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EVALUATION OF PULP REACTION OF NANOCLAY MODIFIED ADHESIVE SYSTEMS USING BULK FILL RESIN COMPOSITE

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Bulk fill, Clay, Dellite® 43B, Dellite® LVF modified Adhesive system, Montmorillonite.

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ABSTRACT

Introduction: Protecting the vital pulp in deep carious lesions possess a great challenge for clinicians in minimal invasive dentistry. **Aim:** To assess the pulp response to two montmorillonite modified adhesive systems (with and without organic modifier) in deep dentine cavities in dog's permanent posterior teeth. **Materials and Methods:** Six-healthy adult mongrel dogs were undergoing general anesthesia through intravenous injection of sodium pentobarbital; a rubber dam was fixed in place; cavities were prepared in labial surface of each tooth. Cavities were divided into three groups according to the restorative materials used: Group1: Prime&Bond universal™ adhesive (Control). Group2: Dellite®43B modified Adhesive system (with organic modifier). Group3: Dellite®LVF modified Adhesive system (without organic modifier). BulkFill material with Flowable consistency was used to fill the rest of the cavity over tested materials. Each group was subdivided into 3 equal subgroups at time intervals: after 1 week, 30 and 60 days. The study design was performed to obtain a total of 90 dogs' teeth, the number of samples for each material was n=7, and total number of samples in the six dogs for each material 42 teeth in each group, the best 30 teeth were examined. Differences between timepoints was evaluated by Friedman's test, and overall differences induced by groups, timepoints and interaction were assessed using two-way ANOVA. **Results:** There was no significant difference in pulp response of the conventional prime and bond adhesive system and Dellite43 modified adhesive. A significant difference between the two mentioned materials and Dellite LVF modified adhesive system. **Conclusion:** Organic modified montmorillonite adhesive produced favorable pulp response and seemed to be a promising biocompatible material comparable to conventional adhesive.

INTRODUCTION

In the adhesive systems development and within the concepts of minimally invasive dentistry, direct resin composite restorations are conservative treatments that are capable of rehabilitating the tooth and should give the tooth sufficient bond strength to withstand in the oral cavity environment [(1,2)].

Composite polymerization creates internal stresses that cause bond loss at the tooth composite interface, cuspal deflection, and enamel crack formation; all are principal factors in the potential restoration failure [(3)].

Bulk-fill composites were developed to address some of the flaws in light cure resin composites. Because of their low polymerization stress and strong responsiveness to light curing, they are appropriate for insertion in a 4mm bulk placement or larger [4].

Clay is a soft, loose, earthy substance with particles of a grain size of <4 μm. It forms due to the weathering and erosion of rocks containing the mineral group feldspar (known as the 'mother of clay') over vast periods [5].

When a tiny quantity of montmorillonite (nanoclay) is added to the polymer matrix, the resultant composite's mechanical, physical, and chemical characteristics are considered to be significantly improved over traditional materials [6]. The mechanical performance (stiffness and strength) was improved significantly by adding a minor quantity of nanoclay (0.5–5.0wt %) to the polymers [7–9].

Furthermore, none of the previous research articles investigated the pulpal response of the studied materials i.e. two nanoclay modified adhesive systems (with and without organic modifier). This study examined the null hypothesis that there was no difference between the two studied nanoclay modified adhesive systems (with or without organic modifier). Accordingly, it was of prime importance to evaluate the pulp response in deep dentine cavities in permanent posterior teeth and evaluate reaction to two nanoclay modified adhesive systems (with and without organic modifier).

MATERIALS AND METHODS

Materials

The materials used in current study include Bulk fill resin composite (SureFill SDR), Prime &Bond Universal™, Etch, Dellite®43B, and Dellite® LVF. Bulk fill resin composite (SureFil SDR) composed of; Modified UDMA, TEGDMA, EBPDMA, Pigment, Photo Initiator, Barium and Strontium, Alumino-Fluoro-Silicate glasses, Silicon Dioxide-Amourphous, Strontium, Alumino-Silicate Glass, Filler load: 68wt%; 45vol%, and the manufacturer is Dentsply Caulk, Universal, 100407, Germany (www.dentsplysirona.com). Prime& Bond universal™ Phosphoric acid modified acrylate resin, Multifunctional acrylate, Bifunctional acrylate, Acidic acrylate, Isopropanol, Water, Initiator, Stabilizer; Manufacturer: Dentsply Sirona, Germany (www.dentsplysirona.com). Etch was used composition: 36% phosphoric acid conditioner, manufactured by Dentsply Detrey Germany, www.dentsplysirona.com. Dellite®43B, composed of Nanoclay deriving from a naturally occurring montmorillonite especially purified and modified with a quaternary ammonium salt (dimethyl benzyl hydrogenated tallow ammonium), manufactured by Laviosa, Italy, www.laviosa.com. Dellite® LVF Nanoclay deriving from a naturally occurring especially purified montmorillonite Laviosa, Italy, www.laviosa.com.

Study setting:

1. Selection of dogs:

After approval of the Ethics Committee of Faculty of Dentistry, Suez Canal University, no. 94/2018. This study was carried on a total of 90 permanent posterior dogs' teeth. A total of six adult mongrel dogs weighing an average of 10 kg, aged between 12 to 18 months, were included as experimental animal models. All dogs were healthy and showed no clinical signs of dental disorder.

2. The housing of Dogs: [5]

Research Ethics Committee of General Organization for Veterinary Services (Dokki) approved

research on dogs at Veterinary Hospital (Abbasia) according to the following guidelines General Organization for Veterinary Services (no. 1396). Dogs were social animals and were better housed in groups than individually. The number of dogs that can be kept in any group depends on age, sex, breed, facilities and capability of staff to manage the group. Floors were be cleaned and yet provide a stable footing for the animal's comfort. Adequate lighting was required to facilitate thorough examinations of the dogs.

Temperature and humidity were managed so that an environment was provided which maintained the dogs in their thermo-neutral comfort zone. For dogs housed indoors, a temperature range of 18 to 29°C was supported. Indoor facilities were adequately ventilated to keep an appropriate range of temperature and humidity. Early feeding was preferable, so the animals were observed for any possible gastric dilation or bloat during the day. Dogs were fed from bowls; each dog had its bowl. Individual stainless-steel food bowls were preferable as they were easier to clean and sterilize. Wastes were removed daily. Enclosures were cleaned daily and disinfected once a week [5]. After doing the experiment, dogs were euthanized, and the jaws were sectioned for obtaining the teeth after their decalcification.

4. Preparation & Premedication of dogs:

Operations were done under general anesthesia, including premedication with a mixture of atropine sulphate (0.05 mg/kg body weights; Atropine injection, injectable solution, B.P., Vet.2003, ADWIA, New Cairo, Egypt)) and diazepam (1.0 mg/kg-body-weight; Valium 10mg; Roche, Germany) intravenously to reduce salivation and resist any sudden arrhythmia.

5. Induction of anesthesia:

Anaesthesia was induced immediately through the intravenous cannula by injecting ketamine (10 mg/kg body weight) (SIGMA TEC Pharmaceutical Industries-Egypt), and xylazine (1.0 mg/kg body weight) (XylaJect, ADWIA, Egypt). The depth of anesthesia was maintained by injection of thiopental sodium intravenously. This technique allowed smooth and delayed induction with deep anesthesia, good degree of analgesia, muscle relaxant and smooth recovery [10]. The dogs were undergoing general anesthesia through intravenous injection of sodium pentobarbital.

6. Cavity preparation:

Each dog was placed on the operating table in a supine position. Standardized buccal pit cavities of 3mm depth (the depth was very close to the pulp but without exposure) and 3mm width were prepared on the facial surfaces of the teeth after application of rubber dam using round and fissure carbide burs (size 010; Dremel Tungsten Carbide Cutter with Ball Tip, 3.2 mm; Dremel, Bosch, Germany) locked to a high-speed hand piece (40,000rpm) connected to the high-speed motor (WisDent Technology co. Ltd., China) with air and water coolant to avoid heat generation [11]. The depth and width of the cavities were 3mm each and checked by the periodontal probe to ensure a uniform cavity.

7. Experimental study grouping:

1. The cavities were divided into three groups (G1, G2, G3) according to the restorative materials used: Group1 Prime Bond universal ™ adhesive &Bulk fill material with Flowable consistency (Control Group). Group 2 Dellite® 43B modified Adhesive system Bulk fill material with Flowable consistency. Group 3 Dellite® LVF modified Adhesive system Bulk fill material with a Flowable consistency.

2. Each group was further subdivided into three equal subgroups at different time intervals (T) where (T1) after one week, (T2) after 30 days and (T3) after 60 days [11]

8. Animal model grouping:

The study was divided into three phases at different time intervals (Table 1). Four premolars, the first and second molar, were selected for the treatment. In each experimental dog; the material were n=42. From every 42 teeth in each group, the best 30 teeth were selected to be examined.

Table (1) *Animal model design*

Guardant	Teeth	Treatment performed
Upper right	Four premolars, first and second molar	G1
Upper left	Four premolars, first and second molar	G 2
Lower right	Four premolars, first and second molar	G3
Lower left	Fourth premolar	G1
	First molar	G2
	Second molar	G3

9. Restoration of cavities:

Restoration kits were properly placed and arranged to be ready for use. The etchant gel and adhesive bonding system were applied according to the manufacturer's instructions. The different types of adhesive systems (according to the grouping of specimens) were used to restore the prepared cavity. The adhesive layer was thinned with a weak stream of air to avoid its pooling and light-cured for 10 seconds at a light intensity of more than 500 mW/cm^{2}with the same curing unit. The restoration was placed according to the manufacture instructions of bulk fill co bulk-fill restoration with 3 mm bulk in the cavity and applied by the suitable composite application. The excess was removed with carver after packing the material finishing strip (3M™ Sof-Lex™ Finishing Strips Refill, 3M, Lebanon) and Mylar strip (Dental Band Matrix Strip Mylar Banda Celuloide 4"x 3/8" Box /1000 Pcs PREHMA, USA) was used to obtain a smooth surface.

10. Finishing polishing of the Resin composite restoration:

The restored teeth were finished using 15μm grit compo shape finishing diamond burs (Intensiv®, Viganello-Lugano, Switzerland) then polished using a Sof-Lex® Finishing disc (3M ESPE, St.Paul, MN, USA).

11. Authunization of dogs and teeth preparation:

The dogs were sacrificed after the testing periods 7 days (T1), 30 days (T2) and 60 days (T3) by intravenous injection of euthanasia solution (20% pento barbitone sodium, 0.7ml\kg). After fixation for ten days, the jaws were dissected and immersed in and 10% formalin for fixation. The hemi sectioned jaws were demineralized in demineralizing solution (50% formic acid+20% sodium citrate) for four months; checking of demineralization was done using blunt needle. After demineralization of jaws, the teeth were extracted from the bone by a specified scalpel. The tips of roots were cut to facilitate the intrusion of demineralizing solution into the tooth. The specimens were placed in the demineralizing solution for two weeks more. After complete decalcification, samples were dehydrated in ethanol and embedded in paraffin and sectioned in longitudinal sections by microtome (5 um). All sections were stained with hematoxylin and eosin stain after placing on glass slides.

12. Images analysis

A pathologist blindly evaluated the sections. The images analysis was done using a light microscope (Leica microscope), including a digital camera mounted on it. All images were captured by the digital camera (BX 200) and transferred to the computer system by Leica software (Leica queen) for images analysis. This was performed in the central research lab in dental services at October University for Modern Sciences and Arts (MSA University). The pulpal response to the investigated materials was histologically evaluated based on a scale from 0-3 according to the following (Table 2).

Table (2) *Pulpal response scoring system*

Score		Criterion
0	Normal pulp	No observable difference compared with control
1	Mild reaction	Edema, slight disruption of odontoblastic layer, vascular dilation, hyperemia, hemorrhage, occasional interstitial inflammation cells
2	Moderate reaction	Necrosis in odontoblastic layer, edema, hyperemia, vascular dilation, hemorrhage, increased number of inflammation cells.
3	Severe reaction	Generalized necrosis of pulp elements, edema, vascular dilation, hemorrhage, increased number of inflammation cells.

Statistical analyses

Data collected, handled, and organized using Microsoft Excel 2016. Data was analysed using IBM-SPSS version 28.0 (Armonk, NY: IBM Corp.). Normality was checked using Shapiro-Wilk at 0.05 level. Differences between timepoints was evaluated by Friedman's test, and overall differences induced by groups, timepoints and interaction were assessed using two-way ANOVA statistic at 0.05 level.

RESULTS

1. Group-I: Control (Prime & Bond)

At 7 days postoperative (G1T1):

By examining sample, the Prime & Bond Elect Universal Dental Adhesive system showed moderate inflammatory reaction with vasodilation for the blood vessels and interstitial edema as well as a moderate distraction of the odontoblastic layer at variable areas, the majority of the samples scored1 (Table, 3; Figure 1A)

At 30 days postoperative (G1T2):

There was a moderate inflammatory reaction and vasodilation but less than the same Group at 7 days. The odontoblastic layer showed sign of healing and less interstitial edema, the majority of samples scored 1 (Table, 3; Figure 1B)

At 60 days postoperative (G1T3)

There was a mild inflammatory reaction, almost complete healing had accrued together with slight vasodilation in some blood vessels and mild interstitial edema. Most of the slide scored 1 (Table, 3; Figure 1C).

2. Group-II

At 7 days postoperative (G2T1)

The organic modified material (Dellite 43 modified Adhesive system) investigated reduction of the inflammation to the considerable degree, moderate vasodilation, necrosis of the odontoblastic layer at variable sites and inflammatory edema were detected similar to group G1T1. Also, after 7 days, most of the slides scored 1 (Table, 4; Figure 1D).

Table (3) *Descriptive statistic of group-I (Control (Prime & Bond), frequency (n, %) and significance using chi-square.*

Group	Time	Group-1: Control (Prime & Bond) frequency				
		Score frequency n (%)				Significance
		Score-0	Score-1	Score-2	Score-3	χ2
Group-1: Control (Prime & Bond) frequency	T1	1 (10.0%)	7 (70.0%)	2 (20.0%)	0 (0.0%)	0.045*
	T2	0 (0.0%)	80 (80.0%)	2 (20.0%)	0 (0.0%)	>0.05 ns
	T3	8 (80.0%)	2 (20.0%)	0 (0.0%)	0 (0.0%)	>0.05 ns
Friedman's test sign.		>0.05 ns				

, **, *, significant at p<0.05, p<0.01, p<0.001; NS, non-sign. at p>0.05*

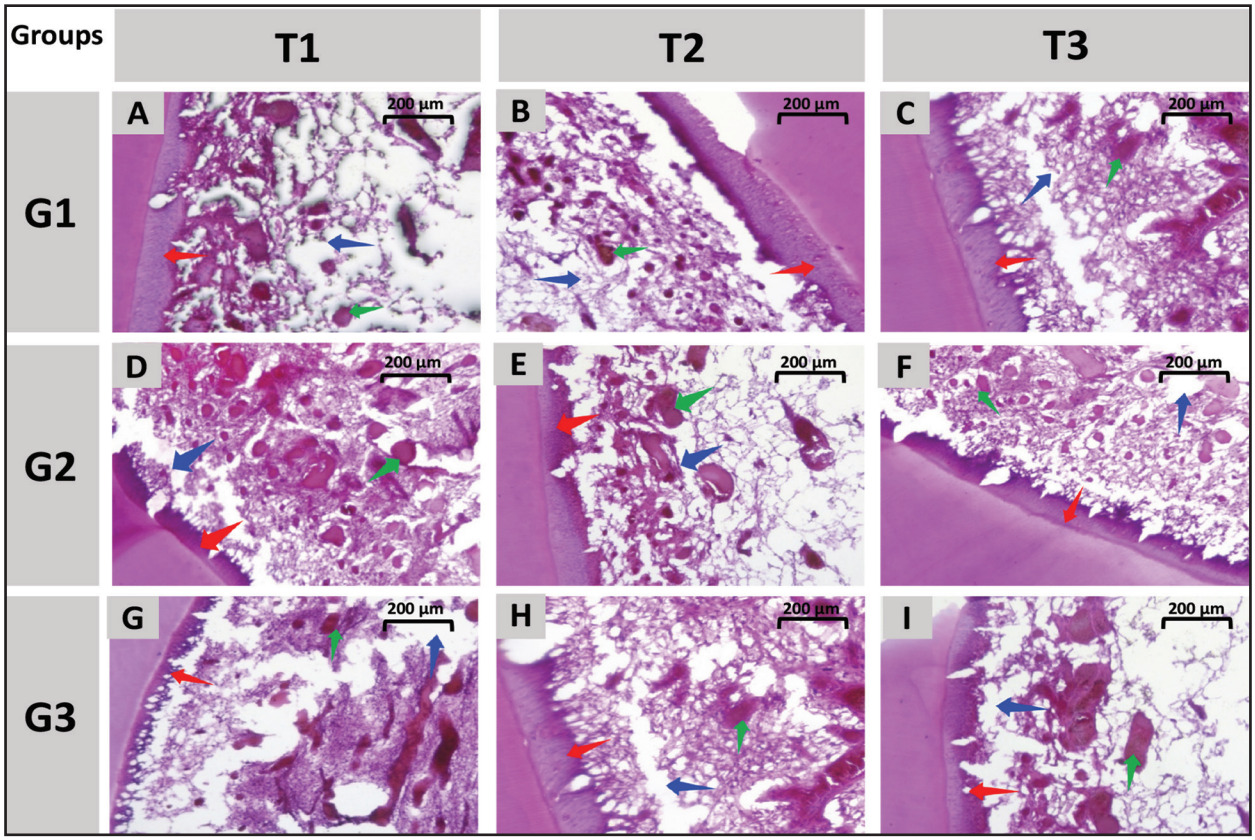

Fig. (1) Photomicrograph of groups G1(A,B,C), G2 (D,E,F), G3(G,H,I), red arrows indicate destructed odontoblastic layer, green arrow indicate dilated blood vessels, and blue arrow indicate edema at a magnification X200. The photomicrographs represent (A) G1T1 showing moderate inflammatory reaction with Vasodilation for the blood vessels. (B) G1T2 showing less vasodilation and healing of the odontoblastic layer with less interstitial edema. (C) G1T3 showing a mild inflammatory reaction, with complete healing with slight vasodilation in some blood vessels and mild interstitial edema (D) G2T1 showing reduction of the inflammation to the considerable degree, moderate vasodilation, necrosis of the odontoblastic layer at variable sites with inflammatory edema similar to group G1T1 also after 7 days. (E) G2T2 showing reduction of the inflammatory reaction and promoted regeneration of the odontoblastic layer. (F) G2T3, showing almost normal odontoblastic layer, absence of vasodilation and no interstitial edema. (G) G3T1 showing diffuse distraction and degeneration of the odontoblastic layer along the dento-pulpal wall in addition to sever vasodilation to the blood vessels, inflammatory edema with haemorrhage. (H) G3T2 showing moderate inflammatory reaction in form of vasodilation and diffuse interstitial edema. (I) G3T3 showing some healing at areas of odontoblastic layer, however moderately interstitial edema was found at the sub-odontoblastic layer together with vasodilation in some of blood vessels after 60 days.

Table (4) *Descriptive statistic of group-II (Dellite 43 modified), frequency (n, %) and significance using chi-square.*

Group	Time	Group-II: Dellite 43 modified				
		Score frequency n (%)				Significance
		Score-0	Score-1	Score-2	Score-3	χ2
Group-II: Dellite 43 modified	T1	2 (20.0)	6 (60.0)	2 (20.0)	0 (0.0)	>0.05 ns
	T2	2 (20.0)	7 (70.0)	1 (10.0)	0 (0.0)	0.045 *
	T3	8 (80.0)	2 (20.0)	0 (0.0)	0 (0.0)	>0.05 ns
Friedman's test sign.		0.003**				

*, **, ***, *significant at p<0.05, p<0.01, p<0.001; NS, non-sign. at p>0.05*

At 30 days postoperative (G2T2)

The Dellite 43 modified Adhesive system after 30 days detected farther healing, significant reduced inflammatory reaction and promoted regeneration of the odontoblastic layer. There were only few detected blood vessels and almost no interstitial edema in most of the slide, so it scored 1 (Table, 4; Figure 1E)

At 60 days postoperative (G2T3)

The organic modifier (Dellite 43 modified adhesive system) shows a great result. The pulpal histology showed almost normal odontoblastic layer, absence of vasodilation and no interstitial edema. Most of the results scored 0 (Table, 4; Figure 1F).

Group-III

At 7 days postoperative (G3T1)

Dellite LVF modified Adhesive system showed a diffuse distraction and degeneration of the odontoblastic layer along the dento-pulpal wall. In addition, sever vasodilation to the blood vessels, inflammatory edema and haemorrhage occurred at the pulp tissue. All those histological changes happed after only 7 days, most of the slides scored 2 (Table, 5; Figure 1G).

Table (5) *Descriptive statistic of group-III (Dellite LVF modified), frequency (n, %) and significance using chi-square.*

Group	Time	Group-III: Dellite LVF modified				
		Score frequency n (%)				Significance
		Score-0	Score-1	Score-2	Score-3	χ2
Group-III: Dellite LVF modified	T1	1 (10.0%)	3 (30.0%)	6 (60.0%)	0 (0.0%)	>0.05 ns
	T2	0 (0.0%)	7 (70.0%)	3 (30.0%)	0 (0.0%)	>0.05 ns
	T3	0 (0.0%)	7 (70.0%)	3 (30.0%)	0 (0.0%)	>0.05 ns
Friedman's test sign.		>0.05 ns				

*, **, ***, *significant at p<0.05, p<0.01, p<0.001; NS, non-sign. at p>0.05*

At 30 days postoperative (G3T2)

The inorganic modified adhesive system detected after 30 days a moderate inflammatory reaction in form of vasodilation and diffuse interstitial edema that extended largely at the subodontoblastic area. Only mild regeneration was detected in the odontoblast. Most of the results scored 1 (Table, 5; Figure 1H)

At 60 days postoperative (G3T3)

The inorganic modified adhesive system (Dellite LVF) showed some healing at areas of odontoblastic layer, however moderately interstitial edema was found at the sub-odontoblastic layer together with vasodilation in some of blood vessels after 60 days, so most of the results scored 1 (Table, 5; Figure 1I)

Table (4) represent the frequency (n, %) of histological scores of groups 1, 2, and 3 at different timepoints (T1, T2, T3). The highest median (IQR) of 2.0 (1-2) was recorded in G3T1, however the lowest median (IQR) of 0.0 (0.00-0.25) was recorded in G2T3 Table (6).

Table(6) *Descriptive statistic of studied groups*

Group	Time	n (%)				Significance	
		Score-0	Score-1	Score-2	Score-3	χ2	Friedman's
Group-1: Control	**T1**	1 (10.0)	7 (70.0)	2 (20.0)	0 (0.0)	0.045*	
(Prime & Bond)	**T2**	0 (0.0)	80 (80.0)	2 (20.0)	0 (0.0)	>0.05 ns	>0.05 ns
	T3	8 (80.0)	2 (20.0)	0 (0.0)	0 (0.0)	>0.05 ns	
Group-II: Dellite 43	**T1**	2 (20.0)	6 (60.0)	2 (20.0)	0 (0.0)	>0.05 ns	
modified	**T2**	2 (20.0)	7 (70.0)	1 (10.0)	0 (0.0)	0.045 *	0.003**
	T3	8 (80.0)	2 (20.0)	0 (0.0)	0 (0.0)	>0.05 ns	
Group-III: Dellite LVF	**T1**	1 (10.0)	3 (30.0)	6 (60.0)	0 (0.0)	>0.05 ns	
modified	**T2**	0 (0.0)	7 (70.0)	3 (30.0)	0 (0.0)	>0.05 ns	>0.05 ns
	T3	0 (0.0)	7 (70.0)	3 (30.0)	0 (0.0)	>0.05 ns	
Two way ANOVA							
Corr.model		<0.001***					
Intercept		<0.001***					
Groups		<0.001***					
Time		>0.05 ns					
Groups x Time		>0.05 ns					

, **, *, significant at p<0.05, p<0.01, p<0.001; NS, non-sign. at p>0.05*

DISCUSSION

The experimental animal's study was selected because the animal model unlike human is easy to work, allow long term of evaluation with the possibility to treat all teeth identically by controlling history, care, and age to provide comparative evaluation of different tested materials.

As teeth in mammals are similar histologically [12], hence the protocol of the current study involved dogs. In addition, the pulp size provides a suitable sample for histological evaluation. Beside that dog's dentition provides a good number of teeth as it has three incisors, four premolars and three molars in each quadrant which allows the comparison of more than one material in the same dog. Mongeral dogs were included as they are prevalent in Egypt. The age of selected dogs ranged between 12 to 18 months to assure that all teeth present in their mouth were permanent. The experiment was carried out on dogs, fourth premolars and the three molars as they are relatively larger in size so large number of specimens could be obtained [13].

Standardized class I (Buccal pits) cavities of 3mm depth and 3mm width (mesio-distal) and 3mm long (occluso-cerviral) were prepared on the buccal surface of the tooth using round and fissure carbide burs with high speed under copious amount of water with intermittent movements to decrease heat generation. Carbide burs were used to provide efficient cutting without excessive heat generation. The bur was replaced after every third preparation to ensure cutting efficiency. Each dog received the three different restorative materials used in the study. After injection of the material finishing stone and Mylar strip were used to obtain smooth surface. Dogs were fed according to specific regimen that contains soft food till the end of testing periods due to impair masticatory function to avoid falling of restoration [14,15].

The pulp response was monitored at three periods. One short period of seven days to monitor the initial inflammatory pulp response to the materials of study. Two longer observation periods of 30 days and 60 days were selected to show progressive or limited extension of the pulpal response [12].

The true "gold standard" of pulp status is the histological analysis. Unfortunately, the true state of pulp health or pathology cannot be determined by clinical signs, symptoms or radiologic appearance. Clinicians have only relatively crude assessments, such as the application of hot or cold temperatures, an electric current, percussion of the tooth, changes in the appearance of associated soft tissues and patient reports of symptoms. However, numerous studies including histological analysis have demonstrated a chronically inflamed pulp, while the patients reported no symptoms; the investigators discerned no signs, and no apical/ radicular pathology were noted on radiographs. It must also be kept in mind that most studies that include histological analysis are of quite a short duration, typically two to four months [16].

In this study, by examination of the samples at 7 days T1 following cavity preparation and restoration using conventional adhesive system G1 (G1T1), The Prime Bond Universal dental Adhesive system showed at the first 7 days moderate inflammatory reaction with vasodilation for the blood vessels and interstitial edema as well as a moderate distraction of the odontoblastic layer at variable areas. Most of the samples showed score 2 (Figure 1A).

At 30 days postoperatively (G1T2) showed moderate inflammatory reaction. It showed vasodilation but less than the same Group at 7 days. The odontoblastic layer showed sign of healing and less interstitial edema due to biocompatibility with the adhesive, most samples scored 2 (Figure 1B).

At 60 days postoperatively control group (G1T3) showed a mild inflammatory reaction, almost completely healing had occurred together with slight vasodilation in some blood vessels and mild interstitial edema. Most of the slides scored 1 (Figure 1C).

By the examination of the samples with the organic modified material (Dellite 43 modified Adhesive system) at 7 days T1 following cavity preparation and restoration with Dellite 43 modified adhesive G2 (G2T1) with showed reduction of the inflammation to the considerable degree, moderate vasodilation, necrosis of the odontoblastic layer at variable sites and inflammatory edema were detected similar to group G1T1 (Figure 2) also after 7 days. Most of the slides scored 2 (Figure 1D)

The inflammatory reaction of The Dellite 43 modified Adhesive system after 30 days postoperatively (G2T2) show farther healing significant reduced inflammatory reaction and promoted regeneration of the odontoblastic layer. There were only few detected blood vessels and almost no interstitial edema in most of the slide, so, it scored 1 (Figure 1E)

At 60 days postoperatively (G2T3), the organic modifier (Dellite 43 modified adhesive system) shows a great result. The pulpal histology showed almost normal odontoblastic layer, absence of vasodilation and no interstitial edema. Most of the slide scored 0 (Figure 1F)

By examination of the samples following cavity preparation and restoration with Dellite LVF modified adhesive (inorganic) G3 at 7 days (T1) (G3T1), Dellite LVF modified Adhesive system showed a diffuse distraction and degeneration of the odontoblastic layer along the dento-pulpal wall in addition to sever vasodilation to the blood vessels, inflammatory edema and hemorrhage occurred at the pulp tissue was detected all those histological changes happened after only 7 days. Most of the slides scored 3 (Figure 1G)

This inflammatory reaction decreased at 30 days postoperatively (G3T2). The inorganic modified adhesive system detected after 30 days a moderate inflammatory reaction in form of vasodilation and diffuse interstitial edema that extended largely at the sub-odontoblastic area were only mild regeneration were detected in the odontoblast, most of the slides scored 2 (Figure 1H).

Smectite-type clay composed of an expandable type of aluminosilicate clay mineral which has a layered structure and relatively high cation exchange capacity. By replacing the natural inorganic exchange cations with organic compound, MMT surface is converted from being primarily hydrophilic to hydrophobic, which enables it to interact strongly with organic compounds dissolved in water [18]. This in agreement with other studies [19,20] which found that, Polymer-nanoclay composites involve the interaction of polymer matrix of polyacrylic acid with the nanoplates of clay and are formed by the dispersion of low weight percentages of nanoclay into polymers improving physical, biological and chemical properties of the resulting composite as compared to conventional materials. Clay minerals can be used as gastrointestinal protectors, antacids, and antidiarrhoeaics. They are administered by the patient orally in the form of pills, powders, suspensions, and emulsions but their prolonged use is inadvisable as they tend to decompose when they come into prolonged contact with hydrochloric acids [21].

In general, clay materials whether organic or nonorganic are biocompatible to the human body. They can be beneficial to human health by serving as active principles or excipients in pharmaceutical preparations, in spas, in beauty therapy medicine[21],

in wound healing [18,22] ability of clay minerals to adsorb and retain harmful and toxic substances and used as drugs. This explains our results that the Dellite 43modified adhesive produces the best results. Still, the follow up period of 60 days in the current study couldn't be An actual clinical representative for long term success of this material. Also, other clinical factors could affect the behavior of the restorative material pulp response such as the thickness and type of remaining dentine (secondary, reactionary, or reparative), nanoleakage, Matrix metalloproteinase enzyme activity and the preoperative pulp reaction to carious lesion [12,23]. Accordingly, further in-vivo studies are required with longer periods of evaluation and different clinical situations to assure the efficiency of the tested materials.

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