATE-I)
- High speed airtor (NSK) (PLATE-II)
- Digital Vernier calliper (WorkZone Hand Tools) (PLATE-II)
- Williams Graduated Probe (GDC) (PLATE-II)
- Amalgam carrier (GDC) (PLATE-III)
- Parallelogram condenser (GDC) (PLATE-III)
- Round condenser (GDC) (PLATE-III)
- Ward carver (GDC) (PLATE-III)
- Hollenback carver (GDC) (PLATE-III)
- Frahm carver (GDC) (PLATE-III)
- Amalgamator (Mixalloy Amalgamator, Rhos Brazil) (PLATE-IV)
- L-Mould (PLATE-IV)
- Composite instruments (GDC) (PLATE-IV)
- Composite finishing & polishing kit (Shofu Dental Corporation) (PLATE-IV)

- LED Light curing gun (Bluephase N MC, Ivoclar Vivadent, Schaan/Liechtenstein) (PLATE-V)
- Incubator (Lyzer) (PLATE-V)
- Precision cutting saw (IsoMet 5000, Buehler) (PLATE-XIV)
- Grinder & polisher (Buehler) (PLATE-XIV)
- Confocal Laser Scanning Microscope (ZEISS with LSM Software ZEN 2007) (PLATE-XIV)

Materials:

- 0.9% normal saline solution (Eurolife healthcare) (PLATE-VI)
- Artificial saliva (Wet Mouth, ICPA Health Products Limited) (PLATE-VI)
- Silver Alloy (DPI) (PLATE-VI)
- Mercury (DPI) (PLATE-VI)
- Cavity varnish (Namuvar) (PLATE-VII)
- Dye Rhodamine B isothiocyanate (Sigma-Aldrich Chemie GmbH, Gillingham) (PLATE-VII)
- Spacer Wax (MAARC) (PLATE-VII)
- Auto polymerized Acrylic Resin (DPI – RR Cold Cure, Dental Products of India Limited) (PLATE-VII)
- Eco-Etch (Ivoclar Vivadent, Schaan/Liechtenstein) (PLATE-VIII)
- Te-Econom Plus (Ivoclar Vivadent, Schaan/Liechtenstein) (PLATE-VIII)
- Tetric N-Bond (Ivoclar Vivadent, Schaan/Liechtenstein) (PLATE-VIII)
- Tetric N-Bond Universal (Ivoclar Vivadent, Schaan/Liechtenstein) (PLATE-VIII)

Sample preparation:

Root portion of each tooth from cemento-enamel junction to root apex was encircled with thin sheet of spacer wax. Further, the teeth were mounted in cold-cure acrylic resin using L-shaped moulds of dimensions $1.5 \times 1.5 \text{ cm}^2$.

Distribution of study groups :

All the samples were randomly divided into a control group which was further divided into two positive control groups: Group Ia and Ib.

And two experimental groups: Group II and Group III.

Group	Sample Distribution	No. Of Samples
I	Class I composite resin restoration using Tetric N-Bond bonding agent.	20
	Class I composite resin restoration using Tetric N-Bond Universal bonding agent.	20
II	Replacement of Class I silver amalgam restoration with composite resins using Tetric N-Bond bonding agent.	20
III	Replacement of Class I silver amalgam restoration with composite resins using Tetric N-Bond Universal bonding agent.	20

Standard class I cavity was prepared using a round diamond point bur BR-45 (MANI, Japan) and TF-20 (MANI, Japan) and the cavity was refined by using a straight fissure bur SF-41 (MANI, Japan). A new bur was employed after every five preparations. Each Class I cavity had a pulpal depth of 2.0 mm measured using Williams Graduated Probe (GDC) and mesiodistal width of 2.0 mm. A digital Vernier calliper (WorkZone Hand Tools, Germany) was used to measure the width of the prepared cavity design. The cavity preparation was cleaned and the samples were distributed in group Ia, Ib, II, III respectively.

Restoration fabrication:

The prepared teeth were randomly divided into the following groups and restored as follows:

Group Ia: Class I composite resin restoration using fifth generation bonding agent (Tetric N-Bond): (n=20)

The cavity preparations were etched with 37% phosphoric acid (Eco-Etch, Ivoclar Vivadent, Schaan/Liechtenstein) for 15 s and followed by a 30 s rinse with water. The excess water was removed from the dentin surface with absorbent paper. Tetric N-Bond adhesive (Ivoclar Vivadent, Schaan/ Liechtenstein) was labelled with 0.1% Rhodamine B dye (Sigma-Aldrich Chemie GmbH, Gillingham) and then applied to the cavity walls using a microbrush and light cured using a Bluephase N MC (Ivoclar Vivadent, Schaan/Liechtenstein) curing light at an irradiance of 800 mW/cm² for 10 s. Te-Econom Plus (Ivoclar Vivadent, Schaan/Liechtenstein), a light cured packable composite resin, was incrementally inserted with each increment being approximately 1 mm in thickness and light cured for 20 s. After polishing all the

restorations with sand paper discs (Shofu Dental Corporation, California), the samples were stored in an incubator at 37°C in 100% humidity.

Group Ib: Class I composite resin restoration using eighth generation bonding agent (Tetric N-Bond Universal): (n=20)

Tetric N-Bond Universal adhesive (Ivoclar Vivadent, Schaan/ Liechtenstein) was labelled with 0.1% Rhodamine B dye (Sigma-Aldrich Chemie GmbH, Gillingham) and then applied to the cavity walls using a microbrush and light cured using a Bluephase N MC (Ivoclar Vivadent, Schaan/Liechtenstein) curing light at an irradiance of 800 mW/cm² for 10 s. Te-Econom Plus (Ivoclar Vivadent, Schaan/Liechtenstein), a light cured packable composite resin, was incrementally inserted with each increment being approximately 1 mm in thickness and light cured for 20 s. After polishing all the restorations with sand paper discs (Shofu Dental Corporation, California), the samples were stored in an incubator at 37°C in 100% humidity.

Group II: Replacement of Class I amalgam restoration with composite resins using fifth generation bonding agent (Tetric N-Bond) without extending the cavity walls: (n=20)

The samples in this group were first restored using silver amalgam (DPI). The restorative procedure included the application of two layers of cavity varnish (Namuvar, India) to all cavity walls then restored using a high copper amalgam (DPI). The samples were stored in artificial saliva in an incubator at 37°C for one-year. At the end of 1 year, the amalgam was carefully removed to avoid extending the original cavity walls. To prevent encroachment on the dentin, the last layer of amalgam was removed with an explorer. The cavities were then etched with 37% phosphoric acid

(Eco-Etch, Ivoclar Vivadent, Schaan/Liechtenstein) for 15 s and followed by a 30 s rinse with water. The excess water was removed from the dentin surface with absorbent paper. Tetric N-Bond adhesive (Ivoclar Vivadent, Schaan/ Liechtenstein) was labelled with 0.1% Rhodamine B dye (Sigma-Aldrich Chemie GmbH, Gillingham) and then applied to the cavity walls using a microbrush and light cured using a Bluephase N MC (Ivoclar Vivadent, Schaan/Liechtenstein) curing light at an irradiance of 800 mW/cm^2 for 10 s. Te-Econom Plus (Ivoclar Vivadent, Schaan/Liechtenstein), a light cured packable composite resin, was incrementally inserted with each increment being approximately 1 mm in thickness and light cured for 20 s. After polishing all of the restorations with sand paper discs (Shofu Dental Corporation, California), the samples were stored in an incubator at 37°C in 100% humidity.

Group III: Replacement of Class I amalgam restoration with composite resins using eighth generation bonding agent (Tetric N-Bond Universal) without extending the cavity walls: (n=20)

The samples in this group were first restored using silver amalgam (DPI). The restorative procedure included the application of two layers of cavity varnish (Namuvar, India) to all cavity walls then restored using a high copper amalgam (DPI). The samples were stored in artificial saliva in an incubator at 37°C for one-year. At the end of 1 year, the amalgam was carefully removed to avoid extending the original cavity walls. To prevent encroachment on the dentin, the last layer of amalgam was removed with an explorer. Tetric N-Bond Universal adhesive (Ivoclar Vivadent, Schaan/ Liechtenstein) was labelled with 0.1% Rhodamine B dye (Sigma-Aldrich Chemie GmbH, Gillingham) and then applied to the cavity walls using a microbrush

and light cured using a Bluephase N MC (Ivoclar Vivadent, Schaan/Liechtenstein) curing light at an irradiance of 800 mW/cm² for 10 s. Te-Econom Plus (Ivoclar Vivadent, Schaan/Liechtenstein), a light cured packable composite resin, was incrementally inserted with each increment being approximately 1 mm in thickness and light cured for 20 s. After polishing all of the restorations with sand paper discs (Shofu Dental Corporation, California), the samples were stored in an incubator at 37°C in 100% humidity.

All the samples were embedded in cold-cure acrylic resin using L-moulds of dimensions 1.5×1.5 cm². The specimens were rinsed and sectioned buccolingually through the centre of restorations with a microtome precision saw (Isomet, Buehler, Germany). The resulting sections of each tooth were 2 mm thick. The sectioned surfaces were polished with a series of silicon carbide abrasive papers (upto 2400 grit) using running tap water as a lubricant on MetaServ 2000 Grinder polisher machine (Buehler, Germany). The samples were kept humid during the whole study.

Confocal Laser Scanning Microscopy (CLSM) was performed with a 'ZEISS Microscope' with LSM Software ZEN 2007. An Ar/Kr mixed gas laser was used as the light source. Excitation light had a wavelength maximum at 568 nm. The intensity of the excitation light as well as the amplification of the photomultiplier was kept constant during the investigation period. CLSM images were recorded in fluorescent mode. The detected light was conducted through a 590 nm long-pass filter, thus, fluorescent light emitted from the specimen was discriminated from reflected and scattered light. The visualized layer was selected 10 µm below the sample surface and images were recorded with an oil immersion objective (40x, numerical aperture 1.25).

The size of the images recorded was $425 \times 425 \mu\text{m}^2$, and the resolution was 512×512 pixel.

Six photographs of each specimen were taken at six random points along the perimeter of the cavity. In order to quantify the thickness of the hybrid layer, the measurements were performed on each image, and a mean was calculated. Thus, only one value (mean) per section entered the statistical analysis. The number of resin tags represented in the standardized images were counted.

According to the recommended scoring criteria the samples would be evaluated as following:⁶⁰

Tags formation into dentin:-

- 0- not detectable;
- 1- few tags visible;
- 2- uniform tag formation but with a few lateral branches;
- 3- long resin tags with lateral branches uniformly evident.

To avoid observer bias, two evaluators received a prior calibration by an experienced researcher, and then they assigned scores to the confocal images. The scoring for the tag formation was done by the evaluators who were blinded about the adhesives used. In cases where the two evaluators disagreed, both re-examined the image with a third evaluator and reached a joint final decision.

The data was collected and tabulated using an excel sheet (Microsoft Office 2010). This data was then subjected to statistical analysis using a licensed version of SPSS 20.0 (IBM Corporation).

Algorithm for methodology

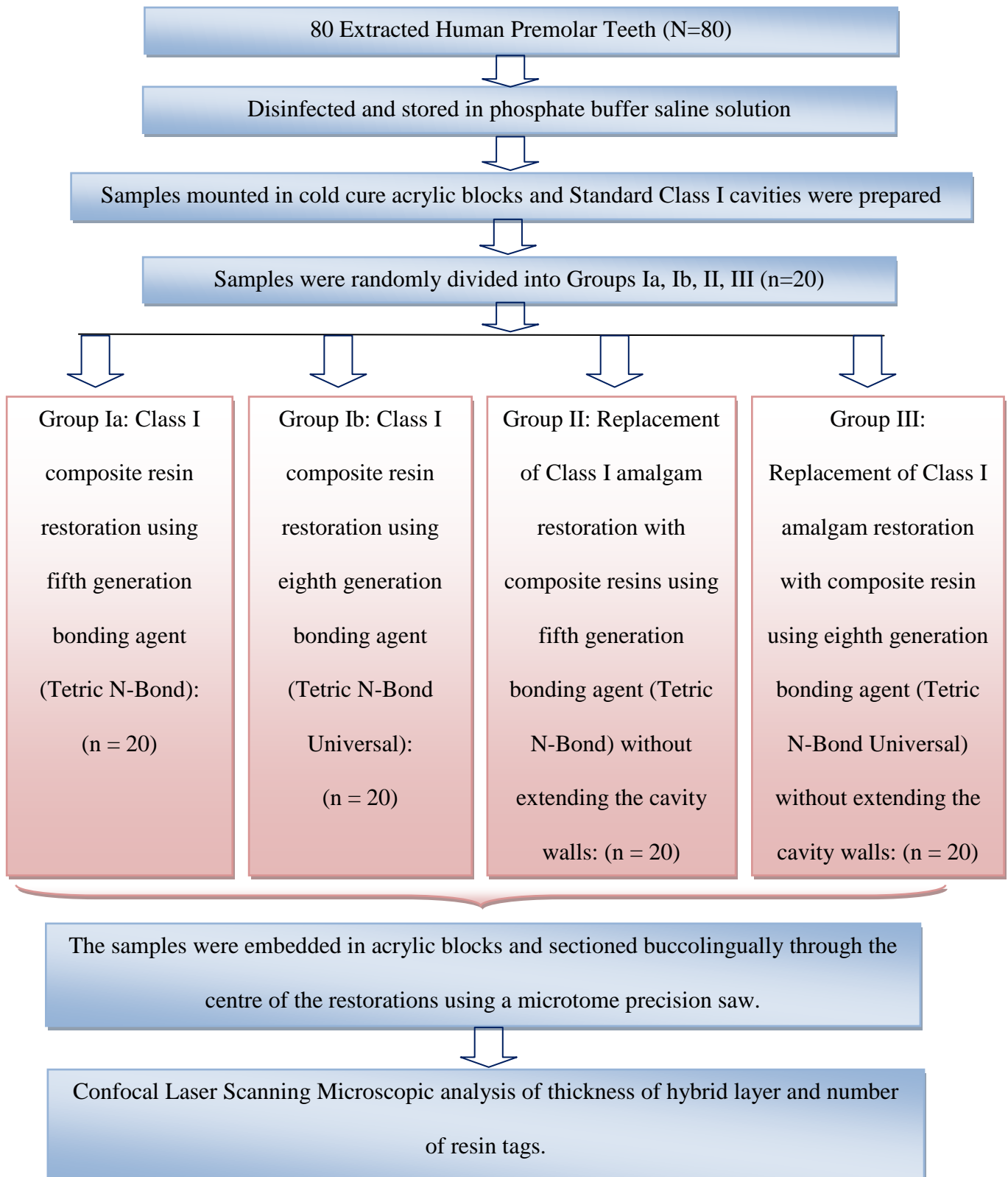


PLATE-I

ARMAMENTARIUM



Hand Instruments (GDC)



Cotton Holder & Waste Receiver (GDC)



Diamond Points (MANI)

PLATE-II

ARMAMENTARIUM



High Speed Airoter (NSK)



Digital Vernier Calliper (Workzone tools)



Williams Graduated Probe (GDC)

PLATE-III

ARMAMENTARIUM



Amalgam Carrier (GDC)



Round & Parallelogram Condenser (GDC)



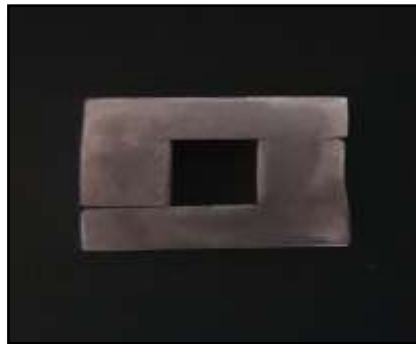
Carvers (GDC)

PLATE-IV

ARMAMENTARIUM



Amalgamator (Mixalloy Amalgamator, Rhos Brazil)



L-Mould



**Composite Instruments
(GDC)**



**Composite Finishing & Polishing Kit
(Shofu)**

PLATE-V

ARMAMENTARIUM



LED Light Curing Gun (Ivoclar Vivadent)



Incubator (Lyzer)

PLATE-VI

MATERIALS



Normal Saline (Eurolife healthcare)



Artificial Saliva (ICPA)



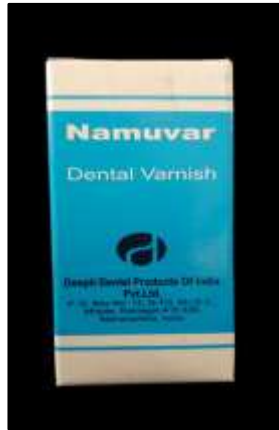
Silver Alloy (DPI)



Mercury (DPI)

PLATE-VII

MATERIALS



Cavity Varnish (Namuvar)



**Dye Rhodamine B isothiocyanate
(Sigma-Aldrich Chemie GmbH,
Gillingham)**



Spacer Wax (MAARC)



**Autopolymerized Acrylic Resin
(DPI-RR Cold Cure)**

PLATE-VIII

MATERIALS



Eco-Etch (Ivoclar Vivadent)



Te-Econom Plus (Ivoclar Vivadent)



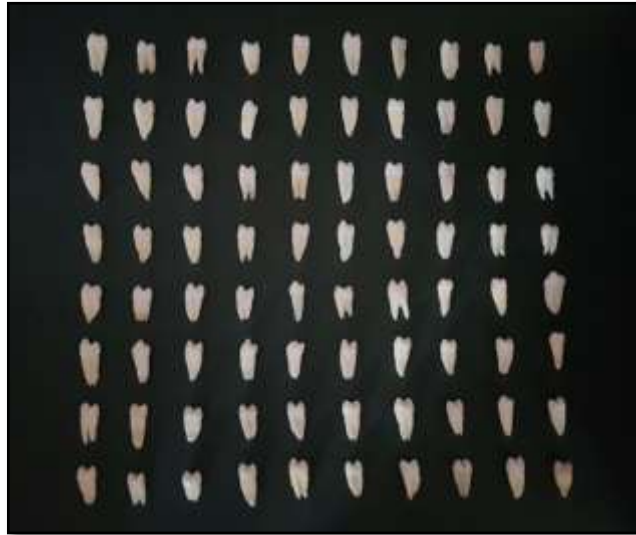
Tetric N-Bond (Ivoclar Vivadent)



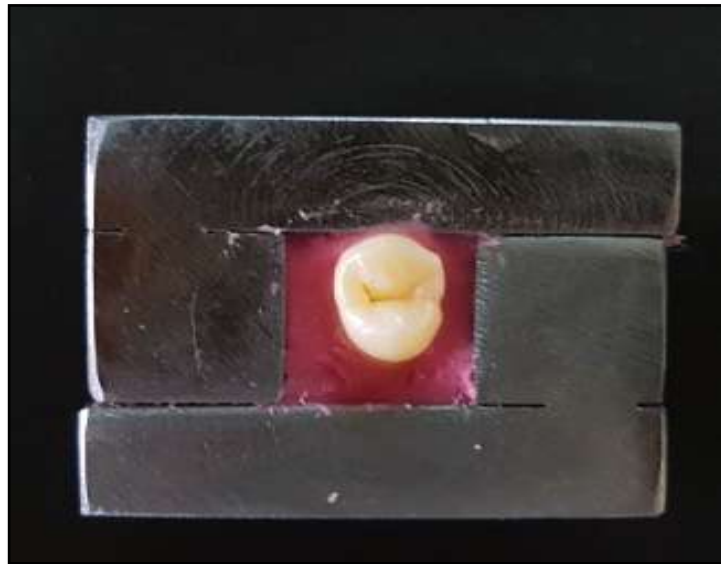
Tetric N-Bond Universal (Ivoclar Vivadent)

PLATE-IX

METHODOLOGY



Sample Size (N=80)



Sample Preparation

PLATE-X

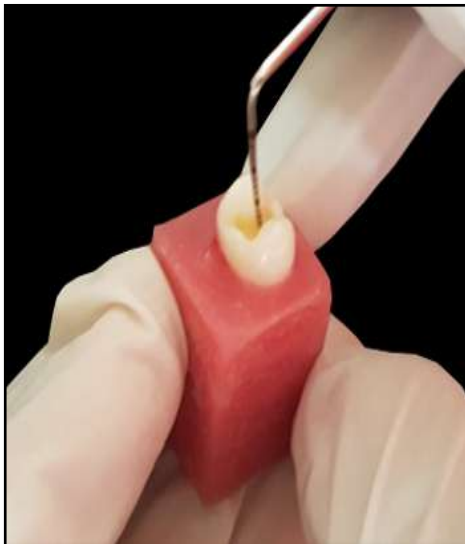
METHODOLOGY



Tooth Preparation



Standard Class I Cavity



Measuring occlusal depth=2mm



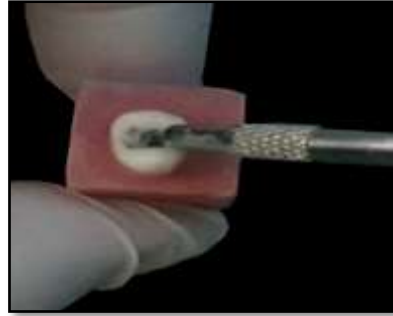
Mesio-Distal Width=2mm

PLATE-XI

METHODOLOGY



Application of Cavity Varnish



Placement of Silver Amalgam



Final Amalgam Restoration



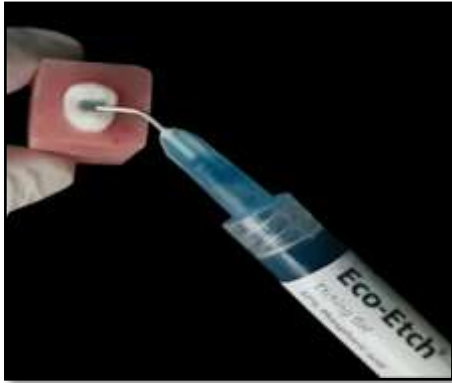
Removal of Silver Amalgam



Discoloured Dentin Under One Year Old Amalgam Restoration

PLATE-XII

METHODOLOGY



Application of Etchant



Labelling with Rhodamine B Dye



Dispensing of Tetric N-Bond



**Dispensing of Tetric N-Bond
Universal**

PLATE-XIII

METHODOLOGY



Application of Composite Resin



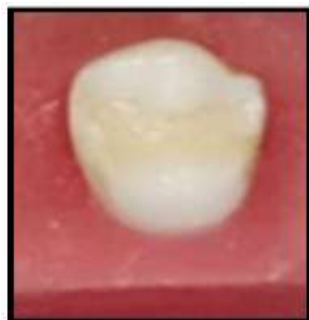
Curing of Sample after Bonding Agent



Application of Composite Resin



Curing of Sample after Composite Restoration



Final Composite Restoration

PLATE-XIV

METHODOLOGY



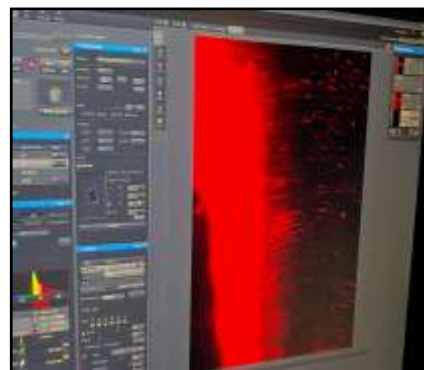
Sectioning of Samples with Isomet Precision Saw (Beuhler)



Finishing & Polishing of Samples on Grinder & Polisher (Beuhler)



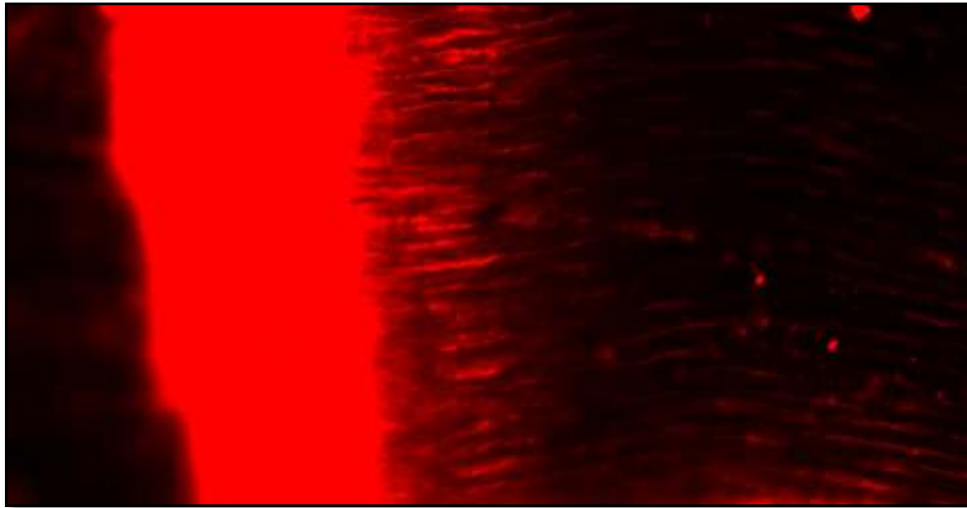
Sectioned Sample



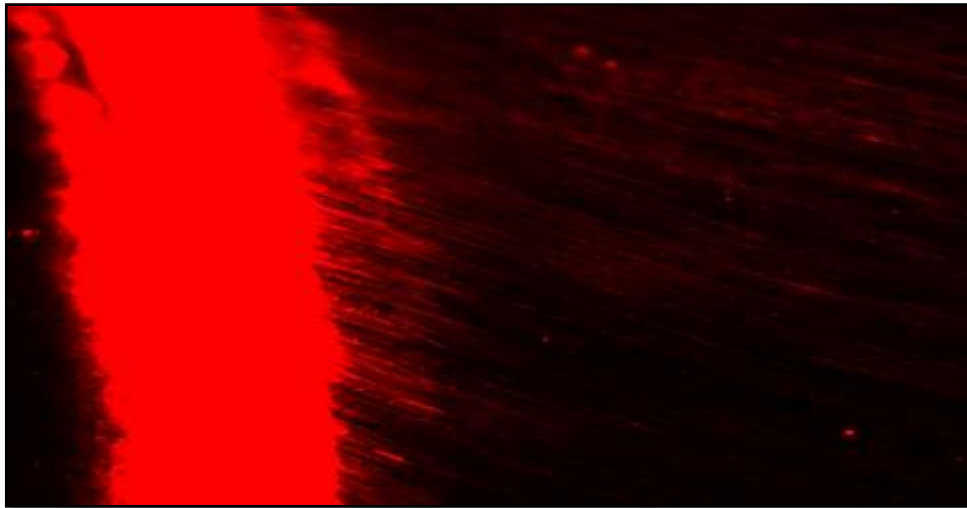
Confocal Laser Scanning Microscope (LSM 510, ZIESS with LSM Software ZEN 2007)

PLATE-XV

CONFOCAL LASER SCANNING MICROSCOPIC IMAGES



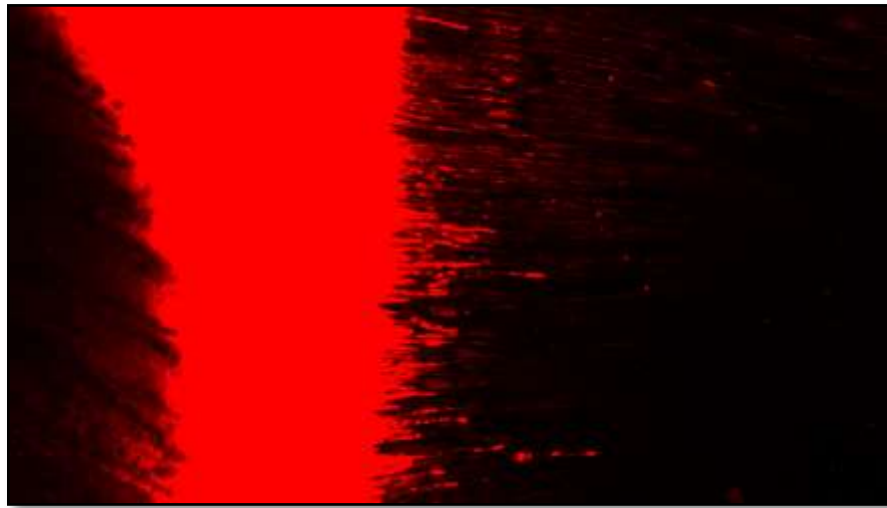
Confocal Laser Scanning Microscopic Image in Group Ia



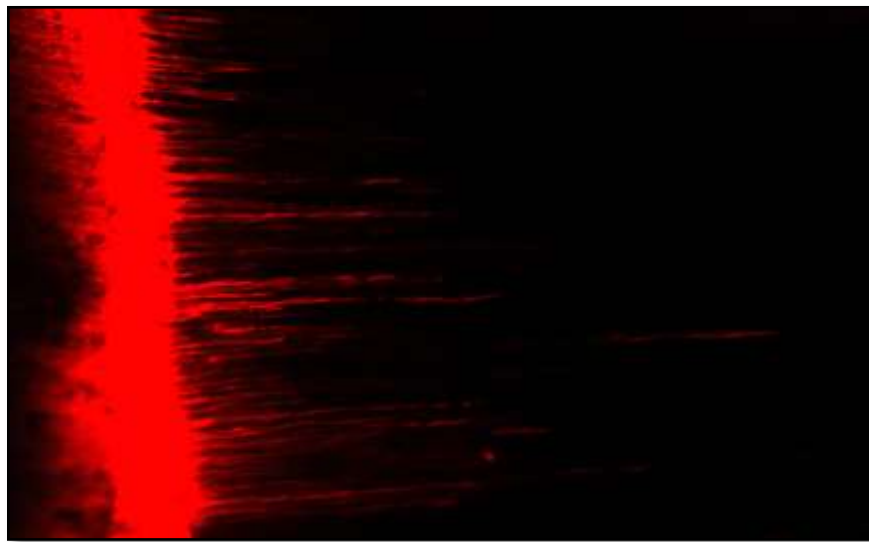
Confocal Laser Scanning Microscopic Image in Group Ib

PLATE-XVI

CONFOCAL LASER SCANNING MICROSCOPIC IMAGES



Confocal Laser Scanning Microscopic Image in Group II



Confocal Laser Scanning Microscopic Image in Group III

Results

The present in vitro study was carried out to evaluate the thickness of hybrid layer and number of resin tags at resin-dentin interface in two different generations of bonding agents using Confocal Laser Scanning Microscope in premolars with Class I amalgam restorations replaced with composite restorations.

Depending upon the generation of bonding agent used, the samples were randomly divided into the following groups:

Group I	a	Class I composite resin restoration using Tetric N-Bond bonding agent.
	b	Class I composite resin restoration using Tetric N-Bond Universal bonding agent.
Group II		Replacement of Class I silver amalgam restoration with composite resins using Tetric N-Bond bonding agent.
Group III		Replacement of Class I silver amalgam restoration with composite resins using Tetric N-Bond Universal bonding agent.

All the specimens were sectioned buccolingually through the centre of restorations resulting in 2mm thick sections of each sample. These sections were evaluated for thickness of hybrid layer (μm) and number of resin tags.

Statistical methods:

Licensed version of SPSS 20.0 (IBM Corporation) was used for statistical analysis. The data on hybrid layer thickness, number of resin tags and the scores was obtained on 20 samples in each of the study groups and was summarized in terms of mean, standard deviation and median. The statistical comparison of mean hybrid layer between specified two groups was carried out using Student's t-test for independent samples. The comparison of resin tags between the same groups was performed using Wilcoxon rank sum test. Further, the comparison of resin tag score distribution was carried out using Pearson's Chi-square test between the groups.

All the analyses were carried out using SPSS ver. 20.0 (IBM Corporation) and the statistical significance was tested at 5% level.

The formulations used in the analysis were:

If x_1, x_2, \dots, x_n are the observations on random variable X, then

A) Sample mean for a set of observations is given by

$$\bar{x} = \frac{1}{n} \sum_{i=1}^n x_i$$

B) Standard deviation for a set of observations is given by

$$s = \sqrt{\frac{1}{(n-1)} \sum_{i=1}^n (x_i - \bar{x})^2}$$

where x_i = observation on each object

n = number of objects

C) Median: It is the middle value of a set of values when arranged in the increasing order of magnitude.

D) Student's t-test for independent samples

The test is used for comparing the statistical significance of difference in the means of two samples. It compares the sample difference between two means in relation to the variation in the data (expressed as the standard deviation of the difference between the means).

It is given by the formula:

$$t = \frac{(\bar{x}_1 - \bar{x}_2) - (\mu_1 - \mu_2)}{s_{(\bar{x}_1 - \bar{x}_2)}}$$

where \bar{x}_1 and \bar{x}_2 are the means of sample observations of two different groups, μ_1 and μ_2 are the means of the respective populations from which the samples are derived, and $s_{(\bar{x}_1 - \bar{x}_2)}$ is the pooled sample standard deviation, which is given by:

$$s_{(\bar{x}_1 - \bar{x}_2)} = \sqrt{\frac{s_{pooled}^2}{n_1} + \frac{s_{pooled}^2}{n_2}}$$

Where,

$$s_{pooled}^2 = \frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2}$$

Here, s_1^2 and s_2^2 are the variance of two samples and n_1 and n_2 are the sample sizes in two groups.

If the test statistic results in a p -value > 0.05 (level of significance), then the null hypothesis H_0 : *There is insignificant difference in the means of two groups* is accepted and the alternative hypothesis H_1 : *There is significant difference in the means* is rejected. On the other hand, if p -value < 0.05 , then the H_1 is accepted and H_0 is rejected.

E) Wilcoxon rank sum test

The test is a non-parametric equivalent of Student's t-test for independent samples, when the assumption of normality is violated. It evaluates the null hypothesis that the two populations are the same against alternative that particular population has larger values than the other. It involves computation of a test statistics based on ranked series. The observations are ranked according to magnitude irrespective of the two groups. The steps involved are as under:

- i) Add the ranks for observations from group 1.
- ii) Since sum of all ranks equal $N(N+1)/2$, the sum of ranks in group 2 is total sum minus the sum of group 1.
- iii) A statistic U is defined as:

$$U_1 = R_1 - \frac{n_1(n_1 + 1)}{2}$$

where n_1 is the size of sample 1 and R_1 is the sum of ranks of sample 1. Equally valid formula for U is

$$U_2 = R_2 - \frac{n_2(n_2 + 1)}{2}$$

The smaller of U_1 and U_2 is for significance testing.

For large sample sizes ($N > 30$), U is approximately normally distributed, and the standardized value is given by

$$z = \frac{U - m_U}{\sigma_U}$$

Where, m_U and σ_U are the mean and standard deviation of U . The significance of z can be obtained from normal probability tables. Here m_U and σ_U are given by:

$$m_U = \frac{n_1 n_2}{2}; \sigma_U = \sqrt{\frac{n_1 n_2 (n_1 + n_2 + 1)}{12}}$$

F) Pearson's Chi-square test

Let X and Y be two variables under study with r and s levels respectively; and the data on $r \times s$ levels be in the form of counts. Let the null hypothesis be that the two variables are independent. That is, knowing the levels of X does not help in predicting the levels of Y ; against the alternative hypothesis that the two factors are not independent. That is, knowing the level of X can help in predicting levels of Y . To decide about the acceptance of hypothesis, the Chi-square test statistic is used which is defined as:

$$\chi^2 = \sum_{i=1}^r \sum_{j=1}^s \frac{(O_{ij} - E_{ij})^2}{E_{ij}}$$

Where, O_{ij} is the observed frequency count for i^{th} level of variable X and j^{th} level of variable Y . E_{ij} is the expected frequency count for same cell. The expected count is given by:

$$E_{ij} = \frac{n_i \times n_j}{n}$$

Where, n_i and n_j are the total counts for i^{th} level of variable X and j^{th} level of variable Y ; and n is the total count. The calculated Chi-square value is compared with the tabulated one for $(r-1) \times (s-1)$ degrees of freedom.

Overall results:

The mean values and standard deviations of thickness of hybrid layer and number of resin tags for the groups Ia, Ib, II and III for two different generations of

bonding agents used namely **Tetric N-Bond** and **Tetric N-Bond Universal** have been described in **Table 1 to Table 9**.

The maximum thickness of hybrid layer (30.74 ± 13.52) was observed in Group Ia whereas the minimum thickness of hybrid layer (9.50 ± 2.57) was observed in Group III. The maximum resin tags (90.10) were observed in Group Ia and minimum resin tags (68.00) were observed in Group III.

Higher values of thickness of hybrid layer and number of resin tags were observed in the control group as compared to the experimental groups irrespective of the generation of bonding agents used.

Analysis for thickness of hybrid layer:

In order to determine the effect of two different generations of bonding agents when replacing old amalgam restoration with composite resins on thickness of hybrid layer, Student's t-test was performed with groups.

The simple main effects were statistically highly significant for each generation of bonding agents when samples were restored with composite resins and when used to replace old amalgam restoration with composite resins as indicated by p-values < 0.0001 . In other words, there was a statistically significant difference in the mean thickness of hybrid layer in the positive control groups when compared to the experimental groups irrespective of the generation of bonding agent used. The mean level for the positive control groups were significantly higher than experimental groups for the two different generations of bonding agents used. (**Graph 1**)

The mean thickness of hybrid layer for samples restored with composite resins using **Tetric N-Bond** bonding agent was $30.74\mu\text{m}$ and for the samples where old

amalgam restoration was replaced using **Tetric N-Bond** bonding agent was 13.25 μ m. The mean hybrid layer in Group Ia (30.74 \pm 13.52) was statistically significantly higher than Group II (13.25 \pm 3.99) with a p-value < 0.0001. (**Table 1**)

The difference in the means of thickness of hybrid layer for **Tetric N-Bond Universal** bonding agent considering the restorations done with composite resins and replacing old amalgam restorations with composite resins was obtained by Student's t test. The mean thickness of hybrid layer for samples restored with composite resins using **Tetric N-Bond Universal** bonding agent was 16.63 μ m and for the samples where old amalgam restoration was replaced with composite resins using **Tetric N-Bond Universal** bonding agent was 9.50 μ m. The mean hybrid layer in Group Ib (16.63 \pm 5.21) was statistically significantly higher than Group III (9.50 \pm 2.57) with a p-value < 0.0001. (**Table 2**)

Irrespective of the restoration done on the cavities, whether restored with composite resins or replaced old amalgam restorations with composite resins, there was a statistically significant difference in the mean thickness of hybrid layer obtained with fifth generation bonding agent i.e. **Tetric N-Bond** compared to eighth generation bonding agent i.e. **Tetric N-Bond Universal**.

The mean thickness of hybrid layer for samples restored with composite resins using **Tetric N-Bond** bonding agent was 30.74 μ m and **Tetric N-Bond Universal** bonding agent was 16.63 μ m. The mean hybrid layer thickness in Group Ia (30.74 \pm 13.52) was statistically significantly higher than Group Ib (16.63 \pm 5.21) with p-value 0.0002. (**Table 3**)

The difference in the means of thickness of hybrid layer for **Tetric N-Bond and Tetric N-Bond Universal** bonding agent considering the restorations when old

amalgam restorations were replaced with composite resins was obtained by Student's t test. The mean thickness of hybrid layer for the samples where old amalgam restoration was replaced using **Tetric N-Bond** bonding agent was 13.25 μ m and for **Tetric N-Bond Universal** bonding agent was 9.5 μ m. The mean hybrid layer thickness in Group II (13.25 \pm 3.99) was statistically significantly higher than Group III (9.50 \pm 2.57) with p-value 0.0013. **Table 4** shows the representation of the same.

Analysis for number of resin tags:

Intraclass correlation coefficient was obtained for deciding the agreement between the resin tag counts between the two evaluators. Two-way mixed model with absolute measurement type were used to obtain the coefficients and the corresponding p-value. The coefficients in all the four groups were more than 0.95 indicating very good agreement between the observers. (**Table 5**)

In order to determine the effect of two different generation bonding agents on number of resin tags, Wilcoxon rank sum test was performed with groups.

The simple main effects were statistically highly significant for each generation of bonding agents when samples were restored with composite resins and when used to replace amalgam restoration with composite resins as indicated by p-values < 0.0001. **Graph 2** shows that there is a statistically significant difference in the mean of number of resin tags irrespective of the generation of bonding agent used. The mean level for the positive control groups were significantly higher than experimental groups for the two different generations of bonding agents used.

The difference in the means of number of resin tags for the samples restored with composite resins using **Tetric N-Bond** bonding agent was 90.10 and for the samples where old amalgam restoration was replaced using **Tetric N-Bond** bonding

agent was 44.50. The median score of resin tags in Group Ia (89.50) was statistically significantly higher than Group II (45.00) with p-value < 0.0001. **Table 6** shows the representation of the same.

The mean number of resin tags for **Tetric N-Bond Universal** bonding agent considering the restorations done with composite resins and replacing old amalgam restorations with composite resins was obtained by Wilcoxon rank sum test. The mean number of resin tags for samples restored with composite resin using **Tetric N-Bond Universal** bonding agent was 68.00 and for the samples where old amalgam restoration was replaced with composite resins using **Tetric N-Bond Universal** bonding agent was 32.65. The median score of resin tags in Group Ib (67.00) was statistically significantly higher than Group III (34.00) with p-value < 0.0001. (**Table 7**)

There was a high statistically significant difference in the mean number of resin tags of fifth generation bonding agent i.e. **Tetric N-Bond** compared to eighth generation bonding agent i.e. **Tetric N-Bond Universal**, irrespective of the restoration done on the cavities, whether restored with composite resins or replaced old amalgam restorations with composite resins.

The mean number of resin tags for samples restored with composite resin using **Tetric N-Bond** bonding agent was 90.10 and **Tetric N-Bond Universal** bonding agent was 68.00. The median score of resin tags in Group Ia (89.50) was statistically significantly higher than Group Ib (67.00) with p-value < 0.0001. (**Table 8**)

The difference in the means of number of resin tags for **Tetric N-Bond** and **Tetric N-Bond Universal** bonding agent considering the restorations when old amalgam restorations were replaced with composite resins was obtained by Wilcoxon rank sum test. The mean number of resin tags for the samples where old amalgam restoration was replaced using **Tetric N-Bond** bonding agent was 44.50 and for **Tetric N-Bond Universal** bonding agent was 32.65. The median score of resin tags in Group II (45.00) was statistically significantly higher than Group III (34.00) with p-value < 0.0001 . **Table 9** shows the representation of the same.

Scores for tags formation into dentin:

Table 10 shows that kappa coefficient was used on scores provided by two evaluators, which ranged between 0.64 to 0.79 indicating substantial agreement between the two evaluators.

Table 11a provides the comparison of resin tags scores between different groups.

The comparison between Group Ia and II (**Graph 3**) shows that maximum proportion of samples showed tag score 3 in Group Ia i.e. 14 (70%) and tag score 1 i.e.12 (60%) in Group II, followed by samples with tag score 2 in both the groups. The difference in the proportion was statistically significant between two groups ($p < 0.0001$).

Further, the comparison between Groups Ib and III (**Graph 4**) showed statistical significance with p-value < 0.0001 , where in Group Ib, the proportion of samples were the same, i.e. 10 (50%) with tag scores 2 and 3 respectively. On the

other hand in Group III, the maximum proportions were observed for samples with tag score 1 i.e. 18 (90%), followed by those with tag score 2.

The comparison between Group Ia and Ib (**Graph 5**) showed statistically insignificant difference ($p > 0.05$). In the comparison between Groups II and III, the distribution of scores between groups was similar suggesting statistically insignificant difference ($p=0.0678$). (**Graph 6**)

Discussion

Ideas run wild without Discussion.

-Serge King

Dental restoration has limited lifespan because of which there is need for restoration replacement.¹⁰ Various factors such as restoration size, aesthetic requirements and occlusion dictate which restorative materials and techniques are the best choices for the patient. A generation of patients have benefitted from the use of amalgam to restore teeth. However, there is a paradigm shift towards tooth-coloured restorative materials. In some cases, the intention behind replacing old amalgam restoration is limited to patient's aesthetic concern.

However, it has been noticed that discoloured dentin adjacent to amalgam restoration contain amalgam corrosion products that can deeply penetrate into the dentinal tubules and thus should be considered as a different substrate for clinical procedures than sound dentin.²³

The bonding principle of dental adhesives is based on the formation of a hybrid layer⁶¹ as well as the penetration of adhesive into dentinal tubules and the formation of resin tags.^{62,63} Formation of hybrid layer and resin tags is dependent on its penetration qualities and surface behaviour of various adhesive systems as well as on the condition and permeability of dentinal surface.²⁸ However, the importance of resin tags and hybrid layer for the quality of bond is still unclear.

The coupling of resin-based cements requires the adjunctive use of dentin adhesives. Various types of adhesives systems have been introduced over the decades. Total-etch resin cements utilize phosphoric acid etching that completely dissolves the smear layer and creates a zone of partially demineralised dentin. Upon rinsing the acid conditioners, adhesive primers and resins are applied to the demineralised dentin to achieve micromechanical bonding. Conversely, self-etch resin cements utilize adhesives containing increased concentrations of acidic resin monomers which simultaneously demineralise and infiltrate the smear layer covering dentin. Recently, the trend in bonding is towards simplification and reducing the procedural steps. Therefore, manufacturers have introduced multipurpose “universal one-bottle adhesives” for use as either self-etch or etch and rinse adhesives.

Bonding to dentin has always been poor compared to enamel because of the morphological, histological, and compositional differences between enamel and dentin. Bonding to dentin is a challenge due to higher water and organic content also due to the presence of dentin smear layer that forms immediately after cavity preparation.⁶⁴ Thus, achievement of reliable bonding and effective adhesion of resin based cements to the tooth structure is still an issue of interest.

The presence of corrosion products, as by-products of old amalgam restoration, on the cavity surface and its effect on composite restoration is still unclear. Further, limited data is available in literature on adhesion to discoloured dentin after amalgam removal. No previous research has conclusively remarked on the effect of presence of corrosion products on different adhesives on hybrid layer and resin tag formation. Hence, the following study was carried out to evaluate the hybrid layer and resin tags at resin-dentin interface in one year old amalgam restorations replaced with composite resins using two different generations of bonding agents under Confocal Laser Scanning Microscopy (CLSM).

Eighty freshly extracted human premolars were selected for the study, which were collected and stored in phosphate-buffered saline for not more than 12 weeks as suggested by **Jameson MW et al. (1994)**.⁶⁵ They observed that storage media and time of the specimen storage affect the tooth after extraction due to water loss with dehydration of dentin. Phosphate-buffered saline shows the best compatibility in maintaining the hydration of the extracted teeth.

Referring to the study by **Pioch T et al. (1997)**,⁴² the authors obtained the mean thickness of hybrid layers for the two experimental adhesives. Using the data after 15 s, the effect size obtained was 0.66. To attain the desired effect with 95% confidence and 80% power, the estimated sample size was 20 per group.

The formulation used was:

$$n = \frac{2 * (z_{\alpha/2} + z_{\beta})^2 * \sigma^2}{e^2}$$

where $z_{\alpha/2}$ and z_{β} are critical values for 95% confidence and 80% power respectively, σ is the pooled standard deviation and e is the estimated effect size.

Premolars present a unique combination of occlusal dynamics, structural loading and anatomical design. The cusps are supported by drastically tapering roots, thin root walls and proximal concavities or invaginations. All these factors predispose the tooth to perforation or fracture.⁶⁶ They often lie in the aesthetic zone and these teeth can be more conservatively restored using direct composite restorations. Therefore, premolars were selected for this study.

In the present study, the teeth were mounted on cold cure acrylic blocks, 1 mm apical to the CEJ using a brass mould. Before mounting the teeth on cold cure acrylic blocks, the periodontal ligament space was simulated using a spacer wax upto the CEJ.^{67,68,69,70,71}

According to **Sturdevant**,⁷² a sufficient depth of 1.5-2.0 mm results in an adequate thickness of the restoration, providing resistance to fracture and wear. For premolars, the distance from the margin of a cavity extension to the proximal surface should not be less than ~1.6 mm. In the present research, standard Class I cavity was prepared by using a round diamond bur (BR-45) and cavity was refined by using a straight fissure bur (SF-41). Each cavity had a pulpal depth of 2.0 mm measured using Williams Graduated Probe and mesiodistal width of 2.0 mm. Thickness of the marginal ridges were maintained to be 2 mm. A digital Vernier calliper (WorkZone Hand Tools, Germany) was used to measure the dimensions of the prepared cavity design.

Application of cavity varnish under amalgam restorations has been a regular treatment over time. **Scholtanus JD et al.** had reported that application of cavity varnish reduces the initial micro-leakage and prevents discolouration of tooth structures by prevention of ion penetration.¹⁰ Thus, in the present study, the cavity

walls were coated with two layers of cavity varnish i.e. Cavity varnish (Namuvar, India) before restoring with silver amalgam.

Silver amalgam is being used in dentistry for more than 150 years. High copper or non gamma-2 amalgam compared to their low-copper amalgam counterparts, exhibits greater strength, less tarnish and corrosion and less creep. Thus, in the present study, high copper amalgam i.e. silver alloy (DPI) has been used to obtain better long-term results.

A previously published research by **Ghavannasiri et al.**¹⁶ stated that the bond strength of 6 month old amalgam restoration when replaced with composite resins keeping cavity walls intact was significantly lower. Also the observation of **Ghavannasiri et al.**²⁵ was that amalgam fillings kept in normal saline for 6 months generated corrosion by-products adjacent to the restoration. So, the tooth samples in the present study with amalgam fillings were stored in artificial saliva in an incubator at 37°C for one year to induce formation of amalgam corrosion products underneath the restoration.

In spite of certain advantages of silver amalgam such as plasticity and ease of manipulation, it possesses inherent defects like darkening of teeth with age.²⁴ Also, cavity preparation for amalgam restoration requires more removal of tooth structure than for composite restorations.⁷³ Excessive removal of unaffected tooth structure is not required for composite restorations. Thus, in the present research, when replacing the old amalgam restorations with composite resins, the cavity walls were kept intact. Extending the cavity walls was avoided by using a sharp explorer to remove the last layer of amalgam.

Lee KW et al.⁷⁴ demonstrated that etch-and-rinse adhesives produced significantly better bond, when compared to self-etch adhesives. Thus, in the current research, two different generation bonding agents i.e. Tetric N-Bond and Tetric N-Bond Universal were used according to manufacturer's instructions. Considering the various contemporary bonding agents in the market for composite resin restorations, current generation dentin bonding agent i.e. eighth generation universal one bottle adhesive (Tetric N-Bond Universal) was compared with a gold standard fifth generation adhesive (Tetric N-Bond).

The nature of restoration work, in terms of its location and size, is decisive in the choice of one material or another. For fillings requiring high mechanical performances and also fulfilling the aesthetic requirements, the most suitable composite is that with the highest inorganic load.⁷⁵ Hybrid composites because of its high inorganic content provides a wide range of advantages like less curing shrinkage, low water absorption, abrasion and wear similar to the tooth structure and a similar thermal coefficient to that of teeth.^{76,77} Thus, for the restoration of the Class I cavity, hybrid composite resins i.e. Te-Econom Plus was used.

Several microscopy techniques are currently used to evaluate the resin dentin interface (RDI), including stereomicroscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM) and confocal laser scanning microscopy (CLSM). In comparison to conventional SEM, CLSM has the advantage of providing detailed information about the presence and distribution of dental adhesives inside dentinal tubules at relative low magnification as 100× through the use of fluorescent Rhodamine–marked primers or bonding agents. The laser scanning microscope scans the sample, sequentially point by point and line by line and assembles the pixel

information into one image.⁷⁸ Use of fluorescent confocal microscopy for analysis of the interface of adhesive materials and tooth structure was first described by **Watson and Boyde**.³⁸ They advocated the use of fluorescent dyes, mixed into components of an adhesive system, to highlight the bonded interface.

Confocal Laser Scanning Microscopy offers improved rejection of out-of-focus noise and provides greater resolution than conventional imaging, yielding greatly enhanced images of biological structures. The specimens required for SEM or TEM analysis are dried, however, this procedure is complicated and not required for CLSM. This advantage leads to a decreased risk of shrinkage or desiccation artifacts and allows the same specimen to be subsequently studied using other, additional microscopic techniques. An additional feature of the confocal principle is that it permits visualization of not only a specimen surface, but also its subsurface.³³ Thus, in the current research CLSM was preferred over SEM and TEM and the sections were observed under the confocal laser scanning microscope.

To reduce the variability, all the samples were prepared and examined by one operator using the standard technique. However, the evaluation and scoring of resin tags were performed by two evaluators who were blinded about the adhesives used.

In the present study, hybrid layer and resin tags were compared between two different generations of bonding agents when applied after removal of one year old amalgam restoration.

The samples were evaluated and scores were allocated to each sample as per the study conducted in **2014 by Fagundes et al.**⁶⁰ The Kappa co-efficient was used on scores provided by the two evaluators which ranged between 0.64 to 0.79. These findings were in accordance with the study done by **Fagundes et al.**⁶⁰ where the

Kappa value for the inter-examiners was 0.77. The inter-examiner correlation for number of resin tags was more than 0.95 indicating very good agreement between the observers.

The results of the study are discussed under the following headings:

1. Effect of adhesives on Class I composite resin restoration:

The mean thickness of hybrid layer (30.74 +/-13.52) and number of resin tags (90.10 +/-10.95) were observed in Tetric N-Bond restored with composite resins [Group Ia]. While the mean hybrid layer thickness (16.63 +/-5.21) and number of resin tags (68.00 +/- 10.02) were observed in Tetric N-Bond Universal restored with composite resins [Group Ib].

A highly significant difference in thickness of hybrid layer and number of resin tags were observed between Tetric N-Bond (total-etch) [Group Ia] and Tetric N-Bond Universal (self-etch mode) [Group Ib] adhesives used (p value <0.001). Better results for Tetric N-Bond were seen which could be justified as phosphoric acid used for total-etch adhesives has pH=0.1-0.4, while the acidity of Tetric N-Bond Universal adhesive ranges from $\text{pH} \leq 2.5$ to $\text{pH} \approx 3$. Accordingly, dentin demineralization is more evident after the application of phosphoric acid because it completely removes the smear layer and opens dentinal tubules.⁷⁹

A well-formed hybrid layer and long resin tags with large number of lateral branches are characteristics of these adhesives with organic solvent: ethanol.⁸⁰ The presence of organic solvent in the composition of adhesives dehydrates acid etched dentin chemically. This leads to the lateral shrinkage of the collagen fibrils, resulting in an increase in the width of interfibrillar spaces and reduction of the hydrophilicity of collagen matrix. Lateral dentinal tubules get filled with organic solvent and the

bonding agent easily penetrates into them. In this way, a very well-formed hybrid layer with long resin tags and an expressed lateral branching is obtained.⁷⁹

In the present study, well formed hybrid layer and resin tags were observed in positive control groups. This is in accordance to **Scott and Thomlinson**,⁸¹ they demonstrated that the organic solvents (ethanol, acetone) remove the watery gel of glycosaminoglycans, thus removing them from the connective tissue. The collagen fibrils in the acid-etched dentin shrink laterally after the application of an adhesive with organic solvents, and the lateral dentinal tubules are filled with an organic solvent (acetone, alcohol), and adhesive resin penetrates easily into them. This is seen as perfectly formed hybrid layer and resin tags.

The decrease in thickness of hybrid layer in Tetric N-Bond Universal can be explained with their weaker acidity when applied as self-etch, which results in less demineralization of dentin, and exposes a small number of dentinal tubules, leading to poorer penetration of adhesive into dentin.⁷⁴ Tetric N-Bond Universal show pH=2.5-3.0 and is a mild etching adhesive.

Mild etching adhesives are not efficient enough to dissolve the smear plugs, which close dentinal tubules, and therefore they remain in dentinal tubules as a part of a hybrid complex. This hybrid complex is characterized by a thinner hybrid layer and less resin tags.

This is in accordance with a research done by **Van Meerbeek et al. (2003)**⁸² and **Inoue et al. (2001)**⁸³ who reported that the exchange intensity induced by etch and rinse adhesives exceeds that of self-etch adhesives. This could explain the small hybrid layer thickness of the Tetric N-Bond Universal adhesive observed in the present investigation.

2. Effect of adhesives on replacing and restoring one year old silver amalgam restoration with composite resins:

Higher values for thickness of hybrid layer and number of resin tags were obtained for groups in which the adhesives were used to restore the teeth with composite resins as compared to those where the adhesives were used to restore and replace one year old amalgam restoration, irrespective of the generation of adhesives used.

The mean thickness of hybrid layer (30.74 +/-13.52) and number of resin tags (90.10 +/-10.95) were observed in Tetric N-Bond adhesive restored with composite resins [Group Ia]. While the mean hybrid layer thickness (13.25 +/-3.99) and number of resin tags (44.50 +/-7.38) were observed in Tetric N-Bond adhesive in one year old silver amalgam restoration and replaced with composite resins [Group II]. A highly significant difference in thickness of hybrid layer and number of resin tags were observed between the groups (p value <0.0001).

The mean hybrid layer thickness (16.63 +/-5.21) and number of resin tags (68.00 +/- 10.02) were observed in Tetric N-Bond Universal restored with composite resins [Group Ib]. While the mean hybrid layer thickness (9.5 +/-2.57) and number of resin tags (32.65 +/-7.88) were observed in Tetric N-Bond Universal adhesive in one year old silver amalgam restoration and replaced with composite resins [Group III]. A highly significant difference in thickness of hybrid layer and of resin tags were observed between the groups (p value <0.0001).

Irrespective of the generation, the root cause to poor bonding of the adhesive agents when used to replace amalgam restorations was probably due to the presence of amalgam corrosion products. These findings are in agreement with those of other

studies^{84,85,86} that showed large amounts of Sn and Zn in dentine (without Cu, Ag, Hg) under amalgam restorations which were strictly related to discoloured and apparently demineralised dentine.

The findings of the present study are in tandem with the findings of **Ghavannasiri et al.**¹⁶ wherein they found a significant difference between the Groups that were Class I light-cured composite restoration and Class I amalgam restoration stored in 37°C normal saline for 6 months and then replaced with composite resins leaving the cavity walls intact. Also, **Applebaum**⁹ suggested, Sn and Zn were found in dentine adjacent to amalgam consistently.

Ellender G et al.²³ supposed that the precipitation of plasma protein into the dentinal fluid caused by amalgam corrosion products may reduce the permeability of the dentin and interfere with the infiltration of the resin monomer of the adhesive. Another probable reason could be that heavy metals decrease the acid solubility of the smear layer, rendering it less etchable than an uncontaminated smear layer.

3. Effect of adhesives in presence of amalgam corrosion products on Class I composite resin restoration:

In the literature, there is insufficient data remarking microleakage of composite resins that replaced high copper amalgam restorations adjacent to discoloured and demineralised dentin. **Harnirattisai et al.**¹⁹ indicated that after removal of amalgam, discoloured dentin showed a lesser degree of bond strength to either self-etch or total-etch adhesives compared with that of surrounding normal dentin. Nevertheless, the two adhesive systems were identical regarding bond strength to discoloured dentin.

These were in contrast with the finding of the present research. In replacing one year old amalgam restoration with composite resins, the mean hybrid layer thickness (13.25 +/-3.99) and number of resin tags (44.50 +/-7.38) were observed in Tetric N-Bond adhesive [Group II]. While the mean hybrid layer thickness (9.5 +/- 2.57) and number of resin tags (32.65 +/-7.88) were observed in Tetric N-Bond Universal adhesive [Group III]. A significant difference in the thickness of hybrid layer was observed between the groups (p value <0.0013). And a highly significant difference in the number of resin tags was observed between the groups (p value <0.0001).

Thus, the hypothesis of the present study that there would be no difference in the penetration of fifth and eighth generation bonding agents for replacement of existing silver amalgam restoration with composite resins under a Confocal Laser Scanning Microscope was rejected. The hybrid layer thickness and the number of resin tags of the bonding agents differed significantly amongst the groups. A complete and deep infiltration of the adhesive system into dentin with numerous resin tags, which has been shown for the adhesive system 'Tetric N-Bond', is more likely to predict a durable bond of the composite resins to dentin than the other systems.

Limitations

1. The extracted teeth were stored according to standard guidelines, but they showed great degree of variations with respect to oral conditions.
2. Exact simulation of the oral conditions was not possible as this was an *in vitro* study. Therefore, complex interactions under intraoral conditions may produce more aggressive responses to the bond structural rigidity, compared with the present *in vitro* test.
3. The study only evaluated morphological aspect of RDI. Further correlation of these morphological aspects with the quality of hybrid layer thickness may establish the validity of claim.

Summary and Conclusion

In the past decades, silver amalgam was the most commonly used restorative material in posterior teeth. At “Minamata Convention”, a treaty was signed proclaiming cessation of all mercury containing products by 2020. The awareness of patients towards mercury poisoning and their aesthetic demands has led to a paradigm shift towards composite restoration as a viable alternative to silver amalgam. Composite restoration not only offers excellent aesthetics but also conserves the tooth structure. Because of patient’s increasing demand for tooth coloured restorations, many amalgam restorations are being replaced with composite resins. However, prospective studies have reported large amounts of amalgam corrosion products under amalgam restorations which were strictly related to discoloured and apparently demineralised dentin.

Thus, the present in vitro study was carried out to evaluate the thickness of hybrid layer and number of resin tags at resin-dentin interface in two different

generations of bonding agents using Confocal Laser Scanning Microscope in premolars with Class I amalgam restorations replaced with composite restorations.

Eighty freshly extracted human premolars, which fulfilled the inclusion criteria were selected for the study. Standard Class I cavity was prepared on the samples.

Depending upon the generation of bonding agent used, the samples were randomly distributed into the following groups:

Group Ia: Class I composite resin restoration using **Tetric N-Bond** bonding agent.

Group Ib: Class I composite resin restoration using **Tetric N-Bond Universal** bonding agent.

Group II: Replacement of Class I silver amalgam restoration with composite resin using **Tetric N-Bond** bonding agent.

Group III: Replacement of Class I silver amalgam restoration with composite resin using **Tetric N-Bond Universal** bonding agent.

There was a statistically significant difference in the mean thickness of hybrid layer in the positive control groups when compared to the experimental groups irrespective of the generation of bonding agent used. (p-value < 0.0001)

It was also found that a highly significant difference existed in thickness of hybrid layer and number of resin tags when the two different generations of bonding agents were compared.

Within the limitations of the study, following conclusions can be drawn:

1. A uniform hybrid layer and continuous resin tags were seen in the groups where fifth generation bonding agent was used.
2. A significantly thicker hybrid layer and higher number of resin tags were obtained when a total-etch system was used for composite restoration or replacing old amalgam restoration with composite resin.
3. The thickness of hybrid layer and number of resin tags were less when eighth generation bonding agent was used in a self-etch mode.

Taking into consideration the findings of the present study, it can be concluded that under experimental conditions, a thicker hybrid layer and higher number of resin tags are formed when a fifth generation bonding agent (total-etch system) i.e. Tetric N-Bond is used. On Confocal Laser Scanning Microscope the penetration of Tetric N-bond was found better than Tetric N-Bond Universal used either for composite resin restoration or after replacing old amalgam restoration with composite resins. However, a correlative study with the quality of hybrid layer formed could give a conclusive remark on the effect of penetration of the bonding agent on the resin-dentin interface.

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Tables & Graphs

Table 1: Comparison of mean hybrid layer thickness between groups Ia and II

Groups	Mean	SD	Median	p-value*
Group Ia	30.74	13.52	25.79	< 0.0001 (HS)
Group II	13.25	3.99	12.95	

*Obtained using Student's t test; HS: Highly Significant

Table 2: Comparison of mean hybrid layer thickness between groups Ib and III

Groups	Mean	SD	Median	p-value*
Group Ib	16.63	5.21	15.68	< 0.0001 (HS)
Group III	9.50	2.57	9.72	

*Obtained using Student's t test; HS: Highly Significant

Table 3: Comparison of mean hybrid layer thickness between groups Ia and Ib

Groups	Mean	SD	Median	p-value*
Group Ia	30.74	13.52	25.79	0.0002 (S)
Group Ib	16.63	5.21	15.68	

*Obtained using Student's t test; S: Significant

Table 4: Comparison of mean hybrid layer thickness between groups II and III

Groups	Mean	SD	Median	p-value*
Group II	13.25	3.99	12.95	0.0013 (S)
Group III	9.50	2.57	9.72	

*Obtained using Student's t test; S: Significant

Table 5: Intraclass correlation between resin tags count between two evaluators

Parameters	Groups			
	Group Ia	Group Ib	Group II	Group III
Resin tags	0.989; < 0.0001	0.984; < 0.0001	0.971; < 0.0001	0.982; < 0.0001

Table 6: Comparison of resin tags between groups Ia and II

Groups	Mean	SD	Median	p-value*
Group Ia	90.10	10.95	89.50	< 0.0001 (HS)
Group II	44.50	7.38	45.00	

*Obtained using Wilcoxon rank sum test; HS: Highly Significant

Table 7: Comparison of resin tags between Groups Ib and III

Groups	Mean	SD	Median	p-value*
Group Ib	68.00	10.02	67.00	< 0.0001 (HS)
Group III	32.65	7.88	34.00	

*Obtained using Wilcoxon rank sum test; HS: Highly Significant

Table 8: Comparison of resin tags between Groups Ia and Ib

Groups	Mean	SD	Median	p-value*
Group Ia	90.10	10.95	89.50	< 0.0001 (HS)
Group Ib	68.00	10.02	67.00	

*Obtained using Wilcoxon rank sum test; HS: Highly Significant

Table 9: Comparison of resin tags between Groups II and III

Groups	Mean	SD	Median	p-value
Group II	44.50	7.38	45.00	< 0.0001 (HS)
Group III	32.65	7.88	34.00	

*Obtained using Wilcoxon rank sum test; HS: Highly Significant

Table 10: Kappa coefficient showing agreement between two evaluators on scores

Scores	Groups			
	Group Ia	Group Ib	Group II	Group III
Resin tags	0.78; < 0.0001	0.700; 0.002	0.798; < 0.0001	0.643; 0.002

Table 11: Number of samples with resin tags scores in each Group

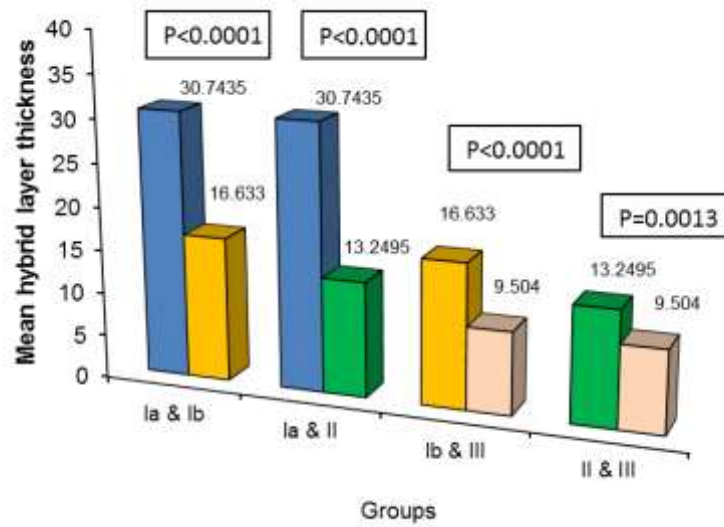
Scores	Groups			
	Group Ia	Group Ib	Group II	Group III
1	0	0	12 (60)	18 (90)
2	6 (30)	10 (50)	8 (40)	2 (10)
3	14 (70)	10 (50)	0	0
Total	20 (100)	20 (100)	20 (100)	20 (100)

Table 11a: Comparison of resin tags scores between different Groups

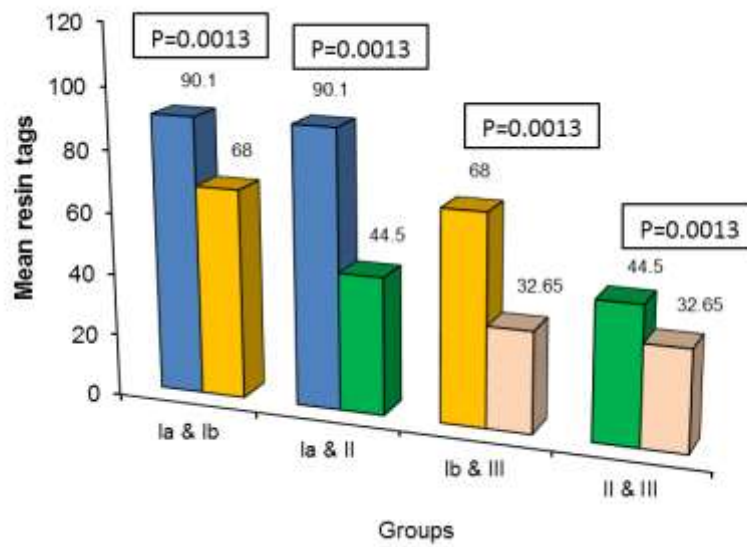
Scores	Groups							
	Group Ia	Group II	Group Ib	Group III	Group Ia	Group Ib	Group II	Group III
1	0	12 (60)	0	18 (90)	0	0	12 (60)	18 (90)
2	6 (30)	8 (40)	10 (50)	2 (10)	6 (30)	10 (50)	8 (40)	2 (10)
3	14 (70)	0	10 (50)	0	14 (70)	10 (50)	0	0
Total	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)
P-value*	< 0.0001 (HS)		< 0.0001 (HS)		0.3329 (NS)		0.0678 (NS)	

*Obtained using Pearson's Chi-Square test; HS: Highly Significant; NS: Non-Significant

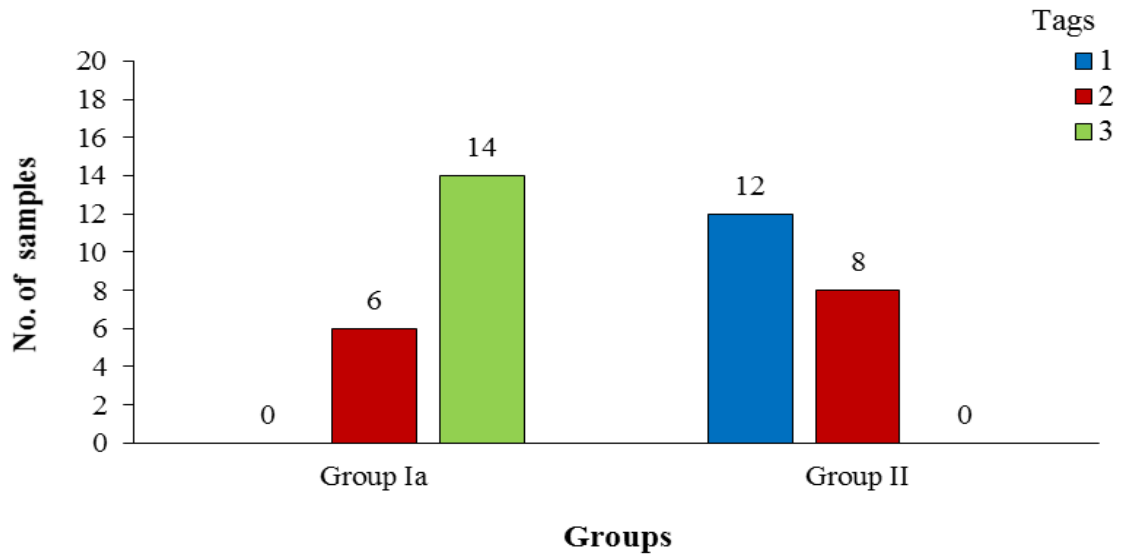
Graph 1: Comparison of mean hybrid layer thickness between different Groups



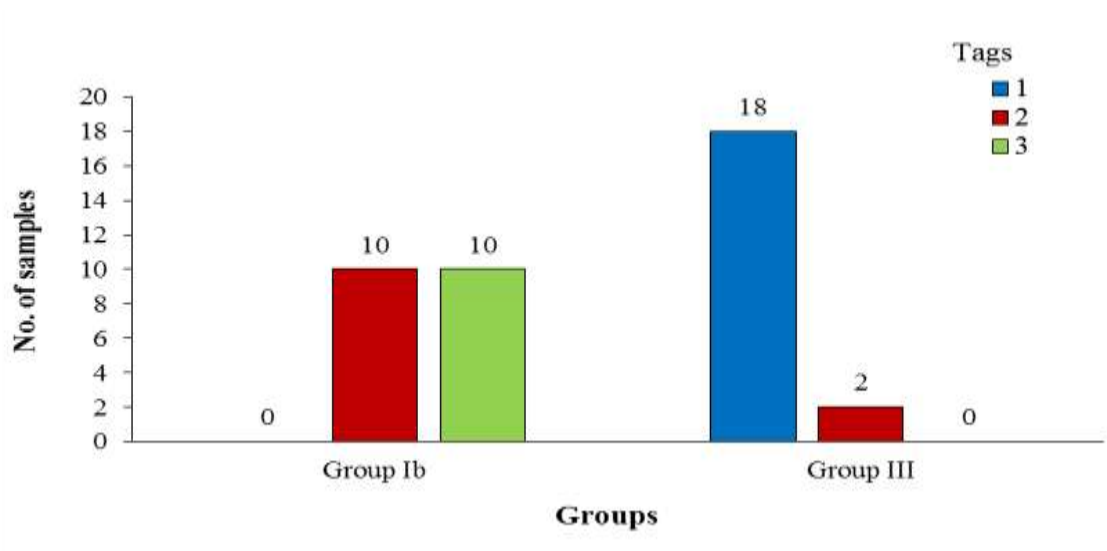
Graph 2: Comparison of mean resin tags between different Groups



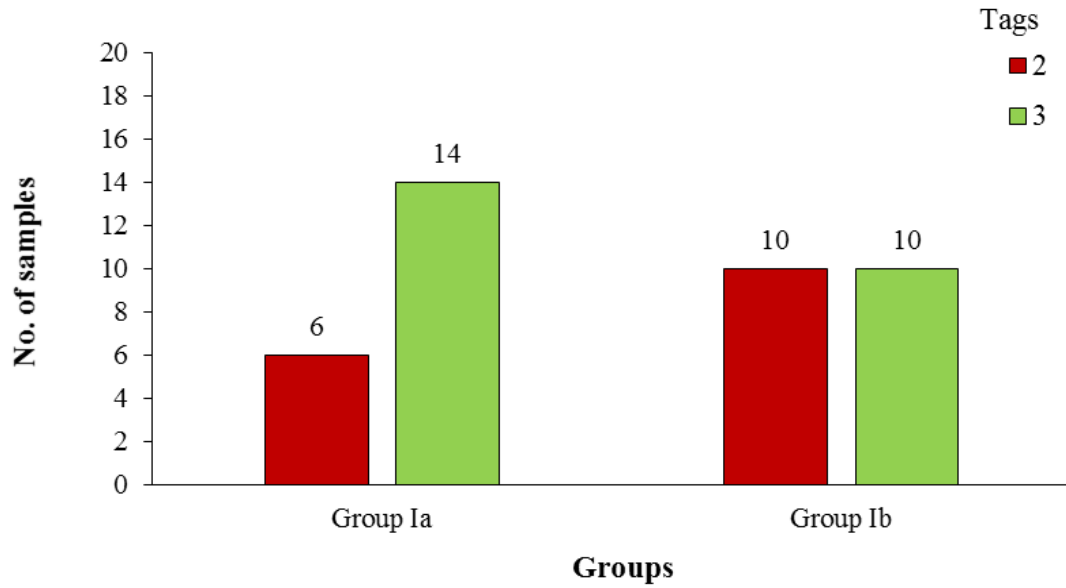
Graph 3: Column chart showing score distribution in samples of Groups Ia and II.



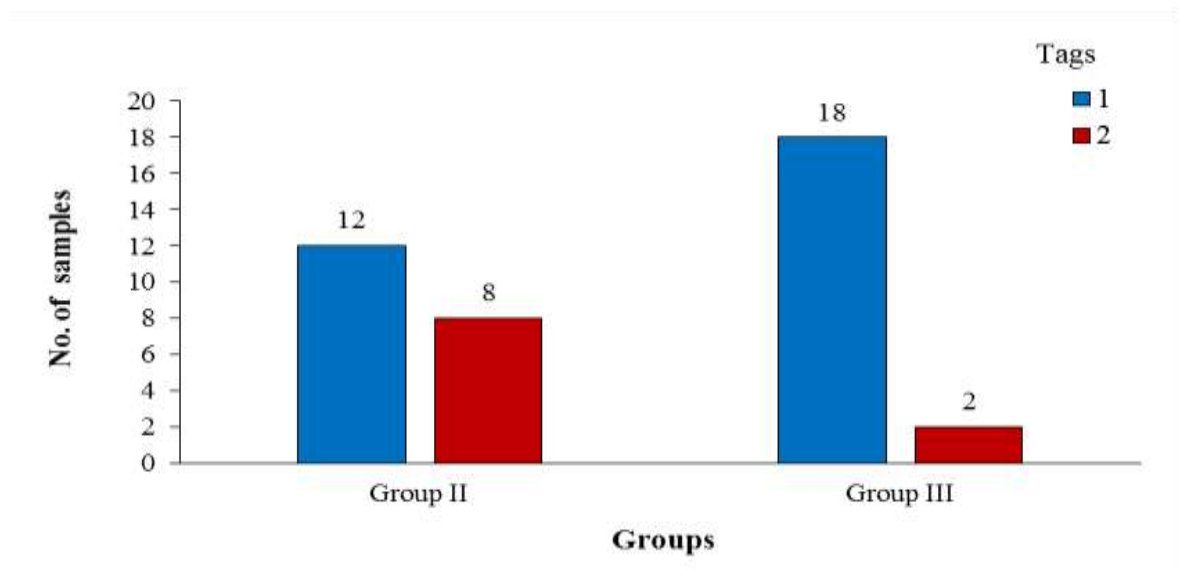
Graph 4: Column chart showing score distribution in samples of Groups Ib and III.



Graph 5: Column chart showing score distribution in samples of Groups Ia and Ib.



Graph 6: Column chart showing score distribution in samples of Groups II and III.



Annexures

Annexure I: Hybrid layer thickness

Sample	Group Ia	Group Ib	Group II	Group III
1	55.73 μm	11.7 μm	14.4 μm	9.85 μm
2	32 μm	15.92 μm	9.6 μm	10.48 μm
3	18.02 μm	12.28 μm	18.01 μm	10.24 μm
4	44.23 μm	7.34 μm	14.4 μm	16 μm
5	36.04 μm	17.34 μm	9.83 μm	8.19 μm
6	29.53 μm	22.29 μm	9.41 μm	9.17 μm
7	21.36 μm	13.72 μm	20.36 μm	8.19 μm
8	15.36 μm	20.58 μm	11.46 μm	10.29 μm
9	17.43 μm	15.43 μm	8.19 μm	6.82 μm
10	15.36 μm	15.36 μm	9.01 μm	7.16 μm
11	25.36 μm	16.34 μm	13.1 μm	9.83 μm
12	17.56 μm	22.4 μm	9.83 μm	10.64 μm
13	24.84 μm	22.7 μm	12.8 μm	10.48 μm
14	24.63 μm	14.08 μm	16 μm	5.24 μm
15	26.21 μm	25.29 μm	20.8 μm	9.6 μm
16	46.31 μm	15.29 μm	19.2 μm	5.73 μm
17	61.75 μm	17.47 μm	14.4 μm	8 μm
18	38.4 μm	13.72 μm	14.74 μm	8.57 μm
19	41.6 μm	25.73 μm	10.24 μm	14.4 μm
20	23.15 μm	7.68 μm	9.21 μm	11.2 μm

Annexure II: Number of resin tags in Group Ia (per 425 micrometers)

Sample	Evaluator I	Evaluator II	Mean of Evaluator I and II
1	103	101	102
2	74	78	76
3	87	85	86
4	89	91	90
5	102	100	101
6	73	71	72
7	92	94	93
8	88	88	88
9	76	74	75
10	103	105	104
11	84	86	85
12	79	79	79
13	91	87	89
14	101	97	99
15	106	104	105
16	72	74	73
17	94	96	95
18	100	100	100
19	88	86	87
20	102	104	103

Annexure III: Number of resin tags in Group Ib (per 425 micrometers)

Sample	Evaluator I	Evaluator II	Mean of Evaluator I and II
1	62	64	63
2	73	71	72
3	60	56	58
4	82	80	81
5	66	70	68
6	75	77	76
7	52	50	51
8	64	68	66
9	78	80	79
10	81	83	82
11	57	55	56
12	60	64	62
13	80	80	80
14	55	53	54
15	72	76	74
16	62	60	61
17	58	60	59
18	70	70	70
19	82	84	83
20	66	64	65

Annexure IV: Number of resin tags in Group II (per 425 micrometers)

Sample	Evaluator I	Evaluator II	Mean of Evaluator I and II
1	48	50	49
2	37	35	36
3	58	54	56
4	47	51	49
5	36	34	35
6	49	53	51
7	37	39	38
8	44	42	43
9	54	50	52
10	34	34	34
11	45	43	44
12	38	36	37
13	45	47	46
14	54	56	55
15	36	36	36
16	49	51	50
17	40	38	39
18	53	55	54
19	48	48	48
20	36	40	38

Annexure V: Number of resin tags in Group III (per 425 micrometers)

Sample	Evaluator I	Evaluator II	Mean of Evaluators I and II
1	35	33	34
2	20	20	20
3	39	37	38
4	44	44	44
5	43	41	42
6	25	21	23
7	42	40	41
8	37	35	36
9	23	25	24
10	28	30	29
11	40	40	40
12	38	40	39
13	35	33	34
14	25	25	25
15	34	30	32
16	21	23	22
17	41	43	42
18	24	22	23
19	37	39	38
20	26	28	27

Annexure VI: Scoring for Group Ia

Sample	Evaluator I	Evaluator II	Evaluator III	Final score
1	3	3	-	3
2	2	2	-	2
3	3	3	-	3
4	3	3	-	3
5	2	2	-	2
6	3	3	-	3
7	3	3	-	3
8	2	2	-	2
9	3	2	3	3
10	3	3	-	3
11	3	3	-	3
12	3	3	-	3
13	2	2	-	2
14	3	3	-	3
15	3	3	-	3
16	2	3	3	3
17	2	2	-	2
18	3	3	-	3
19	3	3	-	3
20	2	2	-	2

Annexure VII: Scoring for Group Ib

Sample	Evaluator I	Evaluator II	Evaluator III	Final score
1	2	2	-	2
2	3	3	-	3
3	2	2	-	2
4	2	2	-	2
5	3	3	-	3
6	2	3	3	3
7	2	3	2	2
8	3	3	-	3
9	2	2	-	2
10	3	3	-	3
11	2	2	-	2
12	2	2	-	2
13	3	3	-	3
14	2	2	-	2
15	2	2	-	2
16	3	2	3	3
17	3	3	-	3
18	3	3	-	3
19	3	3	-	3
20	2	2	-	2

Annexure VIII: Scoring for Group II

Sample	Evaluator I	Evaluator II	Evaluator III	Final score
1	1	1	-	1
2	2	2	-	2
3	1	2	1	1
4	1	1	-	1
5	2	2	-	2
6	1	1	-	1
7	1	1	-	1
8	2	2	-	2
9	1	1	-	1
10	1	1	-	1
11	2	2	-	2
12	1	1	-	1
13	2	2	-	2
14	1	1	-	1
15	2	2	-	2
16	2	1	1	1
17	2	2	-	2
18	1	1	-	1
19	2	2	-	2
20	1	1	-	1

Annexure IX: Scoring for Group III

Sample	Evaluator I	Evaluator II	Evaluator III	Final score
1	1	1	-	1
2	1	1	-	1
3	1	1	-	1
4	1	1	-	1
5	1	1	-	1
6	1	1	-	1
7	1	1	-	1
8	2	2	-	2
9	1	1	-	1
10	1	1	-	1
11	1	1	-	1
12	1	1	-	1
13	1	2	2	2
14	1	1	-	1
15	1	1	-	1
16	1	1	-	1
17	1	1	-	1
18	1	1	-	1
19	1	1	-	1
20	1	1	-	1