An in-vitro evaluation of the effect of preheating of dental composite resin in Class-I Cavity restorations on microleakage using Confocal Laser Scanning Microscopic (CLSM) analysis.

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Abstract:

This study aims to evaluate the effect of preheating of dental composite resin in Class-I Cavity restorations on microleakage using Confocal laser scanning microscopic analysis. 90 extracted human permanent maxillary premolar teeth were included.Class-I cavity preparations were done on the occlusal surfaces of all the teeth with No.245 Tungsten carbide airoterbur in equal dimensions; with the cavity depth of 2 mm, bucco-lingual width of 3 mm and mesio-distal width of 4 mm.All the prepared cavities were then acid etched with 37% phosphoric acid, gently rinsed with waterand air dried. Dentin bonding agent was then applied and light cured for 20 seconds. All the teeth were then randomly divided into three groups with 30 specimens per group.In all the specimens, the prepared cavities were restored with Nano-Hybrid Universal restorative composite resin. In Group A; the prepared cavities were restored with composite resin at room temperature (37^0C), In Group B; composite resin pre-heated to 50^0C and in Group C; composite resin pre-heated to 60^0C. Pre-heating of composite resin was done in a Preheat furnace.Pre-heated composite resin was immediately placed without any time delay into the prepared cavities and light cured for 20 seconds per increment. Thermocycling was done.All the specimens were then subjected to dye penetration test and were then longitudinally sectioned in mesio-distal direction.The presence or absence of microleakage was determined by the extent of dye penetration into the composite resin along the tooth-restoration interface visually observed with Confocal laser scanning microscope using Image J software and the extent of dye penetration was measured and recorded in micrometers (µm). The prepared cavities restored with pre-heated composite resin (50^0C) showed minimal microleakage in the dye penetration test with superior marginal adaptation to the tooth structure compared to pre-heated composite to 60^0C. Cavities restored with composite resin at room temperature (37˚C) showed maximum microleakagewith poor marginal adaptation to the tooth structure.

Key words: Confocal laser scanning microscope; Dental composite resin; Dye penetration; Microleakage; Pre-heated resin.

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Introduction:

Dental composites have been the pinnacle in esthetic restorations ever since its discovery.[1] The use of composite resin as a substitute for dental amalgam as a restorative material of choice in posterior teeth is on the rise since last few decades and this is due to increase in the esthetic demands of patients and concerns about mercury present in the dental amalgam.[2] Dental composite resins represents a unique class of biomaterials with better biocompatibility, curing behavior, esthetics and ultimate material properties, but they are limited by polymerization induced shrinkage stresses, limited toughness, presence of unreacted monomer that remains even after light-cure polymerization. Fortunately, dental composites are of a great deal of research in recent years with the goal of improving their clinical performance by pre-heating of composite resin and changes in the initiation systems, monomers, fillers, coupling agents and also by developing novel polymerization stratergies.[3]

Dental composites set mainly by photo-polymerization, a process that is temperature-dependant, with the rate of conversion increasing with the increase in temperature of the composite resin and higher degree of conversion improves the physical properties of the composite resin.[4]Several studies[4,5,6] have suggested that preheating of dental composite resin showed better marginal adaptation to the prepared cavity walls of the toothstructure by enhancing radical mobility of trapped radicals in the resin resulting in additional polymerization, thus increasing the flowability and reducing the microleakage of composite resin.

In our study, Confocal Laser Scanning Microscope (CLSM) was used for the evaluation of microleakage in the dye penetration test of composite resin at room temperature (37^0C) and pre-heated dental composite resin at (50^0C) and (60^0C) in Class-I cavity restorations. CLSM is a non-destructive technique and it clearly indicates the extent of dye leakage. The advantage of CLSM is the use of lens focus, which can focus a few micrometers beneath the observed surface of the tooth. CLSM scans the sample sequentially point by point and line by line and assembles the pixel information into one image.[7]

Materials and Methods:

Ninty freshly extracted human permanent maxillary premolar teeth were collected in Triveni Institute of Dental Sciences, Hospital and Research Centre. Bilaspur, India. Inclusion Criteria: Non-carious, non-fractured, un-restored, closed root apices. Exclusion Criteria: Carious, fractured, restored, open apex, previously endodontically treated teeth.All the teeth were cleaned of superficial debris, calculus, residual tissue tags with ultrasonic instruments and immersed for 30 minutes in 3% sodium hypochlorite solution for its disinfection, washed with tap water and were then stored in 0.5% thymol at room temperature until used.

Each tooth was then embedded in cylindrical acrylic resin (DPI-RR Cold Cure, Mumbai, India) blocks till the cervical line (cemento-enamel junction) with the crown portion of the teeth exposed, for ease of handling. G.V. Black's Class 1 - Cavity preparations were done in all the teeth along the central groove on the occlusal surfaces in equal dimensions for standaridization; with the cavity depth of 2 mm, bucco-lingual width of 3 mm and mesio-distal width of 4 mm (Figure 1)using No. 245 Tungsten-carbide bur(SS White company, New Jersey, USA) in high speed contra-angled airoter hand piece (NSK, Pana Air, Japan).

Figure 1

Class -1 Cavity preparation on maxillary premolar

William's graduated periodontal probe (API, AshooSons, New Delhi, India) was used to measure the dimensions of the prepared cavities and they were then cleaned with distilled water and air dried. All the prepared cavities were acid etched with 37% phosphoric acid (Prime dental products, Thane, Maharashtra, India) for 15 seconds and wererinsed with water for 10 seconds to completely remove the etchant from the tooth surface and were gently air dried. Dentin bonding agent (3M ESPE Filtek Z250 XT, USA) was applied to all the walls of the prepared cavities with an applicator tip (Reach Global India Private Limited, Pune, India) and was then light cured with LED (Light Emitting Diode) dental composite curing light [Saab, China] for 20 seconds. All the specimens were then randomly divided into three groups with 30 specimens per group.

In all the specimens, the prepared cavities were restored with Nano Hybrid Universal Restorative Composite resin (3M ESPE Filtek Z250 XT, St Paul MN, USA)(Figure 2). In Group A; the prepared cavities were restored with composite resin at room temperature (37^{0}C), In Group B; the prepared cavities were restored with composite resin pre-heated to 50^{0}Cand in Group C; the prepared cavities were restored with composite resin pre-heated to 60^{0}C

Figure 2

Nano Hybrid Universal Restorative Composite resin

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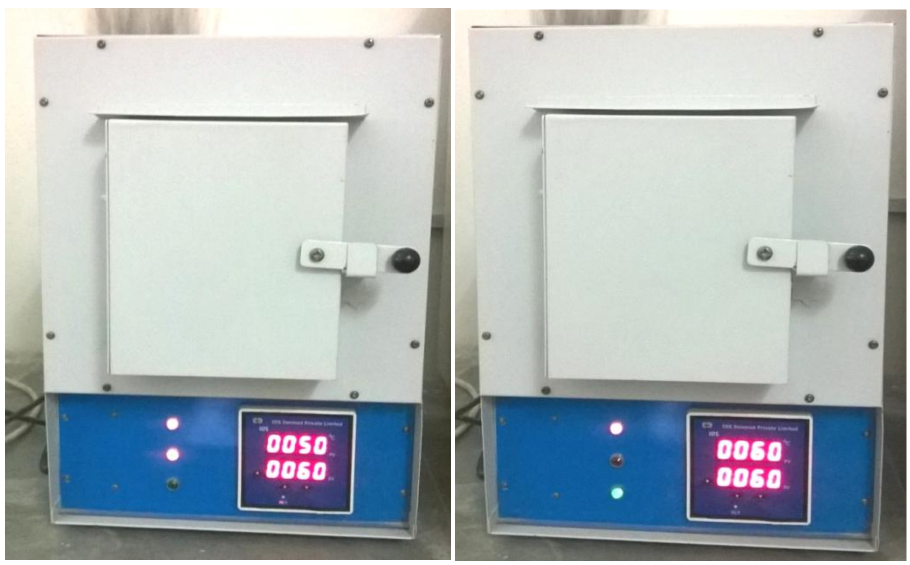
Pre-heating of Nano Hybrid Universal Restorative composite resin to 50^0C and 60^0C of temperature was done in a Preheat furnace (IDS, Denmed Private Limited, New Delhi, India)(Figure 3) and the pre-heated composite resin was immediately placed without any time delay into the prepared cavities in increments of 1mm thickness using a Teflon coated composite carrier instrument (Hu Friedy, Chicago, USA) in oblique layering technique and was then immediately curedusing LED composite curing light for 20 seconds per increment. All the composite restorations were finished using a flame shaped finishing bur (Mani, Inc, Japan) and were polished using medium and extra-thin contouring and polishing Sof-Lex discs (3M ESPE, Dental products, St. Paul, USA). In all the specimens, the cavity preparations and composite resin restorations were done by a single operator to eliminate inter-operator variability.

Figure 3

Preheat furnace at 50^0C and 60^0C



All the specimens were then removed from their acrylic resin blocks and were subjected to thermocycling at $4^0 \pm 2^0$C and $56^0 \pm 2^0$C for 500, 2 minute cycles with a dwell time of 30 seconds as per International Organization for Standardization Standard 11405[8]. All the specimens were then double coated with nail varnish except over the composite resin restorations and 1mm along the tooth-restoration interface.The apical foramen of each specimen was sealed with cyanoacrylate glue (Benson Polymer Limited, India) to prevent the penetration of dye during the dye penetration test from the apical region. All the specimens were then immersed in 0.5% Basic fuschin dye (Thermo Fisher Scientific India Pvt Ltd, Mumbai, India) for 48 hours for the dye penetration test. Later all specimens werethoroughly rinsed under copious running water, air dried, cyanoacrylate glue and nail varnish were completely removed using a surgical scalpel. All the teeth were then longitudinally sectioned in mesio-distal direction with a diamond disc (DFS, Germany) of 0.3 mm in thickness at a speed of 20,000 rpm (revolutions per minute) using a micromotor straight hand piece (Marathon, Saeyang, Korea) and the obtained sectioned specimens of the three groups were examined under Confocal laser scanning microscope.

The presence or absence of microleakage was determined by the extent of dye penetration into the composite resin along the interface of composite resin restoration- tooth surface, visually observed usingConfocal laser scanning microscope [LSM 780, Carl Zeiss MicroImaging, GmbH, Jena, Germany] (Figure 4) with DPSS (Diode Pumped Solid State) 561 nanometers LASER (Light Amplification by Stimulated Emission of Radiation) at a magnification of 10X in the fluorescence mode. Using CLSM, each specimen was then scanned sequentially point by point and line by line and the pixel information is assembled into one image. After the imaging, the fluorescent areas above the background for each 0.5% Basic fuschin-stained sections were selected in Image J software (Java based image processing program) and the default auto-threshold plugin with "ignore black" and "ignore white" boxes checked to account for any under or over-exposed pixels, this algorithm convertedimages to 8-bit depth and the thresholded area was then selected, saved and re-applied to the original 16-bit image. The integrated density (sum of all pixel intensities) of the thresholded area was then measured and recorded in micrometers (µm).

Figure 4

Confocal laser scanning microscope - Zeiss LSM 780

Results:

The recorded readings were tabulated and statistically analysed with computer software; Statistical Package for Social Sciences (SPSS) version 24, Using Analysis of variance (One Way ANOVA) and Tukey's post-hoc test.Analysis of Variance tests the equality of three or more means at one time by using variances. One way ANOVA showed statistically significant difference in the mean values of the dye penetration between the groups as P(Probability) value is < 0.05 (Table 1).

Table 1
Analysis of Variance (One Way ANOVA)

Groups	No. of specimens per group	Dye penetration in Micrometers (µm) (Mean ± SD)	P value
Group A	30	526.92 ± 19.718	**< 0.05**
Group B	30	281.70 ± 17.811	
Group C	30	366.60 ± 14.790	

***P**: Probability
***SD:** Standard Deviation

According to One Way ANOVA between the three groups, Group B; in which the prepared cavities were restored with composite resin pre-heated to 50^0C showed minimal dye penetration in Confocal laser scanning microscopic analysis with the mean value of 281.70µm followed by Group C; in which the prepared cavities were restored with composite resin pre-heated to 60^0C with the mean value of 366.60µm. The maximum dye penetration was seen in Group A; in which the prepared cavities were restored with composite resin at room temperature (37^0C) with the mean value of 526.92µm (Figure 5; Figure 6; Figure 7).

Figure 5
Group A: Evaluation of dye penetration using Confocal laser scanning microscope in fluorescence mode

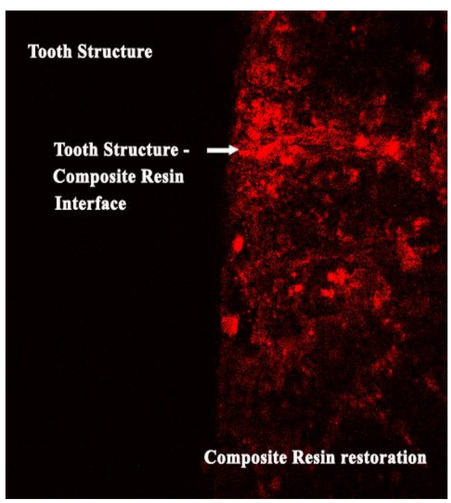


Figure 6

Group B: Evaluation of dye penetration using Confocal laser scanning microscope in fluorescence mode

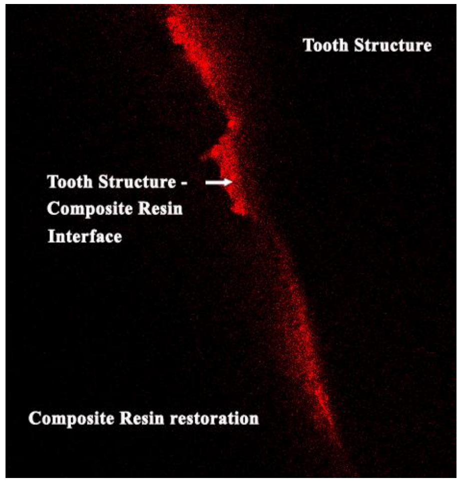
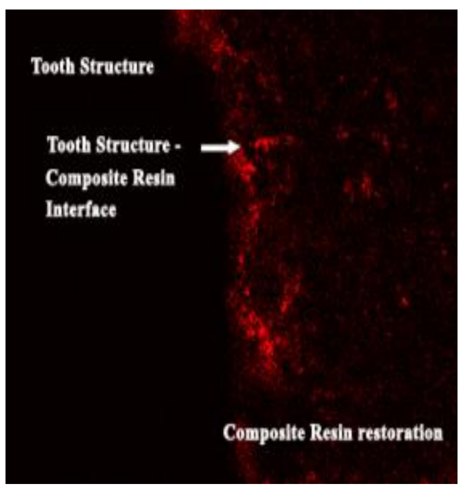


Figure 7

Group C: Evaluation of dye penetration using Confocal laser scanning microscope in fluorescence mode

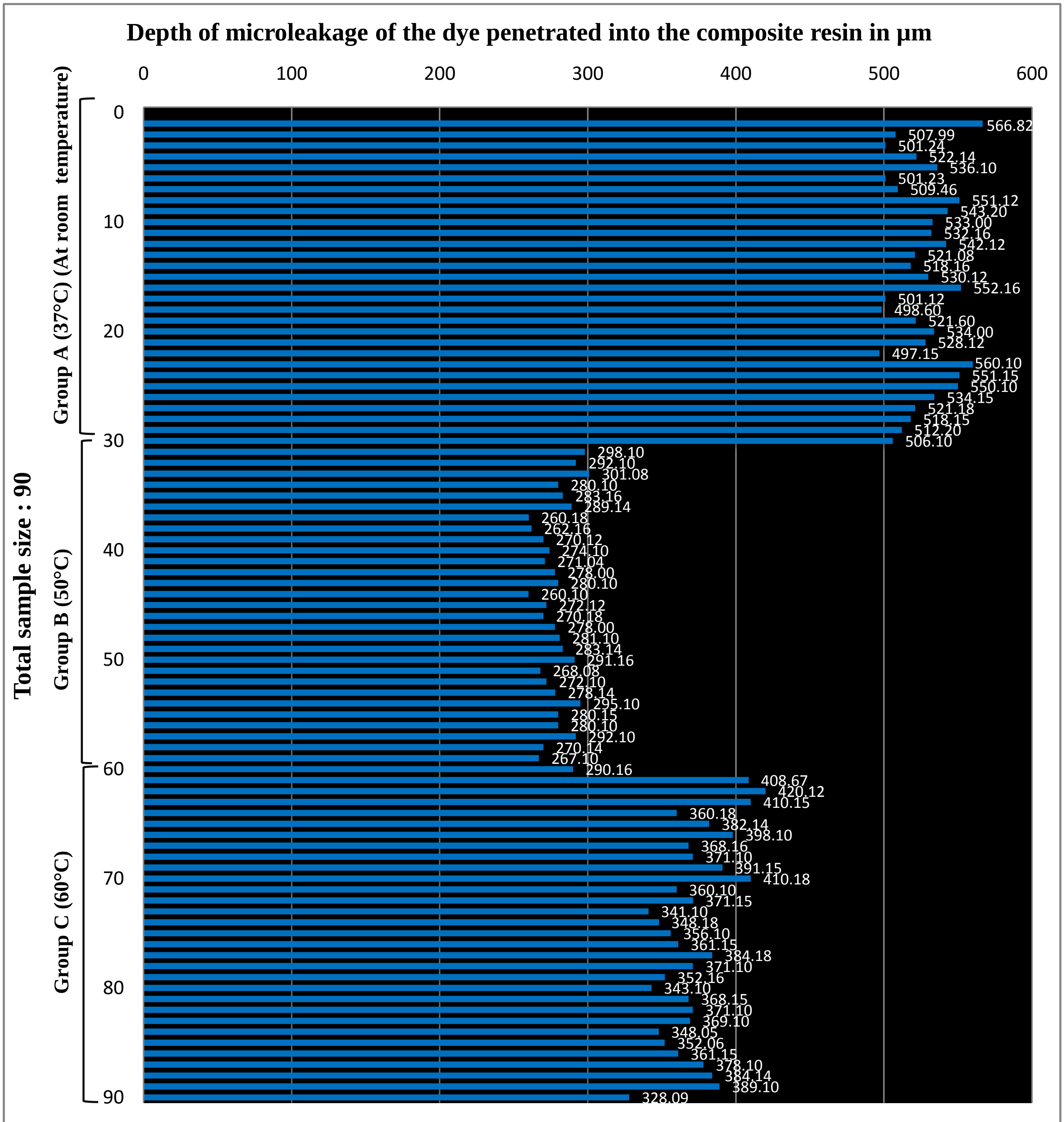


To find where exactly the statistically significant difference is, Tukey's post-hoc test was done for inter-group comparison between the three groups and it was found that, all the inter-group comparisons were statistically significant as the P-value was < 0.05, except in the inter-group comparison between Group B (prepared cavities restored with composite resin pre-heated to 50^0C)and Group C (prepared cavities restored with composite resin pre-heated to 60^0C), as the P-value was > 0.05 and is Non-significant.

Group B(50^0C) and Group C(60^0C); in which the prepared cavities were restored with pre-heated composite resin showed minimal microleakage in the dye penetration test with superior marginal adaptation to the tooth structure and exhibited statistically significant difference in the dye penetration compared to Group A; in which the prepared cavities were restored with composite resin at room temperature (37˚C) and it showed maximum microleakage in the dye penetration test with poor marginal adaptation to the tooth structure (Graph 1; Graph 2)

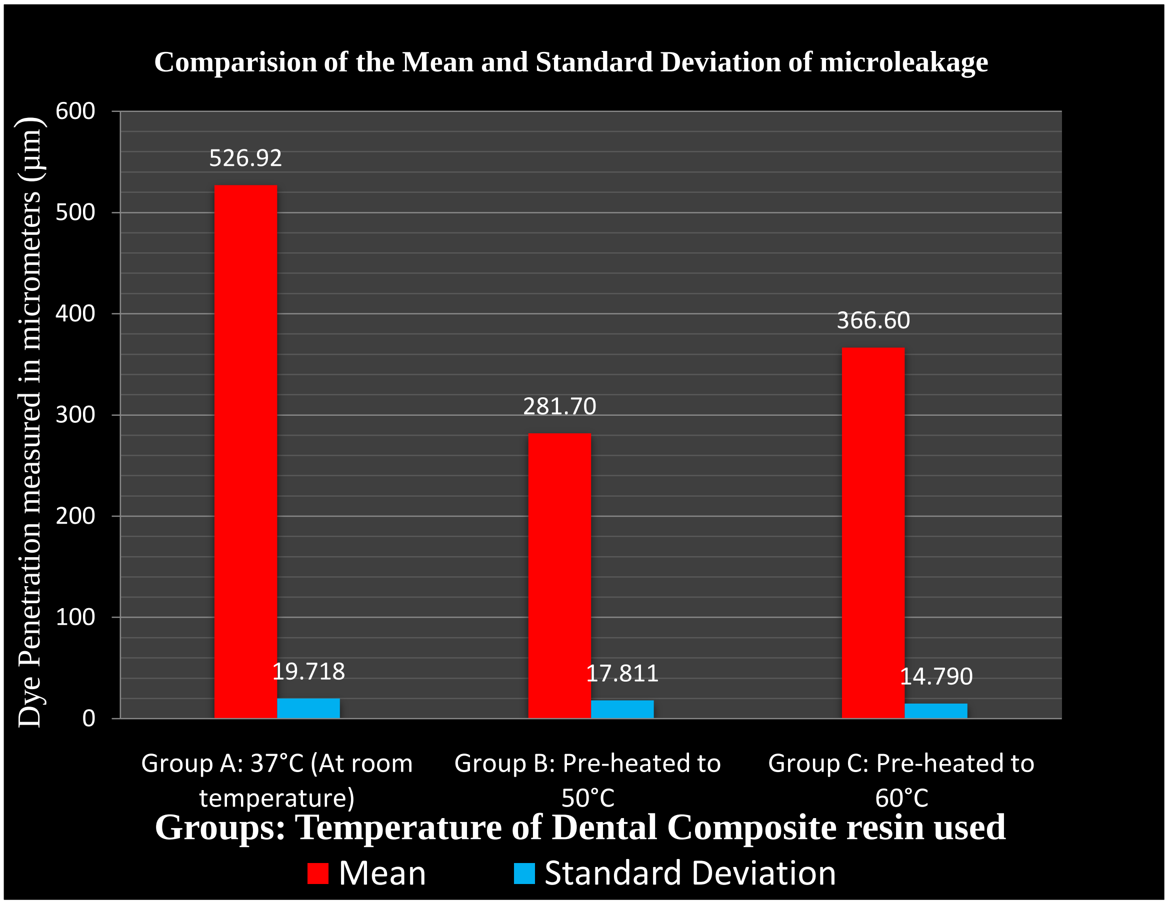
Graph 1

Depth of dye penetration into the composite resin along the tooth-restoration interface



Graph 1 : Group A, Group B, Group C : Dye penetration measured in micrometers (µm)

Graph 2
Vertical Bar Graph



Discussion:

One of the greatest limitations of dental composite restoration is linked to its high polymerization shrinkage. During polymerization, it changes from a Pre-gel phase to Post-gel phase. During the pre-gel stage, the reactive species can rearrange themselves without generating much internal and interfacial stresses to compensate for any volumetric shrinkage.[9,10] However, in the Post-gel stage the resin has partially set and can no longer undergo plastic deformation to compensate for any volumetric shrinkage. As a result, tensile stresses are generated at the resin-tooth interface and causes pulling of the resin material away from the tooth surface.[11,12] Many attempts have been made to overcome this problem including incorporating of fiber inserts or chemicals into the resin and over the recent years there has been growing interest in making composite resin less viscous by preheating them before placing it into the prepared cavity without undermining the properties of the resin.[13,14,15,16,17] Preheating of composite resin reduces its viscosity and increases flowability, which facilitates superior marginal adaptation to the prepared cavity walls of the tooth reducing microleakage. Increase in the temperature of composite resin enchances both radical and monomer mobility, resulting in the formation of highly cross-linked polymer networking with improved mechanical and physical properties of the resin. Superior surface hardness and increased depth of cure are also positive outcomes of preheating of composite resins.[4,18]

Nanohybrid Universal Restorative composite resin was recently introduced in operative dentistry in an endeavour to provide a restorative material with superior esthetics and strength. So in our study, it was used as a restorative material in all the specimens. Nanohybrid composite resin has a unique combination of nano-sized particles, nanoclusters with conventional fillers. The fillers in nanohybrid composite resin contains surface-modified zirconia/silica with median particle size of approximately 3 microns or less, non-agglomerated 20 nanometers surface-modified silica particles with filler loading of 82% by weight (68% by volume) and the resin phase contains Bis-GMA (Bisphenol-GlycidyldiMethAcrylate), UDMA (Urethane DiMethAcrylate), PEGDMA (PolyEthyleneGlycolDiMethAcrylate).[19]

Chohayeb et al[20] reported that the temperature fluctuations can adversely affect the marginal seal or adaptation of any restorative material to the tooth structure and moreover, assessment of the effects of such thermal changes is critical for durability of bond due to difference in the co-efficient of thermal expansion of dental polymers from that of the tooth structure. So to simulate the human in-vivo conditions, in our study all the specimens were subjected to thermocyclingas per International Organization for Standardisation Standard 11405.[8]The purpose of dye penetration test is to evaluate the sealing ability of a restorative material to the prepared cavity walls of tooth structure. Failure of a restorative material to achieve an adequate seal contributes to marginal staining, adverse pulpal response, post-operative sensitivity and recurrent caries. Dye penetration method involves the use of a contrasting dye to stain the areas of microleakage and the tooth-restoration interface is examined for evidence of staining. It has many advantages over the other techniques;no radiation, highly feasible and easily reproducible.[21]The dye penetration test requires an appropriate evaluation tool to determine the true extent of microleakage.In our study, 0.5% Basic fuschin dye was used and the extent of microleakage was measured by the amount of dye penetration into the composite resin along the resin - tooth structure interface using Confocal laser scanning microscope. CLSM is a non-destructive technique for visualizing sub-surface tissue features. One of its main advantages is the accurate detection of microleakagedue to lens focus that occurs some microns beneath the observed surface of tooth or restorative material, as it eliminates the spread of the dye stain caused due to specimen sectioning and also avoids polishing artifacts that exaggerate dye penetration.[22,23]

In our study, pre-heated composite resin was used in Group B(50^{0}C) and Group C(60^{0}C) for the restoration of prepared cavities and it showedleast microleakage in the dye penetration test with superior marginal adaptation to the tooth structure compared to Group A; in which the prepared cavities were restored with composite resin at room temperature (37˚C).The results of our study are in accordance with the previous studies of N.R.F. Salgado et al[24]and M. Daronch et al[25]reported that maximum microleakage in the dye penetration tests was noticed when composite resin was used as restorative material at room temperature(37^{0}C) compared to pre-heated composite resin. When composite resin is used as a restorative material at room temperature(37^{0}C), there is incomplete polymerization of the composite resin and the extent of polymerization is expressed as the degree of conversion of monomeric C-C bonds into polymeric C-C bonds along with the presence of unreacted monomer reducing both the physical and mechanical properties of composite resin.

Preheating of composite resin increases the monomer mobility, decreases the viscosity of the resin thus enhancing the fluidity. When the composite resin is pre-heated, thermal vibration causes further separation among monomers which quickens their slide thereby decreasing the film thickness of the resin and when this resin is placed into the prepared cavities, it easily wets the tooth and shows better marginal adaptationwith the tooth structure, thereby reducing the microleakage.[4,25]Dos Santos RE et al[6] reported that pre-heating of composite resin to 50^{0}C to 60^{0}Cdecreases the viscosity of the resin and enhances radical mobility resulting in additional polymerization with superior marginal adaptation of resin to the tooth structure.

In our study, Group B(prepared cavities restored with composite resin pre-heated to 50^{0}C) showed minimal microleakage in the dye penetration test compared to Group A(37^{0}C) and Group C(60^{0}C). However, there was no significant difference seen in the extent of microleakage between the specimens of Group B and Group C.Friedman J[26] stated, an increase of only 1.6^{0}C when composite resin preheated to 50^{0}C was placed into the prepared cavities causing no pulpal damage.However, Cobb DS et al[27]and BouillaguetS et al[28]found an increase averaging upto 7.9^{0}C when composite resin preheated to 60^{0}C was placed into the prepared cavities causing pulpal damage.

Wagner WC et al[4] reported that pre-heating of composite resin to higher temperature upto 60^{0}C could cause the resin to try to return more rapidly to a previous shape due to its visco-elastic behavior causing it to pull away from the walls of the prepared cavity.DaronchM et al[29] reported that preheating of composite resin to 60^{0}C produces higher conversion and reaction rate resulting in elevated stress formation and accelerate development of the vitrification point causing damage to the integrity of the resin/tooth interfacial bond and the increased stress was seemed to be a consequence of the system thermal contraction rather than an increase in material conversion.

Conclusion:

Within the limitations of this study it was found that;Dental composite resin pre-heated to 50^{0}C and placed into the prepared Class-I cavities, showed minimal microleakage in the dye penetration test with better marginal adaptation to the walls of prepared cavities compared to the composite resin at room temperature (37^{0}C) and composite resin pre-heated to 60^{0}C.With preheated dental composites, there is an ease of composite manipulation as it can be easily injected into a prepared cavity without using hand instruments and it is advised to work at a quicker pace while using preheated composites so as to prevent dissipation of heat.However, further in-vivo studies are recommended to confirm and correlate the findings of this in-vitro study to a clinical scenario.

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