EGYPTIAN DENTAL JOURNAL
www.eda-egypt.org
VOL. 69, 427:438, JANUARY, 2023
PRINT ISSN 0070-9484 • ONLINE ISSN 2090-2360
ORAL MEDICINE, X-RAY, ORAL BIOLOGY AND ORAL PATHOLOGY
Submit Date : 19-11-2022 • Accept Date : 28-11-2022 • Available online: 1-1-2023 • DOI : 10.21608/edj.2022.175677.2354

ASSESSMENT OF MONTMORILLONITE NANOCLAY-MODIFIED GLASS IONOMER BIOCOMPATIBILITY FOCUSING ON PULP RESPONSE

Monaliza Maher Abdelaziz*, Rehab Fawzi Kasem**, Ahmed Gamal Abdelwahed* *and* Amina Fouad Farag***

ABSTRACT

Background and Aim: Incorporation of Montmorillonite (MMT) nanoclay in restorative materials improves their mechanical properties in addition to biocompatibility, yet little is known about effects of MMT nanoclay-modified glass ionomer on dental pulp**.** This study aimed to evaluate pulp response of MMT nanoclay-modified glass ionomer as viable biocompatible material compared to resin-modified and conventional in dog model.

Material and methods: MMT nanoclay was characterized using X-ray Diffraction (XRD) and Fourier Transform Infra-Red Spectroscopy (FTIR). Class V cavities were prepared in 90 dogs' teeth and divided into 3 groups (n=30): G1: conventional, G2: MMT nanoclay-modified, and G3: resin-modified glass ionomer. Then the jaws were dissected and demineralized at three-time intervals of 7, 30 and 60 days to evaluate the pulp response of the extracted teeth of each group microscopically and statistically using Kruskal Wallis and Friedman tests.

Results: MMT nanoclay-modified glass ionomer (G2) group at 7 days was associated with reduced signs of inflammation with lesser interstitial edema, marked thickening of collagen fibers, mild inflammatory cell infiltrate and less destruction of the odontoblastic layer compared to the other two groups. Further healing was observed with nanoclay group after 30 days with significantly reduced inflammation and promoted regeneration of the odontoblastic layer and pulp tissue to reach a normal histological pulp architecture by 60 days.

Conclusion: MMT nanoclay-modified glass ionomer is a promising biocompatible material that shows favourable pulp response over both conventional and resin-modified.

KEYWORDS: Montmorillonite, Nanoclay-modified glass ionomer, Resin-modified glass ionomer, Conventional Glass Ionomer, Pulp response.

* Lecturer of Conservative Dentistry, Faculty of Dentistry, October 6 University, Giza, Egypt
** Professor of Oral and Maxillofacial Pathology, Faculty of Oral and Dental Medicine, Cairo University and Faculty of Dentistry, October University for Modern Sciences and Arts, Cairo, Egypt.
*** Lecturer of Oral and Maxillofacial Pathology, Faculty of Dentistry. October 6 University, Giza, Egypt

INTRODUCTION

Glass ionomer cement (GIC) has gained great popularity in dentistry over the past fifty years with a wide range of applications [1]. It can be used as base, fissure sealant, temporary restoration, especially in patients with high caries activity, and final treatment material [2]. Several characteristics make GIC the material of choice in abovementioned clinical indications as its ability to bond adhesively to tooth structures, particularly to enamel and dentin, fluoride-releasing then recharging capacity over prolonged period and good biocompatibility [3].

GIC biocompatibility is due to some properties such as fluoride release, compatibility of coefficient of thermal expansion with tooth tissues, possibility of its use in deep cavities without the need for intermediary base material [4, 5]. Despite these advantages, GIC has many drawbacks regarding mechanical properties especially insufficient durability and hardness [6].

To enhance mechanical properties of conventional GIC, Resin-modified glass ionomer cement (RMGIC) was introduced. It contains hydrophilic monomers and polymers like HEMA and camphorquinone as an initiator system in addition to basic glass powder as well as water and polyacid; the same essential components of conventional GIC[7]. On one hand, RMGIC has greater strength, esthetics bond and command setting/set compared to conventional GIC [8]. On the other hand, RMGIC biocompatibility is markedly compromised compared with conventional GIC due to release of HEMA monomer in the first 24 hours. This released monomer can diffuse through dentinal tubules and is cytotoxic to the pulp cells [2].

Montmorillonite (MMT) is a crystalline structure formed of layers of clay minerals where each single layer has a central alumina octahedral sheet sandwiched between two silica tetrahedral sheets [9, 10]. Integration of MMT in polymer matrix improves specifications of resulting compound compared to non-modified one. Recent studies that investigated the possibility of clays incorporation in restorative dentistry materials showed improved physical, mechanical and adhesive properties [11, 12].

Nanoparticles are widely used as strengtheners in dental restorations because they have a noticeable effect on their performance when used in small amounts compared to micro fillers [13]. Glass ionomer restorations are modified with MMT nanoparticles by the dispersion of low weight percentages of these clay nanoplates (nanoclay) into polymer matrix. Because of their small size, nanoparticles can penetrate tooth micro-pores, improving the bond layer [14].

Despite these reported benefits of MMT nanoclay incorporation in GIC on its properties, little is known about their adverse effects on dental pulp. Therefore, the current study was conducted to evaluate the pulp response to GIC modified with MMT compared to conventional GIC and RMGIC.

MATERIAL & METHODS

Sample size calculation

Since this study was going to examine 3 different restorative materials at 3 different time intervals, a sample size of 72 (minimum of 24 for each group) according to repeated measures ANOVA design was found sufficient to detect effect size of 0.40, power of 95% and at significant level of 5% ($p<0.05$). For higher significance level ($p<0.01$), final sample size of 90 (30 samples for each group) was selected. The sample size was calculated using G*Power software version 3.1.9.4.

Animals

A total of 6 adult mongrel dogs weighing an average of 10 kg were selected for the current study. Dogs were examined thoroughly and kept under observation for 10 days before being used as experimental animal models to exclude any systemic disease or dental disorder. T in a thermoneutral comfort zone (Supplementary File 1).

Animals were included in the study if their teeth were 1) healthy and sound with no carious lesions and 2) yielded a positive response to pulp vitality testing (vital). The teeth on the other hand were excluded if 1) pulp exposure happened during cavity preparation, 2) contamination of the operative field occurred 3) animal died prematurely, preventing collection of histological data or 4) poor histological quality of collected samples.

Experimental procedures were carried out under the protocol approved by the Research Ethics Committee at the Faculty of Dentistry where all international, national, and institutional guidelines for animal use and care were followed. We present the current article following the ARRIVE Guidelines Checklist for studies on animals (Supplementary File 2).

Classification of teeth

Thirty teeth in each group, including canine, fourth premolar, first and second molars were used, summing up 90 teeth. These teeth were randomly assigned into three equal groups according to the type of restorative material used (n=30) with the aid of a random number table. All selected teeth present in dogs' mouths were permanent as their age ranged from 12 to 18 months to assure that. The experiment was carried out on canines, fourth premolars, and the three molars as they are relatively larger in size and number, so many specimens could be obtained. Each group was further subdivided into three subgroups (10 teeth each) according to the post-operative evaluation periods: Subgroup 1 (T1, 7 days), Subgroup 2 (T2, 30 days), and Subgroup 3 (T3, 60 days).

Characterization of MMT Nanoclay

X-ray Diffraction (XRD): Samples were analyzed using panlytical X-pert pro with cu anode for XRD working at 40 mA / 45 kV. Prior to analysis, these samples were grounded in agate mortar, a flat surface sample was then attached to the sample holder inside the XRD apparatus, and the date was interpreted using software of the XRD machine. Long scan was used, between 2θ angles of 7°–75° at a step size of 0.03° and time per step(s): 100, scan speed (2θ /S): 0.076°, No. of steps 2266.

Fourier Transform Infra-Red Spectroscopy (FTIR): Infrared spectra was obtained in the mid-range using JASCO-6100 *FTIR* spectroscopy, the 1 to 3mg from the samples were ground with 99-97mg of potassium bromide (*KBr)* in an agate mortar,

TABLE (1). Materials' composition, specification and manufactures.

Group	**Material**	**Specification**	**Composition**	**Manufacturer**
G1 Conventional Glass Ionomer	Medifil®	A radiopaque conventional glass ionomer restorative material with chemical adhesive ability to enamel and dentin. Shade A3	*Powder:* fluoroaluminosilicate glass containing Si, Al, Sr, and Na. *Liquid:* polyacrylic acid, copolymers of carboxylic acid and tartaric acid	Promedica (*dental material*), Germany www.promedica.de
G2 MMT nanoclay-modified Glass Ionomer	Dellite® 43B	An off-white nanoclay of 7-9 um derived from a naturally occurring montmorillonite with organic modifier Specific weight 1.6 g/cm^3 and bulk density of 0.40 g/cm^3	Pure nano montmorillonite (nanoclay) consists of a layer made of an inner octahedral sheet of alumina or magnesia sandwiched between two tetrahedral sheets of silica modified with a quaternary ammonium salt (dimethyl benzyl-hydrogenated tallow ammonium)	Laviosa (*advanced mineral solution*), Italy www.laviosa.com
G3 Resin-modified Glass Ionomer	GC Fuji II LC CAPSULE	Light-cured resin reinforced glass ionomer restorative premeasured capsules	The capsule contains (0.33g Powder / 0.085ml Liquid)	GC America . Tokyo, Japan www.gcamerica.com

forming a 3mm diameter KBr pellet, transmittance data was collected in the range of 4000–400 cm–1 with 4 cm–1 resolution.

Preparation of MMT nanoclay-modified glass ionomer

GIC specifications, composition, and manufacturers used in the current study are listed (Table 1). MMT was dispersed in Medifil liquid by the exfoliation-adsorption method. Dellite® 43B (0.375 grams=2.0 wt.%) was weighed on a balance and mixed on hot plate using magnetic stirrer at 100 rpm for 24 hours at 75˚C.

Procedures

All operations were done under general anesthesia including premedication with mixture of atropine sulfate (0.05 mg\kg body weight) and diazepam (1.0 mg\kg body weight) intravenously to reduce salivation and resist any sudden arrhythmia. Anesthesia was induced immediately through intravenous cannula by injection of ketamine (10 mg\kg body weight) and xylazine (1.0 mg\kg body weight). Teeth were isolated using rubber dams and a standardized class V cavities of 1.5mm depth were prepared to be close to the pulp but without exposure and 2mm width on facial surfaces of teeth using round and fissure carbide burs (Komet, 454 South Anderson Road, Suite 14 Rock Hill, SC 29730, USA). Depth and width of cavities were checked by periodontal probe to assure uniform cavity. For G1 and G2 groups, a scope of powder was mixed with one drop of liquid according to manufacturers' instructions on glass slab with spatula and applied by suitable condenser and the excess was removed with a carver. In G3 group, capsules were activated by depressing the bottom button and then triturated in mixing device for 10 seconds. The previously prepared GIC were then applied according to grouping system (Figure 1).

Histopathologic evaluation: Dogs were euthanized according to time intervals by intravenous injection of euthanasia solution (20% pentobarbitone sodium, 0.7ml\kg). Then jaws including teeth were dissected and fixed in 10% formalin for 10 days. These jaws were then demineralized in 50% formic acid+20% sodium citrate for 4 months; checking of demineralization was done using blunt needle. After jaws demineralization, bone was removed from around teeth by scalpel and roots tips were cut to facilitate intrusion of demineralizing solution into teeth. These teeth specimens were again placed in demineralizing solution for another 2 weeks. Following demineralization, teeth were dehydrated in ethanol, embedded in paraffin blocks, and sectioned longitudinally by microtome (5µm thickness). Sections were then stained using H&E staining for histopathologic evaluation.

Blind assessment of stained sections was performed by two expert pathologists independently using an images analysis computer system at magnification of X200 and all captured images were transferred to computer system by Leica Queen

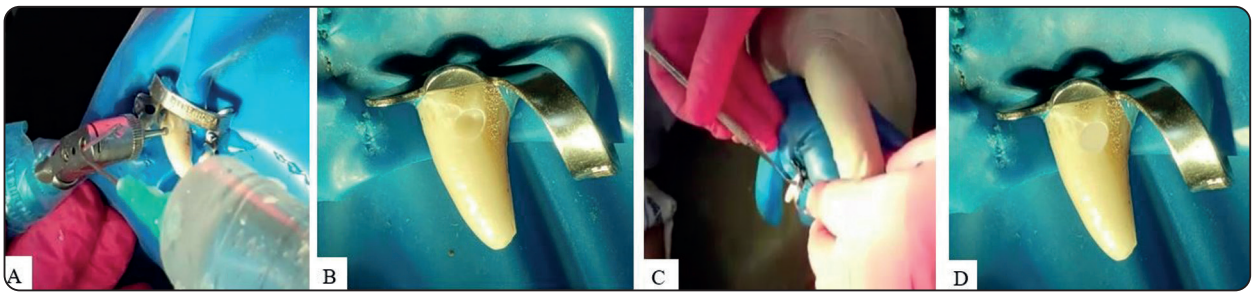

Fig. (1). Cavity Preparation on labial surface of dog`s canine (A) class V cavity preparation, (B) prepared cavity, (C) application of glass ionomer restoration, and (D) restored Class V Cavity.

Software for image analysis. The average scores for pulp response to investigated materials along post-operative evaluation periods were recorded based on a scale from 0-to 3 based on scoring system suggested by Adrian et al. [15] (Table 2).

TABLE (2). Pulp Response Scoring System.

Score	Criterion
0	**Normal pulp tissue**: normal histological architecture with continuous uninterrupted palisading arrangement of odontoblastic layer, absence of vasodilation and no interstitial edema, normal density of collagen fibers within the pulp and no observable other signs of inflammation.
1	**Mild reaction**: slight disruption of odontoblastic layer with mild interrupted palisading arrangement, slight interstitial edema, mild vasodilatation, occasional mild inflammatory cell infiltrate and less collagenization of pulpal tissue.
2	**Moderate reaction:** Moderate distraction of the odontoblastic layer with interrupted palisading arrangement at variable areas, moderate vasodilation, moderate interstitial edema diffuse inflammatory cell infiltrate and marked loss of collagen fibers of pulpal tissue.
3	**Severe reaction:** diffuse generalized distraction of pulp elements and degeneration of the odontoblastic layer with severely interrupted palisading arrangement, severe vascular dilation, multiple enlarged edematous spaces of various shapes and severe diffuse inflammatory cell infiltrate.

Statistical analysis:

Data were presented as frequency (n) and percentage. Statistical analysis was performed using SPSS (Statistical Package for Social Sciences) 26.0 software (IBM, Chicago). Kruskal Wallis test was used to compare between pulp response of different tested materials and the Friedman test was used to compare between different time intervals.

RESULTS

Baseline data: Ninety (90/90) sound vital teeth from 6 adult mongrel dogs were included in the current study (30 samples for each experimental group). All teeth and histological data were included in the analysis as none of the previously assigned exclusion criteria were met and no adverse events had occurred during the trial period.

G1 group: Moderate inflammatory reaction with vasodilation and interstitial edema in the pulp tissue with diffuse inflammatory cell infiltrate at 7 days postoperatively. The moderate distraction of the odontoblastic layer with the interrupted palisaded arrangement was also detected in variable areas, (Figure 2 A). Seven (70%) out of 10 G1T1 samples were given a score of 1, one (10%) case was given a score of 0, and 2 (20%) cases were given a score of 2. At 30 days, the odontoblastic layer showed signs of healing and regeneration with the less interrupted palisaded arrangement in certain areas. Moreover, there was decreased vasodilatation as well as interstitial edema, but diffuse inflammatory cell infiltrate can still be detected, (Figure 2 B). The G1T2 samples scored 1 in 8 (80%) cases and the remaining 2 (20%) cases scored 2. By examination of the samples of the G1T3 group after 60 days, almost complete healing had occurred in the form of mild interrupted palisaded arrangement of the odontoblastic layer at fewer areas and slight interstitial edema, less vasodilatation, marked numerous collagen fibers and mild inflammatory cell infiltrate, (Figure 2 C). Slides of the G1T3 group scored 0 in 8 (80%) cases and 1 in 2 (20%) cases.

G2 group: At 7 days postoperatively, reduced signs of inflammation with lesser interstitial edema, marked thickening of collagen fibers and milder inflammatory cell infiltrate compared to group G1T1 were recorded. However, there was moderate destruction of the odontoblastic layer with interrupted palisaded arrangement at variable areas together with moderate vascular dilatation, (Figure 2 D). G2T1 slides scored 1 in 6 cases, 2 in 2 cases, and 0 in 2 cases. Further healing was observed after 30 days with significantly reduced inflammatory

reaction together with promoted regeneration of the odontoblastic layer where only slight disruption of normal palisaded arrangement at few areas were detected in addition to much lesser interstitial edema, milder vascular dilatation, marked numerous collagen fibers and occasional inflammatory cell infiltrate, (Figure 2 E). Seven G2T2 samples scored 1 and 2 samples scored 0 and 1 sample scored 2. After 60 days, the G2T3 group exhibited normal histological pulp architecture consisting of normal connective tissue with uninterrupted palisaded odontoblastic layer, absence of vasodilation, and no interstitial edema with increased density of collagen fibers within pulp, (Figure 2 F). No signs of inflammation except for the presence of a few subodontoblastic inflammatory cells were noticed in the few slides. These G2T3 samples scored 0 in 8 out of the case while only 2 cases scored 0.

G3 group: G3T1 group showed diffuse generalized destruction of pulp elements and degeneration of the odontoblastic layer along the dental-pulp wall with severely interrupted palisaded arrangement in addition to severe dilation of the blood vessels and edematous spaces of various shapes at multiple areas of the pulp tissue was detected in addition to severe diffuse inflammatory cell infiltrate, (Figure 2 G). All these histological changes happed after only 7 days where the

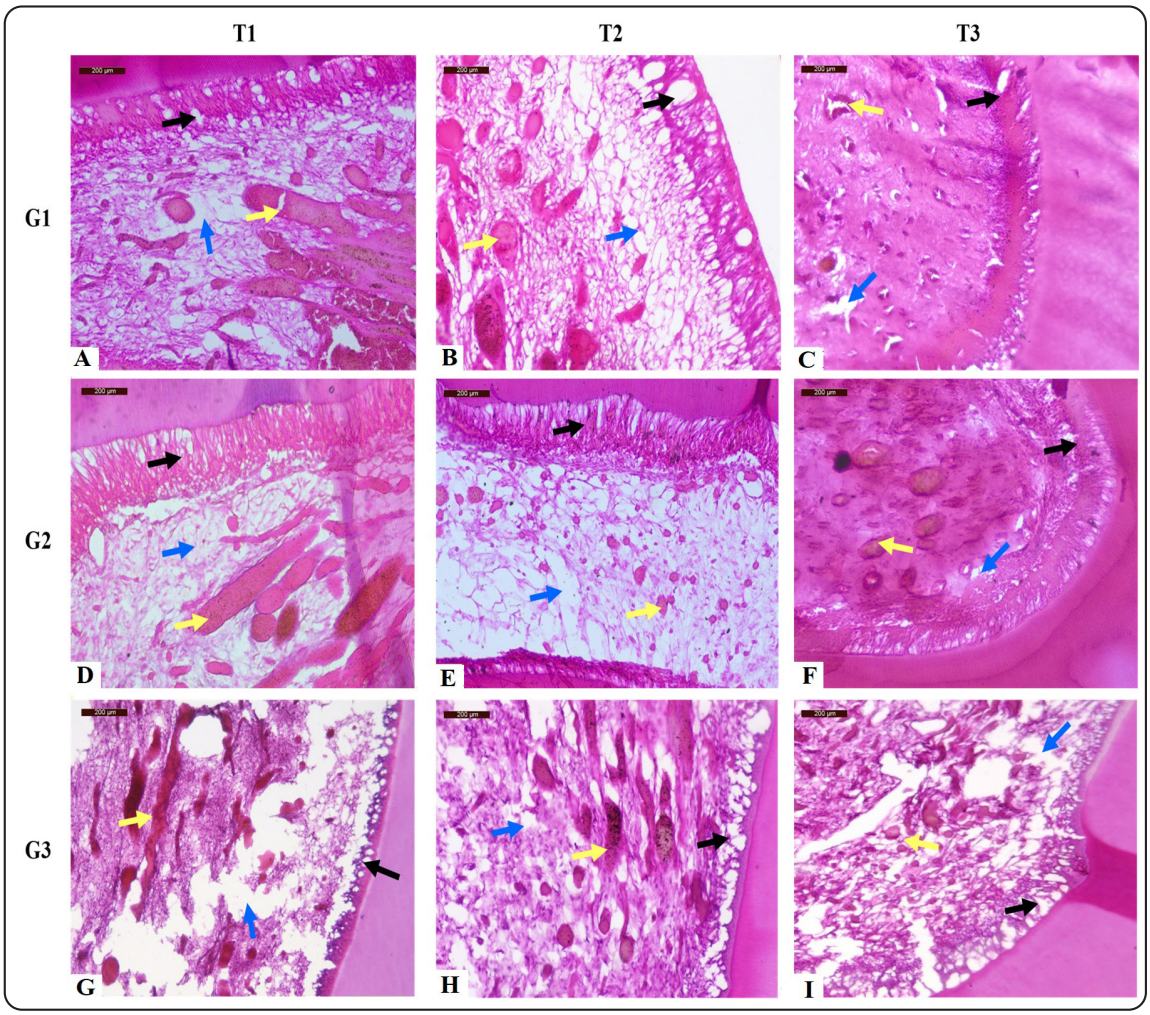

Fig. (2). Photomicrographs of the pulp reaction to different glass ionomer restorative materials showing disruption of odontoblastic layer (black arrow), vascular dilatation (yellow arrow) and interstitial edema (blue arrow). (A,B,C) Conventional G1 group, (D,E,F) Nanoclay-modified G2 group and (G,H,I) Resin-modified G3 group at different time intervals (T1, 7 days; T2, 30 days and T3, 60 days).

slides scored 3 in 6 (60%) out of 10 samples, 2 in 3 (30%) samples, and 1 in only 1 (10%) sample. Samples of G3T2 group at 30 days postoperatively revealed only mild regeneration of the severely interrupted odontoblastic layer and moderate inflammatory reaction in form of vasodilation and diffuse interstitial edema that extended largely at the sub odontoblastic area in addition to moderate inflammatory cell infiltrate, (Figure 2 H). So, scores 2 were given to 7 (70%) cases and 3 (30%) to the other 3 cases. Further healing of pulp tissue was observed at 60 days postoperatively in the G3T3 group in the form of more regenerated areas of the odontoblastic layer with the improved palisaded arrangement, lesser vasodilation, moderate interstitial edema, and reduced inflammatory cell infiltrate compared to G3T2 group, (Figure 2 I). Seven (70%) G3T3 slides scored 2 while the other 3 (30%) scored 3.

Statistical analysis

G1T1 showed lower scores for pulp response than G2T1 and G3T1 after 7 days intervals (T1) but no significant difference between the tested groups was reported (P=0.171). After 30 days intervals (T2), the G1T2 and G2T2 groups showed lower scores than G3T2 but there was also no significant difference between the tested groups (P=0.210). Although the G1T3 and G2T3 showed lower scores compared to G3T3, there was no significant difference between (G1T3, and G2T3) groups but there was a significant difference between the first two groups (G1T3, G2T3) and the third group G3T3 (P≤0.001) (Figure 3, Table 3). G1T3 group recorded lower scores than G1T1 and G1T2. There was no significant difference between (G1T1, and G1T2) groups but there was a significant difference between the first two groups (G1T1, G1T2) and the third group (G1T3) (P ≤0.001). When the pulp response score of the G2 groups was compared at different time intervals, the results showed that G2T2 recorded lower scores than G2T1. The score of the G2T3 group were lower scores than the scores of both G2T1 and G2T2. There was no significant difference between the G2T1 and G2T2 groups, whereas there was a significant difference between the first two groups (G2T1, G2T2) and the third group (G2T3) (P ≤0.008). Scores of G2T2 and G3T3 groups were lower than G3T1 where there was no significant difference between different groups (P=0.508) (Figure 4, Table 4).

TABLE (3). Frequency, and Percentage (%) for pulp response score of different tested materials.

		G1		G2		G3		p-value
		n	%	n	%	n	%	
T1	0	1	10%	2	20%	0	0%	0.171 NS
	1	7	70%	6	60%	1	10%	
	2	2	20%	2	20%	3	30%	
	3	0	0%	0	0%	6	60%	
	Rank	a		a		A		
T2	0	0	0%	2	20%	0	0%	0.210 NS
	1	8	80%	7	70%	0	0%	
	2	2	20%	1	10%	7	70%	
	3	0	0%	0	0%	3	30%	
	Rank	a		a		A		
T3	0	8	80%	8	80%	0	0%	≤0.001*
	1	2	20%	2	20%	3	30%	
	2	0	0%	0	0%	7	70%	
	3	0	0%	0	0%	0	0%	
	Rank	a		a		B		

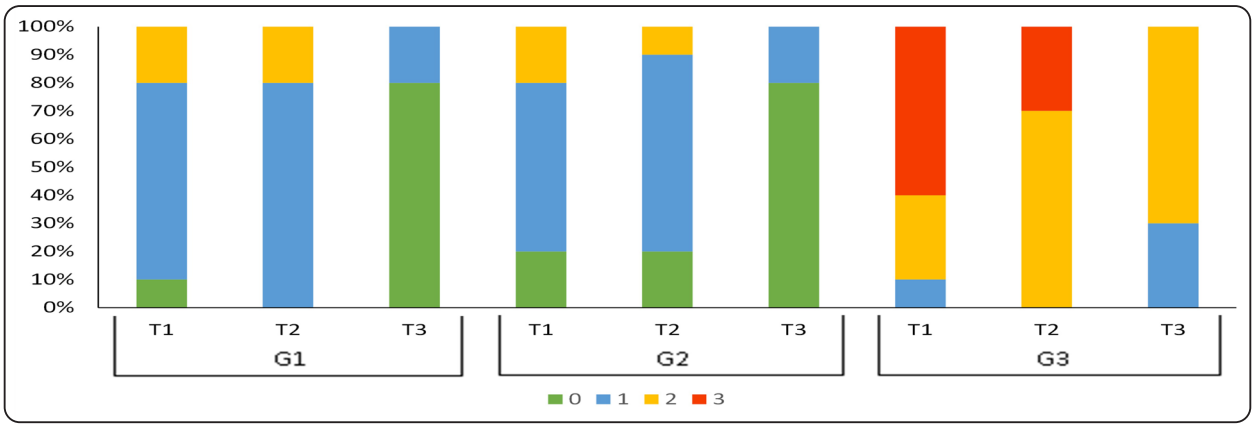

Fig. (3). Stacked Bar chart showing pulp response score of different tested materials.

TABLE (4). Frequency and Percentage (%) for pulp response score of different time intervals.

		T1		T2		T3		p-value
		n	%	n	%	n	%	
G1	0	1	10%	0	0%	8	80%	≤0.001*
	1	7	70%	8	80%	2	20%	
	2	2	20%	2	20%	0	0%	
	3	0	0%	0	0%	0	0%	
	Rank	b		b		a		
G2	0	2	20%	2	20%	8	80%	0.008*
	1	6	60%	7	70%	2	20%	
	2	2	20%	1	10%	0	0%	
	3	0	0%	0	0%	0	0%	
	Rank	b		b		a		
G3	0	0	0%	0	0%	0	0%	0.508 NS
	1	1	10%	0	0%	3	30%	
	2	3	30%	7	70%	7	70%	
	3	6	60%	3	30%	0	0%	
	Rank	a		a		a		

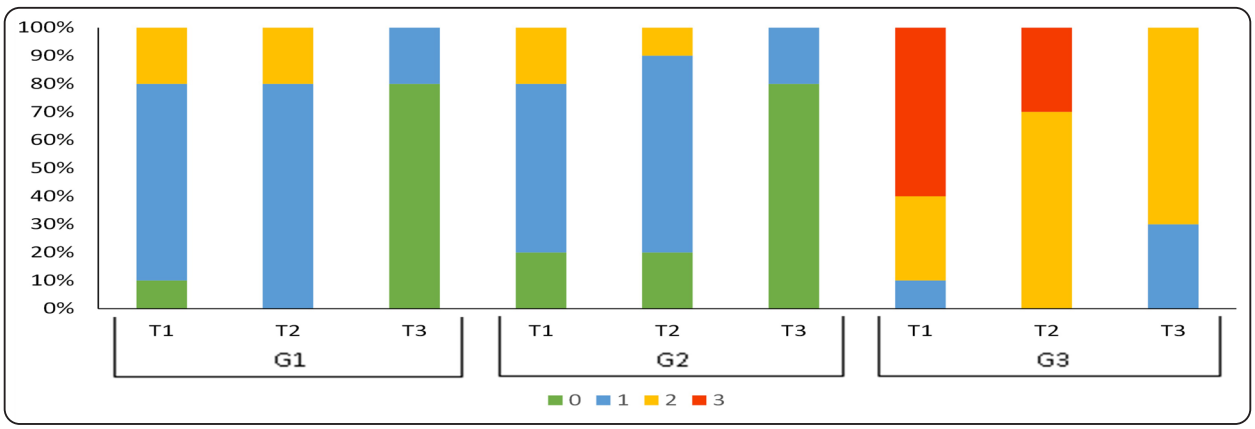

Fig. (4). Stacked Bar chart showing pulp response score of different time intervals.

DISCUSSION

Continuous development of technology provides a wide range of new materials for dental use, with improved mechanical and esthetic properties and biological compatibility. Despite these advances, the need still exists for biomaterials demonstrating high biocompatibility, antimicrobial effects, and ideal mechanical properties. The initial GICs formulation underwent several modifications to enhance handling and physical properties [16]. A remarkable enhancement occurred with RMGIC introduction.

Clay therapy is based on ability of clays and clay minerals to adsorb harmful and toxic substances. Beneficial effects of these materials were proven in the treatment of gastrointestinal disorders such as gastritis due to weakening of mucus-gel barrier. Short-term treatment with clay minerals increases the thickness of adherent mucus. This is ascribed to interactions of mineral particles with mucus components which modify gastrointestinal glycoproteins and enhance their polymerization [17].

Nano-sized MMT has been successfully used as a drug carrier, solubility enhancer with cyclodextrin, catalyst in organic synthesis, tissue engineering material in the manufacture of scaffolds for biomedical purposes, antibacterial effect in dental infections and food packaging materials [18] and to improve mechanical stability of hydrogels used for wound dressings [19]. Because it is non-cytotoxic, MMT has also been reported to be effective for a variety of cell lines, such as human dermal fibroblast [20].

It is suggested that the mechanical properties of GICs after the dispersion of 2 wt% of nanoclays makes these materials suitable for use in load bearing applications. The reinforcement of 2 wt% nanoclay generally resulted in improved mechanical behavior without compromising the acid-base neutralization reaction with a minimal effect on weight of GIC but provides the reinforcement at nanoscale [9]. This also agreed with the study performed by Dowling et al. [13] where the increased interlayer d-spacings for organo modified MMT showed positive reinforcing effect on the GI as expanded layers of this clay had provided an increased opportunity for the polyacrylic acid chains of the GI restorative to diffuse into the MMT galleries.

Pulp response was monitored at three-time intervals. One short interval of seven days to monitor the initial inflammatory pulp response to the materials of study. Two longer ones of 30 days and 60 days were also selected to show the progressive or limited extension of the severe grade of pulp response [21]. The true state of pulp health or pathology cannot be determined by clinical signs, symptoms, or radiologic appearance. Numerous studies including histological analysis have demonstrated chronically inflamed pulp, while the patients reported no symptoms. Therefore, the true "gold standard" of pulp status is histological analysis.

The operative trauma explained the initial slight inflammatory response and tissue organization after 7 days. Decreased inflammatory reactions at 30, and 60 days were due to self-repair processes, thickening of the dentin layer, and vascular remodeling contributing to spontaneous pulp healing. This was in accordance with Six, Costa and colleagues who explained the self-repair of pulp due to reparative dentin formation is seen 30 days postoperatively that was separated from dentin by calcospherites structures as a self-repair layer [22, 23].

GIC bonding ability to tooth by permanent adsorption to hydrophilic surfaces of hard oral tissues, thus offering possibility of sealing margins developed at the tissue interfaces besides the large molecular weight of poly acrylic acid could account for mild inflammatory response of the pulp that was also diminished along different observational periods. Moreover, GICs can prevent diffusion of potentially toxic materials through the dentin to pulp which provides an excellent bacterial seal [24, 25]. However, the findings of study were in contrast with

the study of Hashem and colleagues which reported that earlier clinical studies on GIC placed in non-exposed deep cavities in human teeth showed no symptoms during the observation periods; however, when extracted, an increased inflammatory cell infiltrate in the odontoblast layer was found with more odontoblast aspiration and changes in the odontoblast layer which mostly resolved towards the end of the experiments [26].

RMGIC showed slightly more persistent pulp inflammation than conventional and MMT nanoclay groups observed at the three different time intervals. This inflammatory response may be caused by diffusion of 2-hydroxyethyl methacrylate (HEMA) through dentinal tubules and reaching pulp tissue. Components released from resin-based materials may cause cytopathic effects [27]. HEMA is the main resin monomer in RMGIC, and its toxic potential has been well documented [28]. Presence of HEMA in a culture medium causes apoptosis and genotoxic effects because of its role in inducing DNA strand breaks. This is in agreement with the studies of Ribeiro, Agrawal and colleagues which found that RMGIC has been considered less biocompatible due to the release of unreacted monomers [29, 30]. In addition, HEMA inhibits the release of inflammatory cytokines by immunological cells, compromising the host defense response.

MMT nanoclay- modified glass ionomer showed a better inflammatory response than conventional and RMGIC since MMT is smectite-type clay composed of an expandable type of aluminosilicate clay mineral with a layered structure and relatively high cation exchange capacity. By replacing the natural inorganic exchange cations with an organic compound, the MMT surface is converted from primarily hydrophilic to hydrophobic, enabling it to interact strongly with organic compounds dissolved in water [31]. This is in agreement with findings of two studies which found that the polymer of nanoclay composites involves the interaction of the matrix of polyacrylic acid-based polymer with the nanoplates of clay and is formed by dispersion of low weight percentages of nanoclay into polymers improving physical, biological and chemical characteristics and specifications of resulting composite as compared to conventionally known materials [32, 33].

CONCLUSION

MMT nanoclay-modified glass ionomer produced a favorable pulp response and seemed to be a promising biocompatible material comparable to conventional GIC. Therefore, further studies are recommended to evaluate the effects of incorporation of nano-sized MMT particles on microhardness, fluoride-releasing properties, and biocompatibility of GIC and to assess its clinical application as a pulp capping material in the human.

Declarations

Conflict of Interest: All authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. They completed the ICMJE uniform disclosure form (Supplementary File 3-6).

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The research financial requirements, needs, materials and publication were completely supported and funded at the expense of researchers and paid from researchers' personal funds.

Ethics statement: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The study was approved by Research Ethics Committee at Faculty of Dentistry, October 6 University, Giza, Egypt, with approval number: RECO6U/13-2020 obtained in its meeting held on December 7, 2020. The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of

any part of the work are appropriately investigated and resolved.

Reporting Checklist: The authors have completed the ARRIVE Guidelines Checklist for studies on animals (Supplementary File 2).

ACKNOWLEDGMENTS

The authors would like to acknowledge and offer special thanks to the:

- English language specialized experts from EgyTranscript Translation & Interpretation Agency for their accurate and professional proofreading services of current the manuscript (Supplementary File 7).
- Staff members at Veterinary Hospital (Abbasia), Cairo, Egypt where the experimental procedures of the present animal study were conducted from January 19, 2021 to March 20, 2021 on canine, fourth premolar, first and second molar of adult mongrel dogs commercially purchased from the Al-Fahad Trading Company of Animals (Abu-Rawash, Giza, Egypt).
- Staff members at Oral Dental Research Division Laboratories, National Research Centre, Giza, Egypt for performing the characterization and analysis of MMT nanoclay by X-ray diffraction and Fourier transform infra-red spectroscopy and preparation of MMT nanoclay-modified glass ionomer restorative material.
- Staff members at Dental research laboratory, Faculty of Dentistry, MSA University, Giza, Egypt where histopathologic evaluation of samples was performed.

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