

## THE EFFECT OF POLY (N-ISOPROPYLACRYLAMIDE) BASED MICROGEL APPLICATION ON THE MICROTENSILE BOND STRENGTH TO DENTIN: AN IN VITRO STUDY

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### ABSTRACT

**Objective:** This study was carried out to determine the effect of polyNIPAM microgel particles on the microtensile bond strength ( $\mu$ TBS) of universal adhesive to dentin before and after thermocycling.

**Materials and methods:** Superficial dentin discs were prepared from young human upper premolars. Specimens were divided into three groups G1: All-Bond Universal adhesive was applied directly to etched dentin. G2: 2% polyNIPAM microgel was added to the adhesive before being applied to etched dentin. G3: 2% polyNIPAM microgel was applied on etched dentin before application of the adhesive. In all groups, after light activation of the adhesive, resin composite (Filtek Z350 XT), was applied incrementally on the treated dentin specimens. A total of 120 beams were prepared. Half of these beams were subjected to 5000 cycles of 5°C to 55°C using a PCR thermocycler. Microtensile bond strength testing was performed on all specimens. The fractured dentin side of all specimens was inspected using a scanning electron microscope (SEM) at 200X magnification. Three sticks from each group were gold sputter coated and examined with SEM at 2000X.

**Results:** The statistical analysis showed that before thermocycling, there was a statistically significant difference in the mean  $\mu$ TBS values of all the tested groups  $p < 0.001$ . G1T0 showed the highest statistically significant mean  $\mu$ TBS values. After thermocycling, G1T1 showed the lowest statistically significant mean  $\mu$ TBS values  $p = 0.001$ . Failure mode analysis and SEM analysis confirmed and explained the  $\mu$ TBS results.

**Conclusion:** The addition of polyNIPAM microgel to etched dentin preserved the bond durability, particularly after thermocycling.

**KEYWORDS:** PolyNIPAM microgel, Universal bonding system, bond durability, microtensile bond strength, failure analysis, interfacial analysis, thermocycling.

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## INTRODUCTION

The revolutionary aspect of the adhesive procedure is to achieve a tight adhesive dentin interface that should be stable for years. However, this stability is endangered by several inherent factors as the stress generated by resin composite volumetric shrinkage in addition to the oral environmental factors<sup>(1)</sup>. Masticatory stresses, pH changes, temperature variation, hydrolytic and enzymatic degradations are some of the oral environmental factors that facilitate adhesive bond degradation<sup>(2)</sup>. Another challenge that faces the adhesive interface is the inherent dentin wetness which increases after tooth preparation and acid etching. Different approaches have been attempted to reduce dentin permeability by sealing the dentinal tubules. Strontium chloride, potassium nitrate, iontophoresis/sodium fluoride, glass-ionomer cement, potassium oxalate, dentine bonding agents, and LASER therapy are all methods and materials used for dentinal tubule occlusion<sup>(3)</sup>. Despite the advantage of preserving the smear plugs through self-etching adhesive the acidic content of these adhesives may accelerate the degradation of the adhesive layer<sup>(4)</sup>. Several studies showed that this occurs as a result of the hydrophilicity of these adhesives in addition to the slow diffusion of the acidic monomer through the smear layer resulting in questionable bonding efficacy<sup>(5)</sup>. In the last few years, polymer-based microgels have shown increasing interest in being attractive materials for many technological applications including,

pollution control, drug delivery, and injectable cell scaffolds<sup>(6-8)</sup>.

Microgels are cross-linked polymeric particles based on water-born polymer technology. An extent of swelling and average cross-linking distinguishes these aqueous gels. Their volume varies in response to external stimuli, such as pH and temperature. The first prepared temperature-sensitive aqueous microgel based on a cross-linked polymer is termed poly (*N*-isopropylacrylamide) abbreviated as (polyNIPAM)<sup>(9)</sup>. Aqueous microgels based on polyNIPAM demonstrate temperature sensitivity<sup>(10)</sup>. PolyNIPAM conducts free radical polymerization in aqueous solution, resulting in high molecular weight polymers. They also demonstrate lower critical solution temperatures (LCST) of 32°C, below this temperature the components of the mixture become miscible and swell creating a hydrophilic gel. When the temperature rises the gel expels the water and shrinks resulting in a hydrophobic gel<sup>(11)</sup>. Since the oral temperature is higher than LCST; polyNIPAM can shrink and repel water and become hydrophobic<sup>(12)</sup>. Therefore it was thought that the use of these polyNIPAM in the field of dentistry might be useful as these gels may aid in sealing the dentinal tubules and reduce the water permeability at the dentin adhesive interface. Since the degree of swelling is a unique characteristic of this microgel particles this study was carried out to determine the effect of thermo-responsive polyNIPAM microgel particles on the microtensile bond strength of universal adhesive to dentin before and after thermocycling.

## MATERIALS

TABLE (1) The materials and their composition used in this study:

Materials	Composition	Manufacturer
Select HV Etch w/BAC	35% phosphoric acid containing benzalkonium chloride. pH: < 1	Bisco, INC. IL, USA.
All-Bond Universal adhesive	BisGMA, 2-Hydroxyethyl Methacrylate, 10 Methacryloyloxydecyl Dihydrogen Phosphate (MDP), Ethyl 4-dimethylaminobenzoate, Ethanol, water, initiators, pH: 3.2	Bisco, INC. IL, USA.
Filtek Z350 XT Universal resin composite	The resin matrix: Bis-GMA, TEGDMA, PEGDMA, UDMA, and Bis-EMA resins. Fillers: A combination of non-agglomerated/non-aggregated 20 nm silica filler, non-agglomerated/non-aggregated 4 to 11 nm zirconia filler, and aggregated zirconia/silica cluster filler (consisting of 20 nm silica and 4 to 11 nm zirconia particles)	3M ESPE, St. Paul, MN, USA
PolyNIPAM Microgel	N-isopropyl acrylamide (NIPAM) 97%, acrylic acid, N, N'methylene bis (acrylamide) 99%, potassium persulphate (KPS) 98%	(Sigma Aldrich, UK)

## MATERIALS AND METHODS

### i. Teeth Samples Collection:

The Ethical Committee for Human Studies of the Faculty of Dentistry at October University for Modern Science and Arts University approved this study (reference no:1123). The selected patients were from 18- 30 years old. They had their upper premolars planned for extraction to perform orthodontic treatment. After signing an informed consent, teeth were extracted in the surgical clinic of the Faculty of Dentistry, October University for Modern Science and Arts University. Teeth were then cleaned with periodontal scaler to remove the remaining periodontal tissues and dental calculus. A total number of 90 teeth were preserved for a week in a saturated thymol solution at 5°C for disinfection and then rinsed with deionized water prior to use.

### ii. Preparation of Dentin Specimens:

Teeth roots were sectioned horizontally coronal to the bifurcation using a double-sided diamond disc under water-cooling at low speed using a sectioning machine (IsoMet 4000, Buehler, USA). The pulp tissue was extirpated with an excavator, an endodontic K-file, and irrigated with saline solution.

A mould made of polyvinyl siloxane putty material (ZetaPlus, Zhermack, Italy) was used. It was filled with a mixture of self-activated polyester resin (Polyester resin #2121, Hsien, Taiwan). The apical two third of the sectioned teeth roots were placed in the mould till complete seating of the polyester resin. The occlusal surface of the embedded teeth were then ground flat using the double-sided diamond disc under water coolant. The occlusal surface was first cut to the level of the central groove to remove most of the occlusal enamel. Then the micrometer of the sectioning machine was adjusted to remove 0.5 mm of the occlusal surface to reach the superficial dentin. Dental loops 6X magnification (Ergovision, Lama-medical.org, New Delhi, India) was used to ensure that enamel was only found at the circumference

of the prepared specimens and no remnants were found at the middle where adhesion will take place. In order to achieve a uniform smear layer, 600-grit silicon carbide paper (Grinco waterproof abrasive paper, Italy) was used to polish the specimens under running water for 30 seconds.

### iii. Poly N-isopropylacrylamide (polyNIPAM) Synthesis

A one-liter reaction vessel secured with a three-necked lid was utilized for this reaction. Distilled water (800 ml) was used to dissolve 0.5 gm potassium persulfate (initiator). They were heated to 70°C while constantly stirring using a magnetic stirrer (Regulator hot plate, Gallenkamp & Co. Ltd, England). NIPAM monomer (5gm) was mixed with 0.5 gm N, N'-methylene bis acrylamide (cross-linker) in 200 ml distilled water and poured into the reaction vessel containing the initiator, where they were agitated continuously using the magnetic stirrer for 6 hours at 70°C in an inert nitrogen environment. The formed microgel dispersion (polyNIPAM) was allowed to cool to room temperature at 25°C. It was dialyzed daily using fresh de-ionized water for 7 days, centrifuged in a refrigerated benchtop centrifuge (Sigma 3-16KL, Germany) and at last frozen dried in a Freeze Dryer at -85°C (Christ Alpha 2-4 LD plus Lyophilizer, Germany).

### iv. Preparation polyNIPAM (pNIPAM) Adhesive:

This adhesive was used for G2 group. p(NIPAM) was added to the All-Bond Universal adhesive at a mass fraction of 2%. The amount of p(NIPAM) required was calculated using the following equation<sup>(13)</sup>:

Quantity of material added=

$$\frac{\text{Quantity of solution} \times \text{weight percentage of the material}}{100\% \text{ Weight}}$$

Consequently, 0.12 gm of p(NIPAM) was weighed using a digital balance (Vibra, Shinko Denshi Co., Ltd, Japan). A sterile plastic syringe (10 ml) wrapped with aluminum foil to protect it from

light exposure was used to withdraw the adhesive from its bottle. It was mixed thoroughly with the calculated amount of p(NIPAM) in a test tube that was also wrapped with aluminum foil and placed on a vortex mixer (Stuart Scientific, Bibby Sterilin Ltd, England) for 10 minutes.

#### **v. Restoration of the Dentin Specimens:**

In the control group (G1): The dentin surface was etched with Select HV etchant for 15 seconds and then rinsed with vigorous air/water spray for another 15 seconds. The etched dentin was dried by blotting it with absorbent cotton pellets leaving the dentin surface visibly moist. Then the adhesive was applied according to the manufacturer's instruction using a disposable micro-brush in a rubbing action for 20 seconds, dispersed with oil-free compressed air for 5 seconds and then polymerized using a light-emitting diode of 1200 mW/cm<sup>2</sup> (Elipar™, 3M ESPE, St. Paul, MN, USA) for 20 seconds. In G2 and G3 groups acid etching, rinsing, and dryness steps were performed as in the control group. In G2 group the prepared All-Bond Universal adhesive containing p(NIPAM) microgel was applied in the same manner as in the control group after acid etching. In G3 p(NIPAM) microgel was applied on the etched dentin specimens using a micro brush in a rubbing action for 10 seconds until the microgel appeared dry on the etched dentin specimens. Then the All-Bond Universal adhesive was applied as in the control group. In the three groups, resin composite (Filtek Z350 XT, shade A2) of four mm thickness was applied incrementally on the treated dentin specimens using a teflon cylindrical mould of 7.5 mm diameter and 2 mm thickness.

#### **vi. Preparation of Microtensile Bond Strength Beams:**

Each prepared specimen was divided into several slabs by vertical sectioning using the double-sided abrasive diamond disc underwater-cooling at low speed. Each specimen was rotated 90 degrees and

then sectioned longitudinally to produce several vertical beams of  $0.9 \pm 0.05$  mm<sup>2</sup> and approximate surface area of  $(1.0 \pm 0.1)$  mm<sup>2</sup>. A digital caliber (*Proficraft, Mebschieber, Germany*) was used to confirm the specimen dimensions.

#### **vii. Grouping of the Specimens:**

A total of 120 beams were prepared. They were divided into three groups (G1, G2 and G3) of forty each. These beams were further subdivided into three subgroups of twenty each. The first subgroups (G1T0, G2T0, G3T0) were tested without thermocycling and the second subgroups (G1T1, G2T1, G3T1) were tested after thermocycling.

#### **viii. Thermocycling Technique:**

In the second subgroup; G1T1, G2T1 and G3T1 dentin beams were inserted into polymerase chain reaction (PCR) tubes containing distilled water. They were then placed in heat blocks to be subjected to 5000 cycles of 5°C to 55°C using a PCR thermocycler (Veriti 96 well thermal cycler, Applied biosystems by life technologies, Singapore). Each bath had a dwell period of 30 seconds, with a 10-second transition between each bath. After that,  $\mu$ TBS testing was performed to all specimens.

#### **ix. Scanning electron microscope analysis:**

Three beams from each group were selected for SEM analysis. Specimens were manually polished using 1200, 2500, and 4000 grit silicon carbide abrasive papers. Each specimen was treated for 20 seconds with 37% phosphoric acid, rinsed for 15 seconds with water, and then treated with 2.5% sodium hypochlorite for 60 seconds to allow deproteinization of the dentin interface. The specimens were subsequently rinsed with water, vacuum desiccated, mounted on an aluminium stub, gold sputter coated, and examined under SEM. Characteristic photomicrographs were obtained at 2000× magnification.

**x. Microtensile Bond Strength ( $\mu$ TBS) Test:**

Using a universal Lloyd testing machine (Lloyd material testing, Ametek, England) that traveled at a cross-head speed of 1 mm/minute until failure, each beam was fastened to the attachment using cyanoacrylate adhesive and stretched in tension. Specimens which showed premature debonding before testing were recorded but not included in the statistics. The ratio of the adhesion area in millimeters to the maximum load (N) was used to calculate the values of the microtensile bond strength (MPa).

**xi. Failure mode Analysis:**

The fractured dentin side of all specimens was inspected using a scanning electron microscope (SEM) at 200X magnification (Philips 515 Model, Eindhoven, Netherlands). Failure mode was allocated to one of four categories. The amount of resin that remained on the dentin surface was used to characterize the type of bond failure either (14):

Type 1- Cohesive in dentin:  $\geq 75\%$  of the bonded area is dentin.

Type 2- Cohesive in resin composite:  $\geq 75\%$  of the bonded area is resin composite.

Type 3- Adhesive failure:  $< 25\%$  of the bonded area is resin composite

Type 4- Adhesive/cohesive (mixed failure): Had resin composite from 25% to 75%.

**xii. Statistical Analysis:**

Shapiro-Wilk and Kolmogorov-Smirnov tests were carried out to verify the numerical data distribution. The data were given as mean and standard deviation (SD) values. Three-way mixed model ANOVA and Bonferroni post hoc test were utilized to examine the impact of the various variables under investigation and their relationship to each other. Bonferroni correction was used to compare the main and simple effects. Regarding the failure mode, qualitative data were described using number and percent.. In order to compare categorical variables between groups in the failure

mode, the chi-square test was performed. Fisher's Exact or Monte Carlo correction was employed for chi-square correction when more than 20% of the cells had an expected count of less than five. All tests were conducted with a significance level of  $P < 0.05$ . Statistical analysis was performed with IBM® SPSS® Statistics Version 25 for Windows.

**RESULTS****I-Scanning electron microscope analysis:**

As shown in figures (1 and 5) G1T0 and G3T0 groups showed no gaps between the hybrid layer and etched dentin in addition to the well-formed funnel shaped resin tags and the presence of a thick adhesive layer unlike G2T0 figure (3). After thermocycling G1T1 and G2T1 figures (2 and 4) respectively showed an increase in the thickness of the adhesive layer, deterioration of the bond between the hybrid layer and dentin with weak, short and sometimes absent resin tags. On the other hand, G3T1 figure (6) showed an intimate contact between the hybrid layer and etched dentin with well formed, long and thick resin tags showing lateral branching, in addition, the adhesive layer is the same thickness as that in G3T0 figure (5).

**II-The effect of microgel incorporation, application technique, and thermocycling on the  $\mu$ TBS:**

Table (2) shows the mean and standard deviation (SD) of the  $\mu$ TBS values of the different tested groups before and after thermocycling. The statistical analysis showed that before thermocycling, there was a statistically significant difference in the mean  $\mu$ TBS values of all the tested groups  $p < 0.001$ . G1T0 showed the highest statistically significant mean  $\mu$ TBS values followed by G3T0, while G2T0 showed the lowest  $\mu$ TBS values.

After thermocycling, there was no statistically significant difference in the mean  $\mu$ TBS values of G3T1 and G2T1, but G1T1 showed the lowest statistically significant mean  $\mu$ TBS values  $p = 0.001$ .

When comparing the effect of thermocycling on each group separately, the statistical

analysis showed that thermocycling affected the mean  $\mu$ TBS values of G1T0 and G1T1 only, where G1T1 showed a lower statistically significant  $\mu$ TBS values  $p=0.001$ .

Table (3) shows the effect of the different variables and their interactions on the  $\mu$ TBS. Two-way mixed model ANOVA showed that each variable of the tested groups showed a statistically significant effect. Regarding thermocycling  $p=0.012$ , microgel technique of application and incorporation  $p=0.01$ , while thermocycling with microgel technique of application and incorporation  $p<0.01$ .

### III- Failure mode analysis:

Table (4) presented the failure patterns of each group before and after thermocycling. The statistical analysis revealed that there was statistically significant difference in the type of failure in all

groups before and after thermocycling where  $p=0.026$  and  $p=0.01$  respectively. When comparing each group before and after thermocycling, there was no statistically significant difference in G2 and G3 groups, but G1 group showed a statistically significant difference in the failure pattern before and after thermocycling where  $p_1=0.009$ . In G1T0 55% of the specimens showed cohesive failure in composite, in G3T0 50% of the specimens showed adhesive failure, while G2T0 showed 45% adhesive and 40% cohesive failure in composite. After thermocycling, G3T1 showed 55% adhesive and 35% mixed failure patterns, G2T1 showed 40% adhesive and 35% mixed failure pattern. While G3T1 showed 60% cohesive failure in resin composite. These percentages were confirmed with the representative SEM images of the failure pattern of each subgroup as shown in figures (7-12).

TABLE (2) Mean and standard deviation (SD) of the  $\mu$ TBS (Mpa) of the different tested groups before and after thermocycling

Microgel technique of application and incorporation (The different tested groups)	Thermocycling (mean $\pm$ SD)		P value
	Before (T0)	After (T1)	
Control (G1)	23.81 $\pm$ 7.68 <sup>Aa</sup>	11.77 $\pm$ 4.58 <sup>Bb</sup>	<b>&lt;0.001*</b>
(G2)	12.76 $\pm$ 4.35 <sup>Ac</sup>	15.49 $\pm$ 5.93 <sup>Ab</sup>	<b>0.075ns</b>
(G3)	18.08 $\pm$ 7.63 <sup>Ab</sup>	19.41 $\pm$ 7.35 <sup>Aa</sup>	<b>0.401ns</b>
P-value	<b>&lt;0.001*</b>	<b>0.001*</b>	

Different upper and lower case superscript characters exhibit statistically significant differences within the same horizontal row or vertical column, respectively. \*: significant ( $p < 0.05$ ), ns: non-significant ( $p > 0.05$ ).

TABLE (3) Effect of different variables and their interactions on the  $\mu$ TBS (Mpa)

Source	Type III Sum of Squares	df	Mean Square	F	P-value
Thermocycling	204.10	1	204.09	6.79	<b>0.012*</b>
Microgel technique of application and incorporation	472.19	2	236.10	5.07	<b>0.010*</b>
Thermocycling with Microgel technique of application and incorporation	1237.57	2	618.78	20.59	<b>&lt;0.001*</b>
Error (Thermocycling)	1653.14	55	30.06		
Error (Microgel technique of application and incorporation)	2560.18	55	46.55		

df=degree of freedom\*; significant ( $p \leq 0.05$ ) ns; non-significant ( $p>0.05$ )

TABLE (4) The Failure patterns of each group before and after thermocycling

Aging	Fracture pattern	G1		G2		G3		P-value
		%	n	%	n	%	n	
Before Thermocycling (T0)	Cohesive in dentin	0%	0	0%	0	0%	0	0.026*
	Cohesive in composite	55%	11	40%	8	30%	6	
	Adhesive failure	35%	7	45%	9	20%	4	
	Mixed type	10%	2	15%	3	50%	10	
After Thermocycling (T1)	Cohesive in dentin	0%	0	%	0	0%	0	0.010*
	Cohesive in composite	10%	2	25%	5	60%	12	
	Adhesive failure	55%	11	40%	8	15%	3	
	Mixed type	35%	7	35%	7	25%	5	
p <sub>i</sub> value		MCp=0.009*		0.309		MCp=0.197		

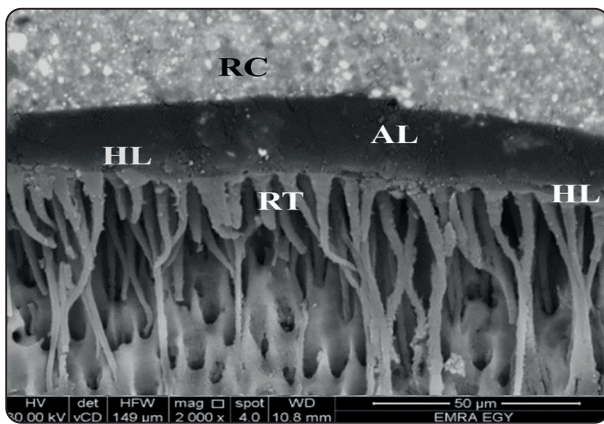


Fig. (1) SEM image of G1T0 group showing a thick and intact adhesive layer (AL). The hybrid layer (HL) is evident and firmly adherent to the short and long funnel-shaped resin tags (RT).

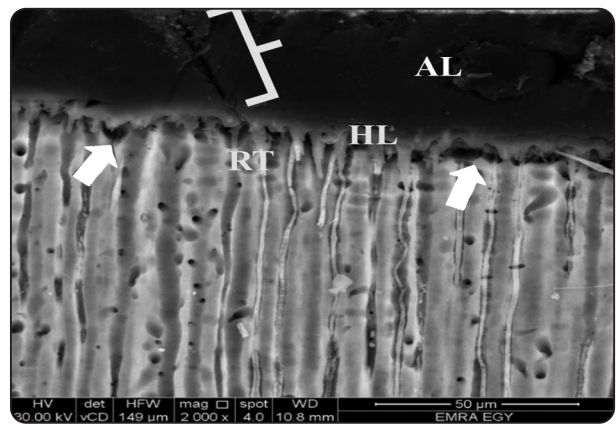


Fig. (2) SEM image of G1T1 group showing gaps (arrows) between the hybrid layer (HL) and the dentin with short, and sparse resin tags (RT). A cleft can be seen in the adhesive layer (AL) identified by brackets.

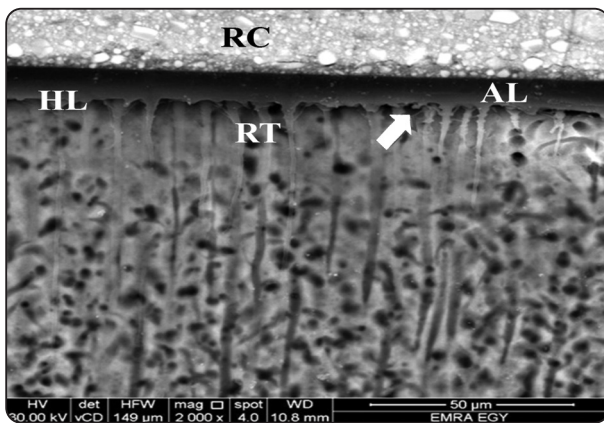


Fig. (3) SEM image of G2T0 group showing a well-defined hybrid layer (HL) with few gaps between the hybrid layer and dentin (arrow). Resin tags (RT) are short and thin.

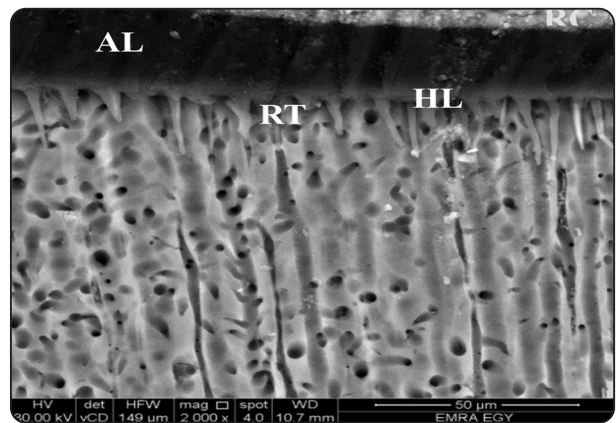


Fig. (4) SEM image of G2T1 group showing a thicker adhesive layer (AL), The hybrid layer (HL) is uniform with thick funnel-shaped resin tags (RT) and less gaps were evident.

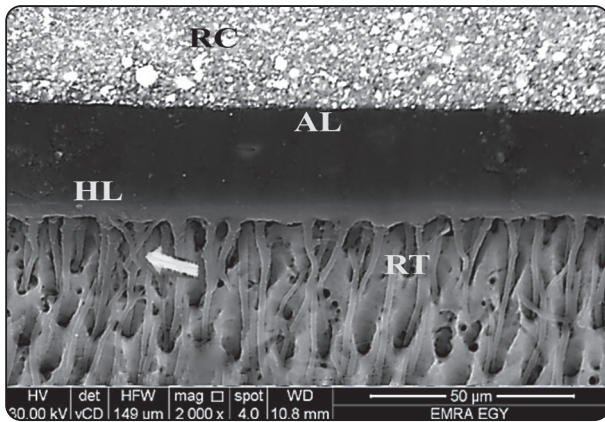


Fig. (5) SEM image of G3T0 showing the thickest and the most uniform adhesive layer (AL). The hybrid layer (HL) is also thick and uniform with long and numerous resin tags (RT) of adequate thickness showing lateral branching (arrows).

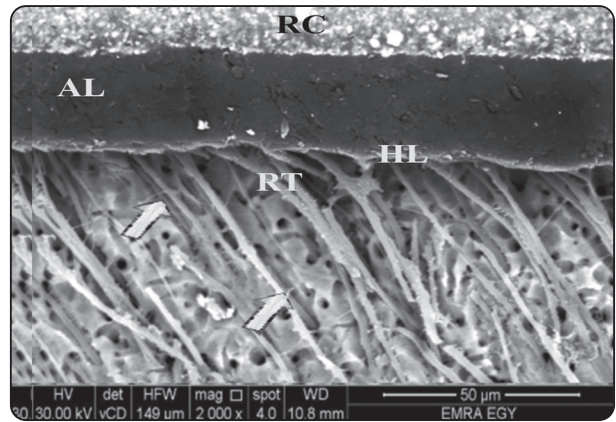


Fig. (6) SEM of G3T1 showing thick adhesive layer (AL). The hybrid layer (HL) is thin and uniform with numerous, very long resin tags (RT) showing lateral branching (arrows).

**a) Representative SEM images showing the most frequent failure modes for G1.**

RC: resin composite, AD: adhesive, D: dentin

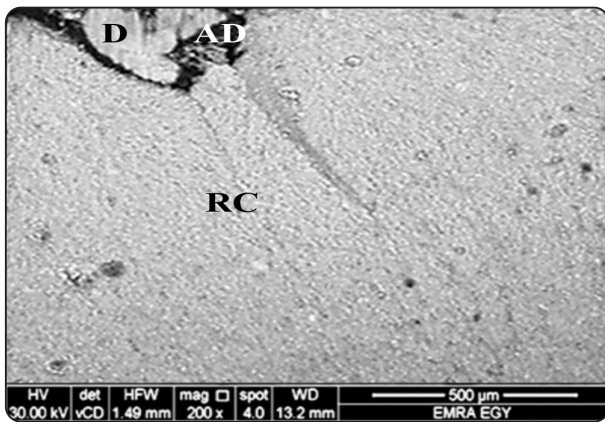


Fig (7) Shows type (2) failure cohesive in resin composite in G1T0.

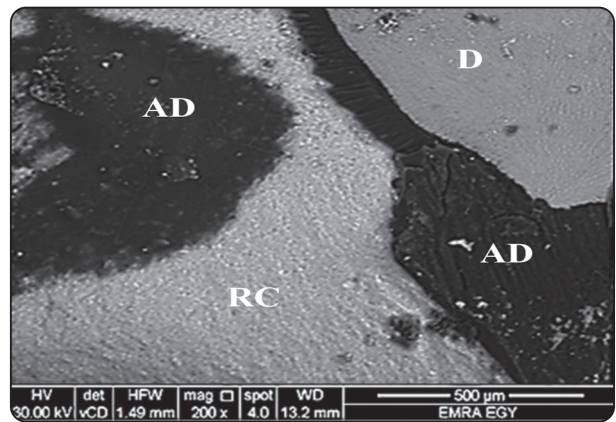


Fig (8) Shows type (4) mixed failure in G1T1.

**b) Representative SEM images showing the most frequent failure modes for G2.**

RC: resin composite, AD: adhesive, D: dentin

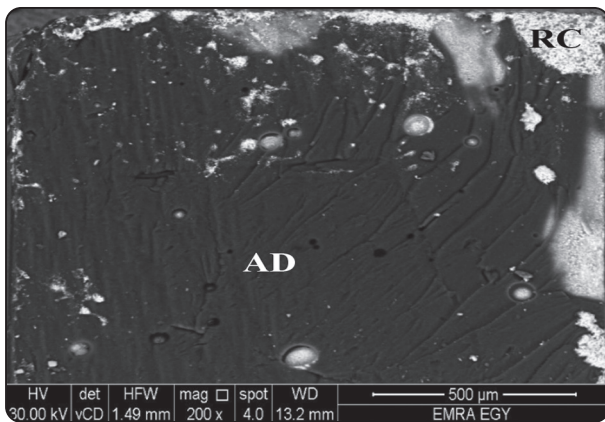


Fig (9) Shows type (3) adhesive failure in G2T0.

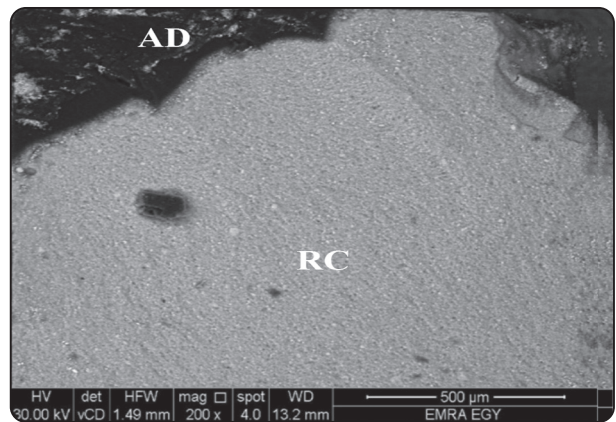


Fig (10) Shows type (2) failure cohesive in resin composite in G2T1.



### c) Representative SEM images showing the most frequent failure modes for G3.

RC: resin composite, AD: adhesive, D: dentin and RT: resin tags

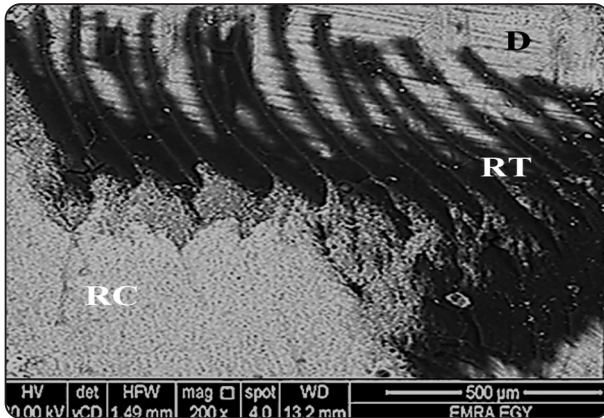


Fig (11) Shows type (4) mixed failure in G3T0.

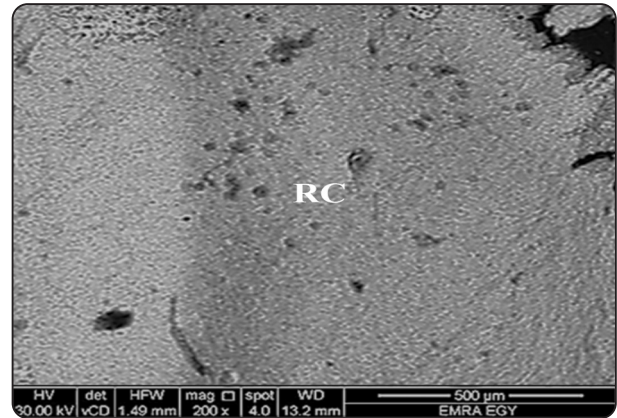


Fig (12) Shows type (2) failure cohesive in resin composite in G3T1.

## DISCUSSION

The endurance and long-term stability of tooth-colored restorations are strongly dependent on the resin-dentin interface, which is produced by the micro-mechanical interlocking of the demineralized dentin collagen network and the infiltrated adhesive monomers. Dentin adhesion is one of the most challenging and unanticipated issues in adhesive dentistry due to its complex histology and dynamic compositional fluctuations<sup>(15,16)</sup>. Evaluating bonding endurance is crucial since the bond between the restoration and the tooth substrate is only considered clinically successful if it lasts long enough.

The simulation of a realistic clinical environment should be taken into consideration to evaluate the durability of the bond. Buonocore (1981) suggested that thermocycling the specimens might be a part of in vitro bond strength tests<sup>(17)</sup>. According to Özcan et al in 2007<sup>(18)</sup>, thermocycling simulates effectively the aging of composites more than other techniques and creates more challenging conditions for resin composite restorations<sup>(18)</sup>. Several studies, demonstrated that 5000 thermal cycles simulate the natural oral environment and affect the composite bond strength<sup>(18,19)</sup>. However, Gale et al., 1999<sup>(20)</sup> proposed that for resin composite restorations, 10,000 cycles would be the equivalent of an entire

year of clinical use, by considering that 20 to 25 thermal cycles occur on average each day<sup>(21)</sup>. Furthermore, an appropriate artificial aging test, as defined by ISO TR 11450 standards, is thermocycling regimen of 500 cycles in water at 5 to 55°C<sup>(17)</sup>. As a result, in this investigation a PCR thermal cycler was used to induce 5000 cycles at two distinct temperatures 5°C and 55°C.

The thermocycling-induced artificial aging could be caused by two mechanisms; first; hydrolysis of the interface components that could be accelerated by the use of hot water, followed by water sorption and the release of unpolymerized resin oligomers and broken-down molecules (Diffusion dependent hydrolysis and elusion). Second, repetitive contraction/expansion leads to thermal stresses by temperature fluctuations. These stresses cause crack propagation at the bonded interfaces, causing in and out flow of oral fluids<sup>(17)</sup>.

Various tests can be used to verify the resinous material's adhesion capability. In the current study micro-tensile bond strength ( $\mu$ TBS) test was performed. It is regarded as one of the most accurate and trustworthy tests. That's because it can measure bond strength with less cohesive dentin failures and more adhesive failures, It also has actual bond strength values and higher interface bond

strength<sup>(17)</sup>. In addition to its usefulness regarding the fabrication of specimens and better-control on bond test sites. Furthermore, the test specimens are small, so multiple specimens can be obtained from a single tooth, leading to more creative study settings, more flexible testing, and improved control of substrate variables<sup>(17)</sup>.

In this study, a universal bonding system was used, which is according to the operator's preferences and the clinical situation, known as a universal bonding system or a single-bottle, no-mix adhesive solution which may be used in total-etch, self-etch, or selective-etch pattern<sup>(22)</sup>. The total-etch mode was chosen in this study according to Matos et al., 2020<sup>(22)</sup> who found that regardless of the level of dentin moisture (wet or dry), universal adhesives, produced an excellent retention rate when applied in etch and rinse (ER) mode in a five-years clinical research and produced significantly lower retention rates and the worst marginal staining when utilized in the self-etched mode.

In the current study, G1T0 showed the highest mean  $\mu$ TBS values and this was supported by SEM analysis that showed the adhesive layer continuously covering the etched dentine surface and entering the funnel-shaped exposed dentinal tubules forming long and thick resin tags. The hybrid layer was easily detected by a light zone at the interface showing proper integration into the intertubular dentin, as shown in Figure (7). It has been suggested that micromechanical interlocking through proper dentine hybridization through resin tags and uniform hybrid layer increase the bond strength of universal adhesives. Etching of dentine with phosphoric acid before applying universal adhesives greatly increases the morphology of the interface infiltration, resulting in longer resin tags and thicker hybrid layers. The removal of smear plugs and smear layer makes the adhesive penetration easier, resulting in longer resin tags particularly with mild universal adhesives as the used All-Bond universal adhesive (pH 2.5-3.5)<sup>(23)</sup>.

Additionally, it was found that an interaction occurs between collagen of demineralized dentin and MDP -containing adhesive through a hydrophobic aliphatic moiety. This occurs because MDP contains a long aliphatic chain (hydrophobic chain) and a dihydrogen phosphate group. Hydrogen bonds also revealed a significant adhesion between collagen and MDP<sup>(24)</sup>. Furthermore, using SEM, Jin et al., 2022<sup>(25)</sup> demonstrated the production of non-uniform deposits and the decrease in the opened tubules diameter following the application of MDP on demineralized dentin. Based on the Energy Dispersive Spectrometer (elemental mapping), the MDP-Ca salt appears to be the primary component of these deposits. Based on these results, the authors proposed that MDP-Ca salt mechanically protects the underlying demineralized collagen by replenishing the water-rich, non-infiltrated resin areas inside the hybrid layer thus ceasing the entry of exogenous MMPs and proteases. Moreover, MDP chemically interacts with the Ca in hydroxyapatite<sup>(25)</sup>.

The microgel groups G2T0 and G3T0 showed a statistically significant lower  $\mu$ TBS when compared to G1T0 ( $p < 0.001$ ). This may be related to the microgel addition which influenced the viscosity, wettability and the ability of resin monomers to penetrate into decalcified dentin<sup>(23)</sup>. It could also be contributed to the hydrophilic nature of p(NIPAM) and being under 34°C leading to its swelling and isolation. This swelling may lead to occlusion of the narrow dentinal tubules of the superficial dentin preventing resin infiltration on immediate testing before thermocycling<sup>(26)</sup>. This was evident at the SEM image of G2T0 Figure (9) revealing shorter, irregular resin tags and the absence of resin tags in some areas indicating incomplete resin infiltration.

After thermocycling G3T1 showed higher  $\mu$ TBS when compared to G1T1. This stability in the bond strength might be explained by many factors. At first, the lower critical solution temperature (LCST) of p(NIPAM) in water is 32°C, below this temperature it swells and cannot easily penetrate the dentinal tubules. Secondly, it shrinks when

the temperature exceeds the LCST<sup>(27)</sup>. That's why during thermocycling the maximum temperature reached was 55°C thus the microgel is in the shrunken form, which might allow it to enter the narrow dentinal tubules and occlude it, and hence decreasing the dentin permeability. This improves the bond strength since moist dentine impact adhesion by encouraging the hydrolytic instability of resin dentine interface<sup>(28)</sup>.

In G3T1 SEM image Figure (6) the adhesive layer thickness remained constant with proper integration of the adhesive into the etched dentin. The length of the resin tags was thick and long showing lateral infiltration indicating deep penetration. Meanwhile, as suggested by Mohsen et al in 2013; this might be related to the flocculation of the p(NIPAM) which is the process that causes individual particles to precipitate into small lumps or aggregate into clot-like masses<sup>(29)</sup>. It occurs when the temperature is raised above the (LCST), which breaks the hydrogen bond between the solvent and the polymer, leaving the particles negatively charged and drastically reduced in size. In this case, positively charged ions attracts negatively charged particles during the flocculation process, removing repulsion forces and enabling the particles to group together to form flocs. Other sources of positively charged ions are either the calcium present in the tooth structure, or the positively charged ions in the used bonding agent, leading to more stability of the bond strength<sup>(30)</sup>.

Regarding thermocycling statistical analysis revealed that there is a statistically significant decrease in the mean  $\mu$ TBS values in G1T1 subgroup and this may be attributed to the interfacial stresses created by thermal aging as a result of the difference in the resins' coefficient of thermal expansion<sup>(31,32)</sup>. In addition, both G1T1 and G2T1 subgroups showed an increase in the adhesive layer thickness as shown in Figures (2 and 4) respectively. Studies explained that this may be related to the hygroscopic expansion that occurs to the adhesive resin materials after thermocycling. In this study the examined all in one adhesive contains

Bis-GMA and HEMA which contribute to water uptake and adhesive layer swelling, bond strength deterioration after thermocycling and these findings agree with Osman in 2019<sup>(17,33)</sup>. Although other studies revealed that this hygroscopic expansion may improve the marginal integrity and compensate for the polymerization shrinkage effects<sup>(34)</sup>.

The failure pattern analysis was confirmed with the  $\mu$ TBS values, the SEM analysis images Figures(1-6) and SEM failure pattern images Figures(7-12). There was a statistically significant difference in the type of failure in all groups before and after thermocycling and when comparing each group before and after thermocycling, only G1 group showed a statistically significant difference in the failure pattern before and after thermocycling indicating that thermocycling showed a significant deteriorating of the bond strength in this group which agrees with several studies<sup>(35,36)</sup>. G1T0 showed 55% cohesive failure in resin composite indicating a high  $\mu$ TBS. The same for G3T1 which showed 60% of the same type of failure. G2T0 and G2T1 subgroups showed almost same percentage of adhesive and cohesive failure in resin composite.

## CONCLUSIONS

The application of p(NIPAM) directly on etched dentin caused an increase in the bond durability after thermocycling. On the contrary its application inside the all-bond universal adhesive did not show much improvement in the bond strength. Thermocycling negatively affected the bond strength of all-bond universal adhesive

### Clinical relevance:

The current study findings are of important clinical relevance; as a strong bond between resin composite and tooth structure is essential to increase the longevity of the final restoration and enhance its stability and performance. However, the current study has some limitations; that it is an in vitro study with limited sample size and the simulation of in vivo conditions is yet unsolved. Moreover,

the effect of microgel on other properties of the adhesive system such as biological, mechanical and physical properties are of great importance and it is recommended to be considered in further researches.

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