



NANOLEAKAGE EVALUATION OF BIOMODIFIED RESIN DENTIN INTERFACE USING CHITOSAN NANOPARTICLES, AN IN-VITRO STUDY

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ABSTRACT

Objective: This study was directed to evaluate the effect of chitosan, grape seed extract and chlorohexidine on resin dentin interface and nanoleakage evaluation of universal adhesives. **Materials and methods:** A total of 60 upper premolars divided into 4 groups, with a total of 15 teeth in each group were used in this study. To study the effect of the three treatments groups with chitosan, grape seeds and chlorohexidine (CHX), ANOVA test was used for comparison of these materials with control group. After scrubbing and disinfection of every tooth by sodium hypochlorite and then distilled water, standardized Class V cavities were prepared. The 0.2% chitosan solution was prepared with 0.2 gram of chitosan. Grape seed was prepared with 6.5% conc. chlorohexidine with 2% concentration. All restorations were placed according to the manufacturer's instructions, using the incremental placement technique and were cured using the light-emitting diode, Scanning electron microscopy is well established technique to examine the interface between dentin restorative material, AgNo₃ dye is used in this study ,as it's proved according to different studies that AgNo₃ is suitable in photography. **Results:** There was a significant difference in nanoleakage evaluation between CHX group and all remaining groups, with no significant difference in nanoleakge evaluation between chitosan group and Grape seed extract group. Nanoleakge increased with time. **Conclusion:** The application of CHX 2% before composite resin application is found to increase chance of nanoleakage in resin dentin interface.

KEY WORDS: Chitosan, chlorohexidine, grape seed extract

INTRODUCTION

Resin composite restoration is the daily use in dental practice to replace the lost tooth tissue⁽¹⁾. Achieving efficient and stable bond between composite and dentin still remains a challenge in restorative dentistry ⁽¹⁾, the major limitations of dentine as a bonding substrate are its heterogeneous composition and hydrophilic nature ⁽²⁾.

The majority of studies for dentin bonding requires acid etching that removes the smear layer and smear plugs and decalcifies the underlying

dentinal structures ⁽³⁾. Peritubular dentin is partially removed, Concomitantly, intertubular dentine is etched up to a depth of 5 μ m that exposes a collagen-based organic matrix ⁽⁴⁾.

The consequent application of the resin monomers results in the so-called hybrid layer or inter-diffusion zone⁽⁵⁾. However, it has been proposed that resins may not penetrate into the exposed matrix⁽⁶⁾. This incomplete penetration of the monomers would result in zones susceptible to hydrolytic degradation and ultimately would produce areas of nanoleakage⁽⁷⁾.

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Nanoleakage throughout the hybrid layer and/or adhesive resin can be described as penetration of any substance into 20- to 100-nm-sized spaces present in the adhesive and/or tooth substrate ⁽⁸⁾.

Although these spaces are too small to allow for bacterial penetration, they are large enough for enzymes of natural dentin to enter these enzymes are matrix metalloproteinases (MMPs). MMPs are a family of zinc-dependent endoproteinases whose enzymatic activity is directed against components of the extracellular matrix and so cause biodegradation of dentin and also affected the stability of long term bond ⁽⁹⁾.

Chitosan is one of the materials that used as MMPs enzymes inhibitors and becomes more popular as a therapeutic agent, due to its antimicrobial properties as well as high biocompatibility ⁽¹⁰⁾.

Also Chlorhexidine (CHX) strongly inhibits the proteolytic activities of MMP-2, -8, and -9, which inhibit the activity of MMPs, so might improve bond durability⁽¹¹⁾. Moreover Proanthocyanidins (PA) has a positively affects the resistance of resin–dentine bonds against enzymatic challenge of (MMPs) after 6 months, consequently improving the bonding durability ⁽¹²⁾

PA are oligomeric flavonoids found in high concentrations in grape seed, pine bark, cranberries, lemon tree bark have received growing attention due to their biocompatibility and many beneficial biological properties, including anticarcinogenic, anti-inflammatory, antibacterial and immunostimulatory effects ⁽¹³⁾.

The formation of nanoleakage structures is not only caused by a discrepancy between the depth of demineralization and resin infiltration but can also be caused by the incomplete polymerization or hydrogel formation of the resin. Nanoleakage structures can be classified into a variety of different shapes, including spot mode, reticular mode, and dendritic shapes (water tree) ⁽¹⁴⁾.So, this study hypothesized that the use of MMPs inhibitors would assist to suppress the long term degradation of bond and decrease the nanoleakage

MATERIAL AND METHODS

Materials

Materials used in this study, their types, compositions and manufacturer are presented in table 1.

TABLE (1) Materials used in this study.

Materials	Types	Compositions	Manufacturer
ENA HRI	Nanohybrid composite	1,4-Butandiol dimethacrylate, UDMA Bis-GMA Filler: nano zirconium oxide (80% weight – 63% volume)	Micerium S.p.A, Italy
Prime & bond	Universal adhesive	PENTA, 10-MDP, Active Guard™ Technology crosslinker	Dentsply sirona, Germany
chitosan	Dentin biomodification	Randomly distributed β-(1-4)-linked d-glucosamine (deacetylated unit) and N-acetyl-d-glucosamine (acetylated unit)	Sigma company, Egypt
Grape seed extract	Dentin biomodification	High content of unsaturated fatty acids, particularly linoleic (18:2) and oleic (18:1) acids. Traces of linolenic (18:3) and palmitoleic (16:1) acids	The key phenolic compounds of all grape variety and seeds were gallic acid, 3,4-dihydroxybenzoic acid, (+)-catechin ve 1,2-dihydroxybenzene
CHX	Dentin biomodification	(1, 1 1-hexamethylene bis [5-(p-chlorophenyl) biguanide] di-D-gluconate) in a base containing water, 11.6% alcohol, glycerin, PEG-40 sorbitan diisostearate, flavor, sodium saccharin, and FD&C Blue	

METHODS

A total number of 60 sound human upper premolars, free from caries, cracks & restorations are collected from Dental surgery clinic in the faculty of dental medicine, Al-Azhar university to be used in this study.

Each tooth was cleaned, polished & examined under a stereo microscope 50x (Discovery V8 Stereo, Carl Zeiss MicroImaging GmbH, Göttingen, Germany) to exclude the teeth with cracks and defects; teeth were stored in distilled water at 37 C until use.

The teeth, after being selected with respect to the inclusion and exclusion criteria. Standardized Class V cavities were prepared on the buccal surface of each tooth at the cement-enamel junction using a tapered fissure carbide bur(ss white,457#USA) in a high speed handpiece, with an air/water spray coolant (2mm height, 2mm width, 2mm depth). The same operator (principal investigator) performed all the cavity preparations and restorations to eliminate interoperator variability

On the tooth surface the outline of the cavity was drawn by a 0.5 pencil by a matrix band for standardization of class V cavity a matrix band with a previously made cut hole of 2 x 2 mm which was secure on the tooth using a retainer in which the gingival floor of the cavity was set at (1 mm) below the cemento-enamel junction of the tooth. The cavity form was finished by round bur in a low speed hand piece by water coolant.

Sixty premolars teeth were divided into four groups, with a total of 15 teeth in each group and then five teeth divided into 12 subgroups. To study the effect of the three treatments groups with chitosan, grape seeds and chlorohexidine while the control group didn't receive any treatment.

- **Group A:** 15 teeth restored without any modification (Control Group).

- **Group B:** 15 teeth treated with 0.2% chitosan solution by means plastic syringe prepared class V, after a certain period (1 minute) then gentle air dried with 3 way chip syringe for 20 sec. Each specimen received the applied pre-treatment solution acc. To the 3 storage periods.
- **Group C:** 15 teeth treated with 2% Chlorhexidine solution was applied to the dentin for 30 s, and then dried with cotton pellet.
- **Group D:** 15 teeth treated with 6.5% Grape seed solution for 10 min and rinsed with distilled water.

Then the adhesive (prime & bond) was applied according to the manufacturer's instructions, microbrush was dipped in the adhesive and rubbing the cavity with the adhesive then wait for 10 sec. for evaporation of solvent after that light curing for 30 seconds (Blue-Lex LD 105 Monitex Co.1200MW, Taiwan),

After each storage period specimens were painted with nail varnish on all the teeth except 2mm away from the restored cavity in order not to be penetrated with silver nitrate solution.

The specimens were placed in a 50% (w/v) silver nitrate solution (pH=9.5) in total darkness for 24 h. The resin-dentin end of the assembly was cut into multiple parallel slabs, then after rotating the assembly about 90°, another series of parallel cuts were made. Scanning electron microscopy is well established technique to examine the interface between dentin restorative material ⁽¹⁵⁾

RESULTS

From intra-group comparison data we can conclude the following:

There were no statistically significant differences in the nanoleakage score distribution between the three time intervals in the control group and chlorohexidine.

There were statistically significant differences in the nanoleakage score distribution between the three time intervals in the grape seed group and chitosan in favor of 6 month which have the highest mean rank.

From inter-group comparison data we can conclude the following:

There were no statistically significant differences in the nanoleakage score distribution between the four groups after one day.

There were statistically significant differences in the nanoleakage score distribution between Control group and Chitosan group after 3 months, while there was no significant difference between the other groups. And the Kruskal-Wallis P-value was not statistically significant.

There were statistically significant differences in the nanoleakage score distribution between Control group and Chitosan group as well as Control and Grape seeds while there was no significant difference between Control and Chlorohexidine as well as Chlorohexidine and Chitosan. And the Kruskal-Wallis P-value was statistically significant in favor of Chitosan group which have the highest mean rank.

The silver nitrate uptake expressed by scores method suggested by Yuan Y et al.⁽³⁵⁾, no leakage (score 0), slight leakage (score 1), or distinctive leakage (score 2) with respect to the penetration depth.

Statistical analysis for Nanoleakage score distribution results were performed by Kruskal-Wallis and Mann-Whitney Test ($p < 0.05$). Data were analyzed using the statistical software SPSS (version 25, IBM Co. USA).

TABLE (2) Comparison of nanoleakage score distribution between the four groups at different time intervals.

		Score 0	Score 1	Score 2	P-value**
Day1	Control	66.70%	33.30%	0%	0.350 ^{NS}
	Cholorexidine	33.30%	66.70%	0%	
	Grape speed	0%50.0%	50.0%	0%	
	Chitosan	16.70%	83.30%	0%	
3 months	Control	33.30%	66.70%	0%	0.066 ^{NS}
	Cholorexidine	0%	66.70%	33.30%	
	Grape speed	16.70%	50.0%	33.30%	
	Chitosan	0%	33.30%	66.70%	
6 months	Control	16.70%	66.70%	0%	0.032 ^S
	Cholorexidine	16.70%	33.30%	50.00%	
	Grape speed	0%	33.30%	66.70%	
	Chitosan	0%	16.70%	66.70%	

** $P \leq 0.05$ - P-value from Kruskal-Wallis test - NS= Non significant - S= Statistically significant at $P \leq 0.05$

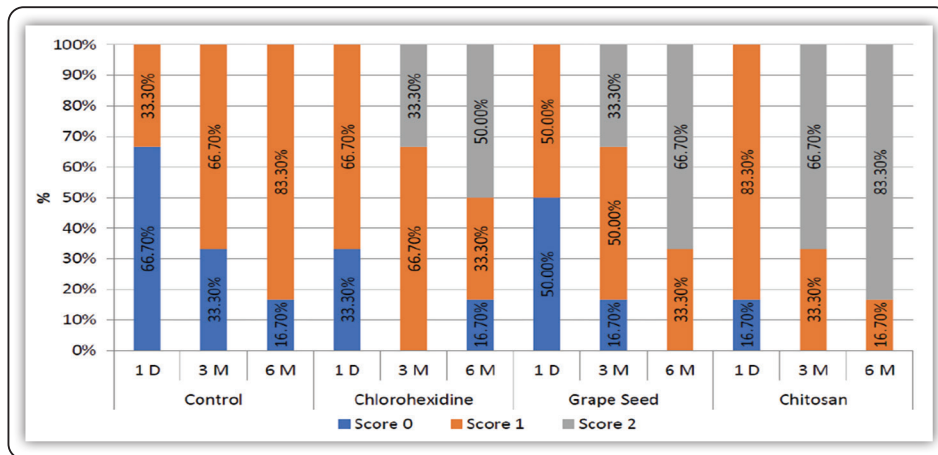


FIG (1) Nanoleakage Score distribution (in %) between the four groups at different time intervals

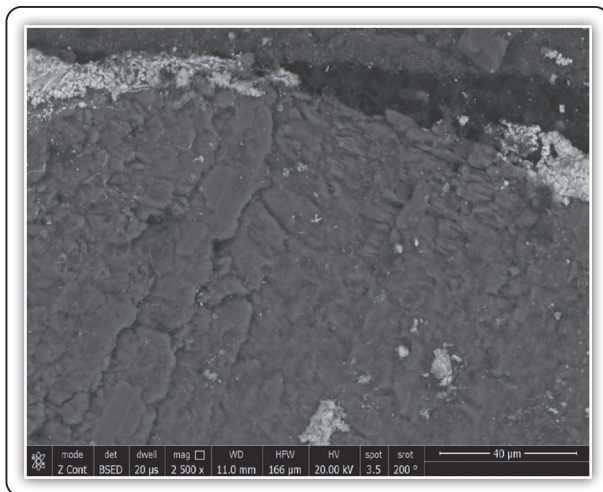


FIG (2) Backscattered image SEM(2500x) chitosan 6 months storage showing nanoleakage phenomena as seen by silver nitrate depositions along the resin dentin interface and dentinal tubules.

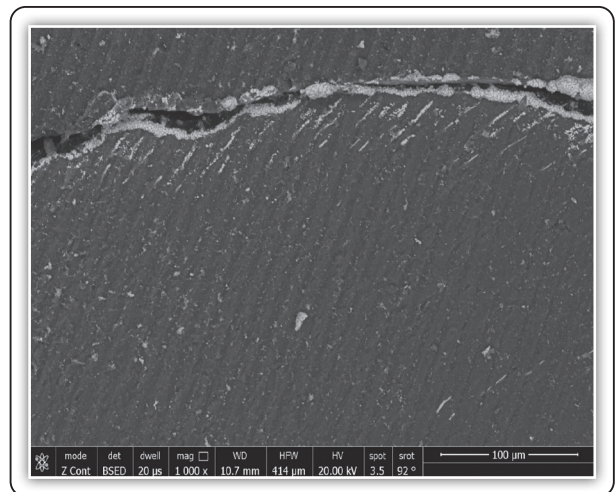


FIG (3) SEM image after Three months storage of Grape Seed Extract (1000x) showing silver nitrate deposition along resin dentin interface and between resin tags and dentinal tubules that indicated nanoleakage phenomena(Backscattered image)

DISCUSSION

Dental composite was widely used as restorative material. The main cause of loss of stability or longevity of the composite is microleakage. Microleakage has been defined by Sidhu and Henderson as “the clinically undetectable passage of bacterial fluids, molecules and/or ions between the cavity wall and the restoration material applied to it”⁽¹⁷⁾.

Many investigators have identified microleakage as the primary cause for recurrent or secondary dental caries, pulpal inflammation and necrosis.⁽¹⁸⁾

The type of leakage, which occurs within the hybrid layer in the absence of gap formation, should be called “nanoleakage”, to distinguish it from microleakage compared with the 10 μm to 20 μm width of gaps causing microleakage.⁽¹⁹⁾

Two modes of nanoleakage expression could be recognized within resin-dentin interfaces: reticular and spotted types. The reticular type consisted originally of discontinuous islands of silver deposits that were randomly distributed within the bulk of the unstained hybrid layers, another type

of nanoleakage is known as water treeing seen as silver deposits oriented perpendicular to the surface HL extending the upper resin bonding layers. Thus far, all marketed products permitted some amount of nanoleakage and water-tree formation. Our goal was to minimize or diminish nanoleakage at the resin/dentin interface. ⁽²⁰⁾

Many methods have been used along the years to measure nanoleakage including using tracer silver dyes- silver nitrate (which was used in this study), silver methenamine and, ammonical silver nitrate, with conventional light microscopy⁽⁹²⁾ and SEM. SEM was selected in this study, as it records 3-dimensional images with high resolution, enhanced magnification, and depth of focus ^(21, 22).

According To Paulose and Fawzy ⁽²³⁾ showed that the highest value of bond strength was recorded at chitosan 0.2% and the lowest value was recorded by chitosan 2.5%. These results show that when the concentration of chitosan increases the bond strength value decrease while the bond strength increased by time after six months

In approval with our study Lukram Nivedita et.al. ⁽²⁴⁾ stated that chitosan showed less degree of microleakage that control group Due to presence of large number of free hydroxyl and amino groups, chitosan has the ability to form a microfibrillar and nanofibrillar network with superior mechanical properties including higher resistance to collagen degradation.

In agreement with our study Perchyonok, Souza⁽²⁵⁾ they stated that: Incorporation of chitosan or nanodiamond or combination of Chitosan/ nanodiamond into the conventional GIC provides a promising multi-dimensional functional biomaterial with excellent properties such as increased shear bond strength, certain degree of microbiological properties, good bio-adhesion without compromising important properties such as microleakage

In agree with our study Diolosà, Donati ⁽²¹⁾ they suggested the addition of chitosan to an adhesive system containing HEMA and ethanol could

present good stability; there would be electrostatic interactions between the chitosan and the organic component of demineralized dentin composed of collagen and glycosaminoglycans, increasing the stability of the hybrid layer. Reduction in nanoleakage could be observed at the interface of systems containing chitosan because of its chemical and physical interaction with the dentin substrate, an event not observed in systems without chitosan in their formulation ⁽²⁶⁾.

In agree with our study Nivedita, Prakash⁽²⁷⁾ stated that Application of Chitosan and GSE improved the shear bond strength to dentin as compared to the control. However, no significant difference in shear bond strength and microleakage was found between them. and this result go with agreement with our study

GSE have remarkable dentine-specific protective effects by decreasing biodegradation rates and enhancing the mechanical properties of the organic matrix. PAs have been reported to strengthen collagen-based tissues by increasing collagen cross-links. There is evidence to prove that GSE increases collagen synthesis and accelerates the conversion of soluble collagen to insoluble collagen ⁽²⁸⁾

Paulose and Fawzy⁽²⁹⁾ showed that the use of 6.5%w/v GSE pre-treatment with any of the adhesive groups after 24 h and 1 year all groups show a significant increase in the degree of nanoleakage after a year of storage. and this go with agreement with our study

Srinivasulu, Vidhya⁽³⁰⁾ stated that GSE application with 6.5% proanthocyanidin increased the resin-dentin shear bond strength than control group this go with agreement with our study

Scarabello Stape, Menezes ⁽³¹⁾ showed that When CHX pretreatment was performed, specimens showed a non-homogenous resin tag surface. This difference in the resin tag surface morphology may be due to the CHX amphipathic properties which might interfere with resin infiltration in the demineralized dentin areas. It has also been reported

that the use of CHX with self-etch adhesives reduces bond strength due to the limited penetration of adhesive into the dentin. This goes with agreement with our study⁽³²⁾

In disagree with our study Nik, Naseri⁽³³⁾ showed that Conditioning the dentin surface after acid etching with ethanol or chlorhexidine, can be effective in decreasing the leakage, especially over time.

In agree with our study Kimyai, Pournaghi-Azar⁽³⁴⁾ showed that the use of CHX in the cavity on composite resin restorations before the application of two one-bottle total-etch adhesives (Syntac and Prime & Bond) and concluded that use of CHX solution resulted in a significant increase in dentin microleakage in deciduous teeth.

CONCLUSION

Within limitations of this study, the following conclusions could be drawn:

1. All groups showed nanoleakage.
2. Nanoleakage phenomena is time dependant phenomena.
3. Chitosan nanoparticles have a positive effect on Nanoleakage.
4. Dentin biomodification give a better effect on nanoleakage in long term storage.
5. MMPs inhibitors preserve hybrid layer & decrease degradation.

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