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THE EFFECT OF CHITOSAN INCORPORATION ON SOME PROPERTIES AND ANTIBACTERIAL ACTIVITY OF DENTAL ADHESIVE

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ABSTRACT

OBJECTIVE: To evaluate the antibacterial activity of adhesive resin incorporating chitosan as well as the adhesive characteristics. **METHOD AND MATERIALS:** Modified adhesive was prepared by adding 0.05%, 0.1%, and 0.2% (w/w) chitosan solution to Heliobond adhesive resin. The solution of chitosan was prepared by dissolving 2 g of chitosan powder in 1 liter of 1% (v/v) acetic acid. Heliobond without chitosan was used as a control. The antibacterial activity was evaluated using a direct contact test against Streptococcus mutans. The viscosity, degree of conversion, pH, and microshear bond strength values of modified adhesive to enamel and dentin were evaluated. Data were analyzed using the ANOVA and Tukey's tests. Statistical significance was set at the .05 probability level. **RESULTS:** The antibacterial properties of adhesives incorporating chitosan were found to exhibit an inhibitory effect on the growth of Streptococcus mutans compared with the unmodified adhesive resin ($P < .05$). The viscosity of adhesives increased with increasing the concentrations of chitosan incorporation into the adhesive. However, the degree of conversion and pH values and microshear bond strength values of modified adhesive to enamel and dentin decreased with increasing the concentrations of chitosan incorporation into the adhesive. **CONCLUSION:** Under the limitations of the present investigation, the following conclusion can be drawn: modified Heliobond adhesive resin with 0.2 wt % chitosan exhibit the best antibacterial activity. The antibacterial properties of modified Heliobond adhesive depend on their pH value. By increasing the concentration of chitosan into Heliobond adhesive system, pH, degree of conversion and bond strength significantly decreased.

INTRODUCTION

The primary aim of dental adhesives is to provide retention to composite fillings. Inadequate sealing at the tooth-restoration interface may lead to microleakage, allowing penetration of microorganisms related to the onset and progression of caries [1].

Heliobond is hydrophobic, light-curing, bonding resin for optimizing the enamel-etch technique in combination with light-curing restorative materials. Heliobond is not exhibited any antibacterial [2, 3]. Chitosan is a deacetylated derivative from the biopolysaccharide chitin which is present in insects' exoskeletons, crustaceans' shells and fungi cell walls.

It is generally regarded as biocompatible, nontoxic, biodegradable, and is inherently antibacterial in nature [4,5]. Chitosan water insoluble but it is soluble in dilute aqueous solutions of various acids, the most widely used is acetic acid. Chitosan is a weak base and has one primary amine group NH_3^+, it is clearly a cationic biomaterial so it produces antimicrobial effect by degrading the cell wall structure and the cell membrane of bacteria [6, 7]. The incorporation of chitosan in experimental adhesive systems associated with: methacrylate monomers have been suggested as a way to enhance antibacterial activity by means of ionic interactions between chitosan and the bacterial cells [8]. The present study was carried out to evaluate antibacterial effect of chitosan modified adhesive and their relation with

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some adhesive properties e.g. viscosity, ph., degree of conversion, micro-shear bond strength to enamel and dentin.

MATERIAL AND METHODS:

One type of adhesive resin Heliobond (Ivoclar, Vivadent, Inc. Amherst, USA, lot # M35797), chitosan powder (Oxford Lab Chem., Neminath Ind., Eastate, Mumbal, Maharashtra, India.lot#9012-67-4) with different concentration (0.05%, 0.1% & 0.2% by weight), CharmEtch (DENTKIST.Inc., Korea lot#1413005)and light cured hybrid resin composite(Te-Econom plus)(IvoclarVivadent, Inc. Amherst, USA lot# U55005) were selected for this study.

Preparation of Chitosan Solution:

About 200 mg of chitosan was weighed separately and dissolved in 0.1 N acetic acid and made up to 100 ml to get 2 mg / ml chitosan solution. [9]. Then 0.5, 1 or 2 mg of chitosan solution was added to 1 gm Heliobond adhesive resin in hourglass to obtain 0.05%, 0.1% and 0.2% (wt / wt) chitosan / Heliobond modified adhesive.

Antibacterial Activity Test:

The antibacterial activity of each group of adhesives was evaluated using blood agar disc-diffusion test against S. mutans. Random samples of soft carious dentin were directly taken from carious cavities by a sterile excavator from randomly selected patients. [10]

Preparation of the Tested Materials

Filter paper discs were used to be coated with the tested adhesives. These discs were wrapped in aluminum foil and sterilized in the hot air oven* at 160°C for 30 min. [11]

A volume of twenty microliter (µl) of each adhesive were impregnated into a sterile filter paper disc (diameter: 6 mm, thickness: 1.5 mm) and cured for 20s using a light-emitting diode (LED) light-curing unit with power output of 1000 mW/cm^2.

The filter paper discs containing the tested adhesives were seated on the Petri dish that containing S. mutans microbial colonies. Each Petri dish plate was labeled with the names of the tested group of adhesive, then the Petri dish plate were incubated at 37°C for 24 h. [12]

After incubation period is completed, the diameters of inhibition zones were measured at three different points and sizes of inhibition zones were calculated by subtracting the diameter of the specimen from the average of the three measurements of the halo, for each tested adhesive group.

Viscosity Measurement:

The viscosity of chitosan modified adhesive groups and control group were measured by using rotary viscometer.

One drop of each adhesive group is placed in the plate of viscometer .The values of torque (S) were determined and speed value (N) was maintained at 256 rpm and viscosity is calculated from the following equation:

$\eta = G. S/N$ where G: Instrumental factor (14200 Mpa.s).

Five specimens were prepared for the measurements for each adhesive group and the mean was calculated.

pH Measurement:

The pH of each chitosan modified adhesive groups and control group was measured with a pH meter.

The pH values of chitosan modified adhesive groups and control group were measured at room temperature with a pH meter connected to a solid-state pH and a reference electrode. Prior to measuring

the adhesive, the pH electrodes were calibrated with buffer solutions. Then, five specimens of each group were prepared for pH measurements and the mean was calculated.

Degree of Conversion Test (DC):

The percentages of DC were calculated for both cured and uncured conditions of all tested adhesive groups. This was performed using Fourier Transformation Infrared Spectroscopy (FTIR).

Each uncured adhesive specimen was smeared onto a potassium bromide disk.[13] FTIR analyses of each tested adhesive group were evaluated before curing. Additional FTIR spectra were obtained immediately after 10-s light-curing and after sample storage in dark, dry environment for 24 hours [14].

The frequency of the infrared region used was between 4000 to 400cm^{-1} wave number and resolution was 4 cm^{-1}.

The percentage of DC for each specimen was calculating from the following equation :[15, 16]

$$DC\ \% = \frac{(1-(C{=}C\ /\ C\ ...\ C)\ \text{after curing})}{(C{=}C\ /\ C\ ...\ C)\ \text{before curing})} \times 100$$

C=C is the aliphatic carbon=carbon bond; while C….C is the aromatic carbon… carbon bond. Where the aliphatic carbon-to-carbon double bond absorbance peak intensity located at 1638 cm^{-1} and that for the aromatic component located at 1608 cm^{-1}, and both of them were compared in each spectrum before and after the polymerization reaction.

Micro-shear bond Strength:

A total of 80 freshly extracted, sound non carious human premolars teeth, free of cracks and any developmental defects .The teeth were cleaned with hard tooth brush under running water and then stored in distilled water. The teeth were then randomly divided into 4 groups (n = 10) for both of enamel and dentin bond test [17].

Especially designed cylindrical Teflon molds were machine milled to fabricate the acrylic blocks. The teeth roots were sectioned 2 mm from the cemento-dentinal junction (CEJ) with a slow-speed diamond saw in a sectioning machine and crown is embedded in acrylic resin .For enamel; the outer surface of the enamel specimens was then ground flat with water-cooled sandpaper discs of decreasing grit (400, 600) in order to produce a clinical relevant and standardize smear layer.

For dentin; the occlusal surface of each embedded tooth was grinded with 120- grit silicon paper to expose flat surface of dentin, and subsequently, polished the exposed dentin with 600- grit silicon paper for 20 s to obtain a uniform smear layer.

The tested adhesive systems were applied to the enamel and dentin surfaces according to manufacturer's instructions. The etching procedure is done by using 37% phosphoric acid etchant which applied for 20s on the flat enamel and dentin surfaces, which were then rinsed thoroughly with an oil-free stream of water for 10s. Then, the excess water was removed using an absorbent pellet, leaving surfaces moist. Then adhesive was applied, gently dried and light polymerized for 20s using LED light-curing unit.

Several Tygon tubes with an internal diameter of 0.75 mm and length of 1mm were positioned over enamel and dentin surfaces of the teeth and then resin composite was carefully packed inside the tubes. The resin composite was light cured for 20s using LED light curing unit. Tubes were removed with a sharp blade.

Each specimen is placed in the lower attachment of the universal testing machine for micro-shear bond testing. A thin wire (diameter 0.2 mm) was looped around each cylinders of resin composite, making contact through half of composite base and was placed as close as possible to the resin-enamel and dentin interface. A shear force was applied to each specimen at a cross-head speed of 0.5mm / min until failure.

Statistical Analysis:

One-way ANOVA tests were used to compare the antibacterial activity, viscosity, degree of conversion and micro-shear bond strength of the different adhesive groups. For all analyses, F - test was used for pair wise mean comparison among the tested groups. Calculations were handled by the software PASW Statistics 17 (SPSS Inc., Chicago, IL, USA), and all of the tests' accuracy was set at a significance level of 0.05.

RESULTS

Antibacterial Activity Examination:

The results of statistical analysis showed that; control group (Group 1) recorded the lowest antibacterial activity means value (1.27 ± 0.61) while; (Group 4) recorded the highest mean value (23.50 ± 2.07). Pair-wise comparisons among the groups revealed that; all groups were statistically significant difference as shown in table (1)

Viscosity of Adhesives Measurements:

The results of statistical analysis showed that; (Group 4) showed the highest statistically significantly viscosity mean value (909.17 ± 62.12). While; (Group 1) recorded the lowest viscosity means value (836.27 ± 9.46). Pair-wise comparisons among the groups revealed that; there was no statistically significant difference between (Group1 and Group 2) and also between (Group 2 and Group 3) as shown in table (2)

pH of adhesive groups:

The results of statistical analysis showed that; (Group 1) showed the highest statistically significantly pH mean value (5.55 ± 0.04). Pair-wise comparisons among the groups revealed that; there was statistically significant difference between all adhesive groups as shown in table (3)

Degree of Conversion (%) of Adhesives Measurements:

The results of statistical analysis showed that; (Group1) showed the highest statistically

TABLE (1): Means± SDs of inhibitory zone for all investigated groups

Antibacterial	**Group 1**	**Group 2**	**Group 3**	**Group 4**
Range	0.5 – 2	14 – 19	18 – 24	20 – 26
Mean ± SD	1.27 ± 0.61	16.50 ± 1.45	20.26 ± 1.78	23.50 ± 2.07
F test	6.007			
P value	0.002*			

P1 (G1 , G2)	P2 (G1, G3)	P3 (G1, G4)	P4 (G2, G3)	P5 (G2, G4)	P6 (G3,G4)
0.001*	0.001*	0.001*	0.001*	0.001*	0.001*

TABLE (2): Viscosity measurements (mpa.s) results (Means ± SDs) for all investigated groups

Viscosity	**Group 1**	**Group 2**	**Group 3**	**Group 4**
Range	822.53 – 853.72	832.31 – 870.11	800.12 – 920.78	812.23 – 981.79
Mean ± SD	836.27 ± 9.46	856.91 ± 10.36	868.46 ± 18.07	909.17 ± 22.25
F test	6.007			
P value	0.002*			

P1 (G1 , G2)	P2 (G1, G3)	P3 (G1, G4)	P4 (G2, G3)	P5 (G2, G4)	P6 (G3,G4)
0.251ns	0.007*	0.001*	0.518ns	0.006*	0.027*

significantly DC (%) means value (24.83 ± 3.18). Pair-wise comparisons among the groups revealed that; there was no statistically significant difference between (Group 1& Group 2) as shown in table (4)

Micro-shear Bond Strength for Enamel:

The results of statistical analysis showed that; control group; adhesive (Group1) recorded the highest mean value (44.98±1.47). Pair-wise comparisons among the groups revealed that; all groups were statistically significant difference as shown in table (5)

Micro-shear Bond Strength for Dentin:

The results of statistical analysis showed that; control group (Group 1) recorded the highest mean value (31.74 ± 1.60). Pair-wise comparisons among the tested adhesive groups revealed that; all groups were statistically significant as shown in table (6)

TABLE (3): pH measurements results (Means ± SDs) for all investigated groups

PH	**G1 (Control)**	**G2 (0.05)**	**G3 (0.1)**	**G4 (0.2)**
Range	5.5 – 5.62	5.26 – 5.43	5.01 – 5.13	4.56 – 4.91
Mean ± SD	5.55 ± 0.04	5.35 ± 0.05	5.07 ± 0.05	4.71 ± 0.11
F test	286.898			
P value	0.001*			

P1 (G1 , G2)	P2 (G1, G3)	P3 (G1, G4)	P4 (G2, G3)	P5 (G2, G4)	P6 (G3,G4)
0.001*	0.001*	0.001*	0.001*	0.001*	0.001*

TABLE (4): Degree of conversion (%) results (Means ± SDs) for all investigated groups

Degree of Conversion	**Group 1**	**Group 2**	**Group 3**	**Group 4**
Range	16.6 – 28.1	20.1 – 25.3	16.2 – 20.8	11.6 – 18.8
Mean ± SD	24.83 ± 3.18	22.80 ± 1.73	19.05 ± 1.41	15.52 ± 2.53
F test	31.659			
P value	0.001*			

P1 (G1 , G2)	P2 (G1, G3)	P3 (G1, G4)	P4 (G2, G3)	P5 (G2, G4)	P6 (G3,G4)
0.057ns	0.001*	0.001*	0.001*	0.001*	0.002*

TABLE (5): Micro- shear bond strength (MPa) with enamel results (Means ± SDs) for all tested groups

Enamel bond strength	**G1 (Control)**	**G2 (0.05)**	**G3 (0.1)**	**G4 (0.2)**
Range	42.99 – 47.22	33.7 – 44.87	25.11 – 35.33	19.11 – 28.33
Mean ± SD	44.98 ± 1.47	37.93 ± 3.19	30.98 ± 3.07	23.51 ± 3.16
F test	106.986			
P value	0.001*			

P1 (G1 , G2)	P2 (G1, G3)	P3 (G1, G4)	P4 (G2, G3)	P5 (G2, G4)	P6 (G3,G4)
0.001*	0.001*	0.001*	0.001*	0.001*	0.001*

TABLE (6): Micro- shear strength (MPa) with dentin results (Means ± SDs) for all tested groups

Dentin	**G1 (Control)**	**G2 (0.05)**	**G3 (0.1)**	**G4 (0.2)**
Range	29.87 – 34.52	19.99 – 30.22	17.23 –22.53	12.99 – 19.1
Mean ± SD	31.74 ± 1.60	23.85 ± 3.07	19.79 ± 1.62	16.62 ± 1.98
F test	92.201			
P value	0.001*			

P1 (G1 , G2)	P2 (G1, G3)	P3 (G1, G4)	P4 (G2, G3)	P5 (G2, G4)	P6 (G3,G4)
0.001*	0.001*	0.001*	0.001*	0.001*	0.002*

DISCUSSION

Complete sealing at the bonded interface (between restorative material and tooth structure) is a prerequisite for successful restorations. There were many recognized causes for restoration failure after restoration placement as, residual bacteria after removal of a carious lesion and microleakage that may cause pulp inflammation and/or increases its sensitivity and may also cause secondary caries.[8,14]. The antibacterial activity of the dentin bonding systems was important factor in prevention of the harmful effect that resulted from bacterial microleakage [8]. Agar-disc diffusion test method is a simple direct inhibition test and it has been most frequently used as antimicrobial test with S. mutans which is associated with the initiation of human dental caries. [18]

The cell membrane of bacteria is surrounded by a cell wall composed of peptidoglycan layers that are precise made of N-acetyl-glucosamine, N-acetylmuramic acid and amino acids which link the positively charged amine groups of chitosan oligomers to glycine in the peptidoglycan structure. Thus, this material disrupts the cell wall and exposes the cell membrane to osmotic shock. Consequently, cytoplasmic contents are extruded and cell death occurs. Chitosan oligomers in the oral environment can have bactericidal and/or bacteriostatic properties[19]. The 0.2% chitosan / Heliobond modified adhesive (Group 4) has the higher antimicrobial activity among all investigated groups where this finding was attributed to the lower pH of acid-soluble chitosan, where the antimicrobial activity increasing by decreasing of pH and increasing the concentration of chitosan. Heliobond has no or very poor antibacterial activity may be because it does not contain HEMA in its composition, since resin adhesives that contain HEMA exhibit substantial antibacterial activity[20,21].

The low viscosity of Heliobond adhesive may be due to its TEGDMA content were its composition is based on TEGDMA / Bis-GMA.Where an increase TEGDMA will result in the decreases of the adhesive viscosity[22] . Increasing of viscosity of chitosan modified adhesives with increasing their concentration from 0.05% to 0.2% may be due to increasing the cross- linking content between chitosan and adhesive. [23]

At the time of incorporating chitosan into the adhesive system, the pH must be acid, because chitosan is insoluble at pH values higher than 7.0 (basic mean) and extremely soluble in an acid medium[24]. Heliobond adhesive represent a pH results around 5.5, which facilitate the incorporation of chitosan. The addition of chitosan statistically increases the acidity of the Heliobond adhesive from (5.55 to 4.71), this may be due to the acetylation of chitosan in acetic acid during preparation of chitosan solution [5].

This might be linked to the increase viscosity and the reactants cross-linking agent with concentration increases of chitosan, which may interfere with the mobility of the monomers in the system [5, 25]. Restricted mobility of monomer may decrease the polymerization rate and the conversion of double bonds so the lower viscosity of resins (Group 1 and 2) allows better monomeric mobility and distribution of free radicals inside the material, which can enhance the polymerization process leading to a greater monomer conversion.

Recently, the micro-shear bond strength test was introduced as a substitute for the conventional shear test where the stress distribution is more concentrated at the interface in the micro-shear bond test which reduces the chance of cohesive failure in the material that does not represent the ''true'' interfacial bond strength.[26] . Shimida et al., [27] modified the micro-shear bond test by replacing the blade with a looped orthodontic wire.

In enamel and dentin there were statistically significant difference in bond strength, were the control group demonstrated the higher bond strength with decrease in bond strength of the chitosan modified groups as the concentration of chitosan increased from (0.5%, 0.1% and 0.2%) respectively. This may be due to increase the viscosity of modified adhesive that have effect on better flow of adhesive resin into enamel and dentin surfaces.

CONCLUSIONS

Under the limitations of the present investigation, the following conclusion can be drawn:

1- Modified Heliobond adhesive resin containing chitosan possess different degree of antibacterial activity, modified Heliobond adhesive resin with 0.2 wt % chitosan exhibit the best antibacterial activity.

2- The antibacterial properties of modified Heliobond adhesive depend on their pH value.

3- By increasing the concentration of chitosan into Heliobond adhesive system, pH and bond strength significantly decreased.

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