AL-AZHAR
Dental Journal
For Girls

The Official Publication
of The Faculty of Dental
Medicine For Girls,
Al-Azhar University
Cairo, Egypt.

Print ISSN 2537-0308 • Online ISSN 2537-0316

ADJ-for Girls, Vol. 8, No. 3, July (2021) — PP. 343:353

The Ultra-morphological Evaluation of Conventional and Resin-Modified Glass Ionomer Cements Modified with Nano-Hydroxyapatite Particles at Sound and Caries-Affected Dentin Interfaces

Zeinab M. Zaki[1]*, Maha A. Niazy[2], Mohamed H. Zaazou[3], Shaymaa M. Nagi [4], Dina W. Elkassas[5]

Codex : 01/21.07

azhardentj@azhar.edu.eg

http://adjg.journals.ekb.eg

DOI: 10.21608/adjg.2021.22941.1219

Restorative Dentistry
(Removable Prosthodontics, Fixed Prosthodontics, Endodontics, Dental Biomaterials, Operative Dentistry)

ABSTRACT

Purpose: To assess the effect of incorporation of 5 wt% nano-hydroxyapatite particles (Nano-HAp) on the ultra-morphological characteristics of sound and caries-affected dentin substrate/ conventional glass ionomer cement (CGIC) and resin-modified glass ionomer cement (RMGIC) interfaces. **Materials and Methods:** Occlusal surfaces of 16 extracted permanent human caries-free molars were ground to expose flat dentin surfaces. Half of the specimens (n=8) were subjected to pH-cycling to create artificial caries-affected dentin. Specimens were randomly restored with one of the tested restorative materials either control (CGIC and RMGIC) or experimental (CGIC and RMGIC modified with Nano-HAp). Different restorative materials were mixed according to manufacturer instructions and then built-up on the sound or caries-affected dentin surfaces. All specimens were stored in artificial saliva either for 24 hours or 9 months. Specimens were then prepared and observed under field emission scanning electron microscope (FESEM) for ultra-morphological evaluation of dentin-restoration interfaces. **Results:** FESEM observations showed no marked ultra-morphological changes in Nano-HAp modified groups/ dentin interfaces in compared to control groups. There was significant increase in microcracks and pores within the matrix of all GIC groups. Moreover, there were multiples microcracks extended all over the restorations matrix with microgaps formation at dentin-restoration interfaces for all caries-affected dentin and 9 months aging period groups. **Conclusion:** The unique properties of glass ionomer cements were greatly diminished with bonding to caries-affected dentin and gradually declined with aging process. While there was no additional effect was observed from addition of Nano-HAp to these tested cements regarding their ultra-morphological characteristics features under FESEM.

KEYWORDS

Nano-Hydroxyapatite,
Resin-Modified Glass Ionomer,
Ultra-morphology.

- Paper extracted from PhD thesis titled "Effect of nano-Hydroxyapatite Incorporation into Conventional and Resin-Modified Glass Ionomer Cements on Shear Bond Strength to Sound and Caries-Affected Dentin (An in vitro/ in-vivo study)".

1. Assistant researcher of Operative Dentistry, National Research Centre (NRC), Cairo, Egypt.
2. Professor of Operative Dentistry, Faculty of Dental Medicine for Girls, AL-Azhar University, Cairo, Egypt.
3. Professor of Operative Dentistry, National Research Centre (NRC), Cairo, Egypt.
4. Assistant Professor of Operative Dentistry, National Research Centre (NRC), Cairo, Egypt.
5. Professor of Operative Dentistry, Misr International University (MIU), Cairo, Egypt.

* Corresponding author email: zeinabzaki7@gmail.com

INTRODUCTION

Nowadays, modern operative dentistry focuses on the conservation of tooth structure; through minimal surgical intervention and application of aesthetic adhesive restorative materials that possibly perform a therapeutic action on the demineralized dentin. Glass Ionomer Cements (GICs) were first introduced by Wilson and Kentin at the "Laboratory of the Government Chemist, United Kingdom" in the early 1970s. GICs are water-based cements with unique characteristics that make them clinically attractive dental material. They are the only material that bond chemically to enamel and dentin, have a cariostatic effect, remineralization potential due to gradual fluoride ions release for a long period, have similar coefficient of thermal expansion and elastic modules to tooth structure, beside its biocompatibility with pulp tissue and easy handling properties [1,2].

Despite of its wide applications and various advantages, unfortunately conventional GIC's suffers from various shortcomings such as; poor physical properties, brittleness, decreased fracture toughness, inferior mechanical properties, moisture sensitivity and poor aesthetics which prevent their uses in high stress-bearing area [3]. To overcome these drawbacks, new modifications and improvements were introduced into conventional GICs. Among these modifications resin- modified glass ionomer cements (RMGICs) are becoming increasingly popular due to their easier application, extended working time, superior mechanical properties and immediate finishing and polishing procedures following light curing. At the same time, it still keeps the same advantages of the conventional GIC types [4]. However, the mechanical properties and aesthetic appearance of RMGICs were found to be less favourable than resin composite [4].

In order to overcome some of drawbacks of GIC different materials various nano-fillers have been incorporated into glass powder such like; silver cermet, zinc, titanium dioxide, gold and zirconia in an attempt to improve the poor mechanical properties of these materials. Although theses modified reinforced materials exhibited high strength properties and reduced abrasion, their showed poor aesthetics limits their use. Thus, the materials that mimic both the structure as well as mineral composition of teeth are much preferred for clinical application [5]. Therefore, nano-hydroxyapatite particles (Nano-HAp) are considered an excellent candidate as a filler material for both GICs and RMGICs [6, 7].

Bonding to caries-affected dentin (CAD) represents a common substrate for bonding in clinical practice, and it might assume that the structural and morphological alterations within caries-affected dentin (CAD) may negatively impact the performance of dental materials applied to it. Glass ionomer cement (GIC) is one of the best materials for this purpose due to its properties as anti-cariogenic activity, fluoride release and low shrinkage during its setting that lead to lower degree of marginal microleakage in compared to composite [8]. However, most of researchers involve the sound dentin as bonding substrate, and only few studies are available on bonding of GICs to caries-affected dentin (CAD).

Field emission scanning electron microscope (FESEM) is used as a method to assess the variations in surface topography and to evaluate the quality of restoration-bonded surface interface [9]. The strong durable bond for glass ionomer cements (GICs) can be evaluated through FESEM by observing uniform gap-free junction at bonded interface.

Thus, it seems to be of value to conduct a research to study the effect of adding nano-hydroxyapatite particles (Nano-HAp) on the ultra-morphology of CGIC and RMGIC/sound and caries-affected dentin interface. This study was conducted to accept or reject the null hypotheses that, there are no differences in ultra-morphological characterization between Nano-HAp GIC and Nano-HAp RMGIC/sound or caries-affected dentin interface in comparison with CGIC and RMGIC/ sound or caries-affected dentin interface.

MATERIALS AND METHODS

Selected materials:

One conventional glass ionomer cement (Fuji II, GC gold label 2) and one Resin-modified glass ionomer cement (Fuji II LC, improved, GC) were used in this study. Materials brand name, manufacturers and their composition are presented in Table 1.

Preparation of nano-hydroxyapatite particle modified glass ionomer cements:

Nano-hydroxyapatite particles (Nano-HAp) were synthesized for this study by the wet- chemical precipitation method at National Research Centre, Egypt [10]. Synthesized Nano-HAp was then characterized by Scanning Electron Microscopy (SEM), Elemental Dispersive X-ray (EDX) and Transmission Electron Microscopy (TEM) to study the powder purity, surface area and the particle size which affect their bioactivity, mechanical and biological properties.

Modified cements "Nano-HAp GIC & Nano-HAp RMGIC" were prepared by addition of Nano-HAp to the powder component of cements prior to mixing the cements according to manufacturer's specifications [11, 12]. Five percent by weight (5% w/w) of CGIC or RMGIC powder were replaced by Nano-HAp and then mixed using ball mill machine (LFJS, Hunan, China) with 200 rpm for 2 hours to produce a homogenous mixed powder [13].

Selection of teeth and specimens preparation:

After protocol approval from the "Ethical Research Committee" of the Faculty of Dental Medicine for Girl, Al-Azhar university, Cairo; sixteen freshly extracted caries-free permanent human molars were selected to be enrolled in this study. The selected teeth were thoroughly cleaned, scaled and disinfected with 0.2% thymol solution. Teeth were then stored in distilled water, at 4°C to be used within a maximum period of 3 months after extraction with a weekly change of distilled water [14].

Each molar was sectioned precisely, 2mm below the cemento-enamel Junction (CEJ) to separate the coronal portion from the root portion using diamond disc. Each specimen was embedded in the acrylic resin with its occlusal surface facing outward. The occlusal surface of each molar was wet grinded to expose superficial flat dentin surface using #240-grit silicon carbide (SiC) abrasive paper (3M™ Silicon carbide paper sheet, 426) mounted in bench grinding machine (Rajlaxmi bench grinder, RGL- 01,USA) under copious water coolant [15]. The exposed superficial dentin surface was further polished wet with #600-grit silicon carbide paper for 60 seconds to create a standardized uniform smear layer.

Table (1): *Materials brand name, manufacturers and their composition:*

Materials	Composition
• **CGIC: Fuji II, GC Gold Label 2. (GC Corporation, Tokyo, Japan)** • **Nano-HAp CGIC: Nano-HA Fuji II, GC Gold Label 2.**	• Powder (10g): acid soluble "calciumfluoroalumino-silicate glass" composed of: silica, alumina, calcium fluoride, sodium fluroide, aluminum phosphate and aluminum fluoride. • Liquid 7g (5.6ml): Poly acrylic acid (40%), Tartaric acid (5-15%), Maleic acid, Itaconic acid and distilled water.
• **RMGIC: Fuji II LC, improved, GC. (GC Corporation, Tokyo, Japan)** • **Nano-HAp RMGIC: Nano-HAp Fuji LC, improved, GC.**	• Powder (15g): same composition of chemically cured CGIC powder " • Liquid 8g (6.8ml): polyacrylic acid, 2HEMA (HEMA: hydroxyethyl-methacrylate), 2,2,4 TEGDMA (TEGDMA: Triethlene-glycol dimethacrylate) **, TMHMDC (TMHMDC: Trimethyl-hexamethylenedicarbonate.)***, Camphorquinone and distilled water.

Experimental design:

The teeth were randomly allocated into four main groups (G) according to type of tested restorative material; G_1: conventional glass ionomer cement (CGIC) [Fuji II, GC gold label 2], G_2: conventional glass ionomer containing nano-hydroxyapatite particles [Nano-HA Fuji II, GC Gold Label 2] , G_3: resin-modified glass ionomer (RMGIC) [Fuji II LC, improved] and G_4: resin-modified glass ionomer containing nano-hydroxyapatite particles [Nano-HA Fuji LC, improved]. Each group was further divided into two equal subgroups according to tooth substrate either sound dentin (D_1) or caries-affected dentin (D_2). Then, each subgroup was further divided into two equal classes according to aging period either 24 hours (T_1) or 9 months (T_2).

Artificial caries-affected dentin induction:

Half of specimens (n=8) were subjected to cariogenic challenge by pH-cycling to create artificial carious lesion using the following protocol: each specimen was cycled at room temperature for 8 hours in (10 ml) of demineralizing solution (2.2mM $CaCl_2$, 2.2mM NaH_2Po_4 and 0.05M acetic acid at pH 4.4), and then followed by 16 hours in (10 ml) of remineralizing solution (1.5mM $CaCl_2$, 0.9mM NaH_2Po_4, and 0.15mM KCL at pH 7.0). This procedure was repeated for 14 days with the both remineralizing and demineralizing solutions were renewed [16].

Application of different tested restorative materials:

Prepared specimens were randomly assigned to the previously mentioned main groups. All tested restorative materials were used in accordance to manufacturer's instructions as follows:

First Ketac™ conditioner was applied for 10 seconds on the prepared flat occlusal superficial dentin surface, rinsed for 10 seconds with copious amount of water and gently dried with a cotton pellet.

For CGIC and Nano-HA GIC groups; a standard powder/liquid ratio (2.7g /1.0g) "one level scoop of powder to one drop of liquid" was mixed up to 30 seconds. While for RMGIC and Nano-HA RMGIC groups; powder/liquid ratio (3.2g/1.0g) "one scoop of powder to two drops of liquid", was mixed mix up to 20-25 seconds. Each tested restorative material was then build up in 1.5-2mm thick increment on the prepared flat sound or caries-affect dentin surfaces and then light-cured for 20 seconds in RMGIC and Nano-HA RMGIC groups.

EQUIA® Coat was then applied on the exposed surfaces and light cured for 20 seconds. After placement of the restorations, all specimens were stored in artificial saliva in an incubator at 37 ºC with 95% humidity either for; 24 hours or 9 months aging periods.

Preparation of specimens for interfacial analysis of the bonded interface:

Prepared specimens were sectioned longitudinally perpendicular to tooth-restoration interface using "Isomet™ Low Speed Precision Cutting Machine (Buehler Ltd.,Lake Bluff, IL; USA). Serial cutting was done in bucco-lingual direction using a diamond disc (Buehler, IL; USA) at 2000 rpm; under copious coolant to obtain slices of 2 mm thick. Each section was polished with waterproof carbide paper (3M Silicon carbide paper sheet; USA) under running water to obtain uniform smooth surface. All specimens were then placed into ultrasonic cleaner in distilled water for 30 minutes to remove all debris and smear layer (9). Specimens were then observed under Field emission scanning electron microscopy (EESEM) (Quanta 250 FEG, FEI company; Netherlands) at magnification 1000x for ultra-morphological evaluation of dentin-restoration interface for each tested class.

RESULTS

Results of Nano-hydroxyapatite particles characterizations:

Synthesized Nano-HAp for this study was characterized by SEM, EDX and TEM. The results of the prepared Nano-HAp characterization were illustrated from Figure (1 to 3). Scanning electron

microscope (SEM) photomicrograph showed that the shape of nano-hydroxyapatite particles synthesized for this study were almost rod shape with the same average size. Also, individual fine particles with cuboids and hexagonal shapes were observed (Fig. 1).

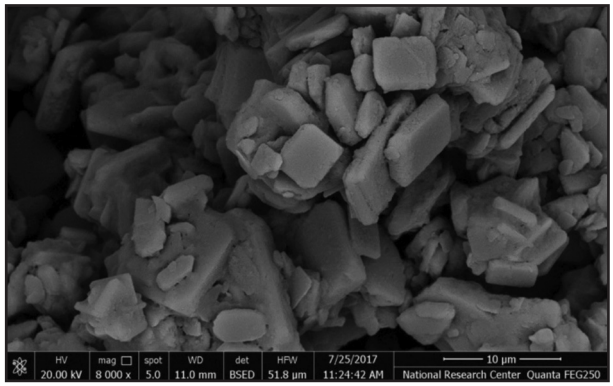

Figure (1): SEM photomicrograph for the prepared Nano-HAp.

The EDX spectra can clearly showed that the prepared Nano-HAp constitutes mainly calcium and phosphate groups. While, the weight percentages of calcium (Ca), phosphorous (P) and oxygen (O) element were found to be 17.46%, 16.03% and 66.3%, respectively and are illustrated in (Fig. 2).

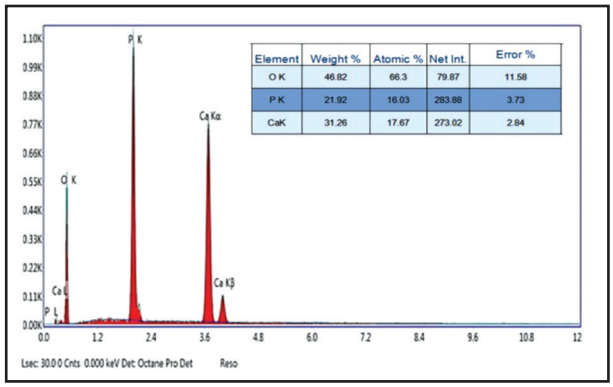

Figure (2): EDX spectra for the prepared Nano-HAp.

Transmission electron microscope (TEM) photomicrograph for the prepared Nano-HAp showed that the morphology of HAp appears in nanostructure form and uniform rod shape. The crystalline size of the prepared Nano-HAp has an estimated crystalline size about (20-80) nm in length and (10-25) nm in width (Fig. 3).

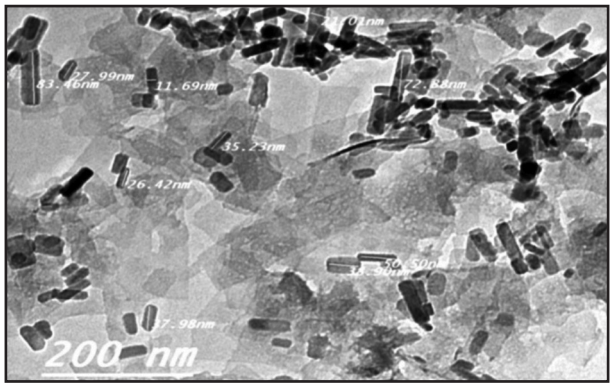

Figure (3): TEM photomicrograph for the prepared Nano-HAp.

Results of Field emission scanning electron microscope (FESEM) observations:

Analysis of the specimens in cross section showed varies morphological characterizations depended upon the type of tested restorative material, tooth substrate (either sound or caries-affected dentin), and aging periods (either 24 hours or 9 months). No differences were observed in the photomicrographs appearance of the restoration-dentin interface between control groups (CGIC and RMGIC) and their modified experimental groups (Nano-HA GIC and Nano-HA RMGIC) (Fig. 4- 11).

FESEM photomicrogrphs at sound dentin- CGIC and Nano-HAp GIC restorations interfaces after 24 hours aging period show gap free junction with intact interface. Small cracks were observed around the fillers within the CGIC martix. Many pores could be also observed within the cement matrix (Fig. 4a& 5a). While FESEM photomicrographs at caries-affected dentin - CGIC and Nano-HA GIC restorations interfaces after 24 hours aging period show small microgaps at restoration-caries affected dentin interface for tested cements. Numerous pores and microcracks could be easily detected around the filler within all the GIC restorations matrix. These microcracks appear to extend from the restorations interface and propagate upwards (Fig. 4b& 5b).

FESEM photomicrogrphs at sound dentin- RMGIC and Nano-HAp RMGIC restorations interface after 24 hours aging period shows a gap free junction and homogenous restorations matrix with no crack formation. A few pores can detected within cement matrix (Figure 6a& 7a). While FESEM photomicrogrph at caries-affected dentin- RMGIC and Nano-HAp RMGIC restorations interface after 24 hours aging period shows a very small gap at restoration- caries-affected dentin interface. No cracks and few porosity can be observed within the homogenous RMGIC matrix (Fig. 6b& 7b).

A large wide gaps extending along the restorations interface with numerous microcracks and pores within the cement matrix were detected in the FESEM photomicrogrph for all caries-affected groups after 9 months aging period (Fig. 8b- 11b).

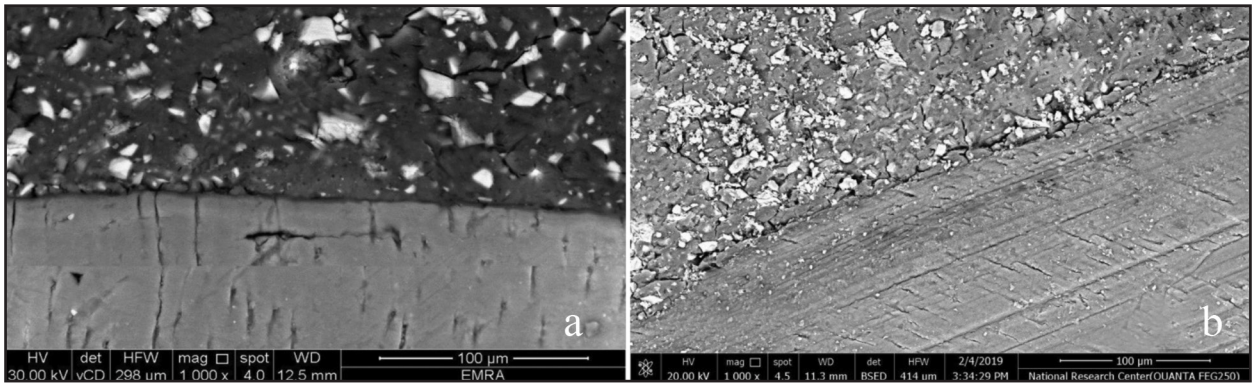

Figure (4): FESEM photomicrographs at dentin _ CGIC restoration interfaces after 24 hours aging period (a) Sound dentin, (b) Caries-affected dentin.

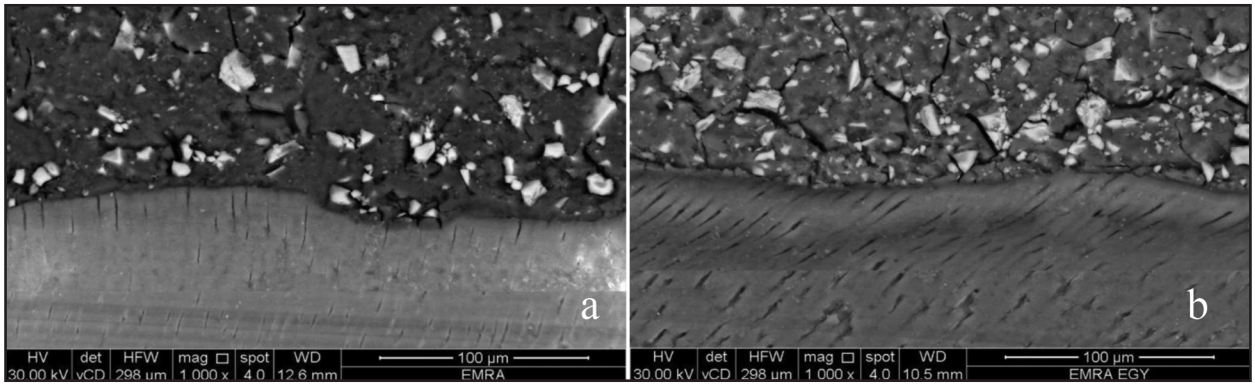

Figure (5): FESEM photomicrographs at dentin _ Nano-HAp GIC restoration interfaces after 24 hours aging period (a) Sound dentin, (b) Caries-affected dentin.

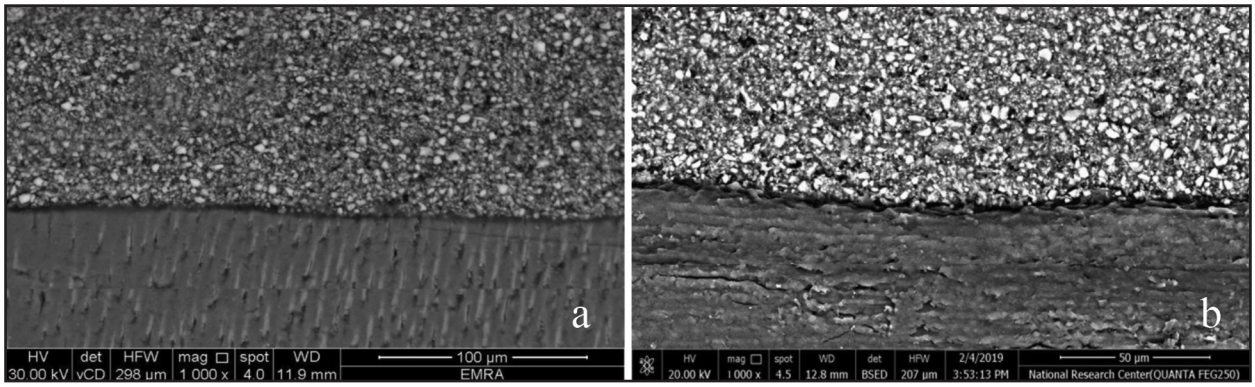

Figure (6): FESEM photomicrographs at dentin _ RMGIC restoration interfaces after 24 hours aging period (a) Sound dentin, (b) Caries-affected dentin.

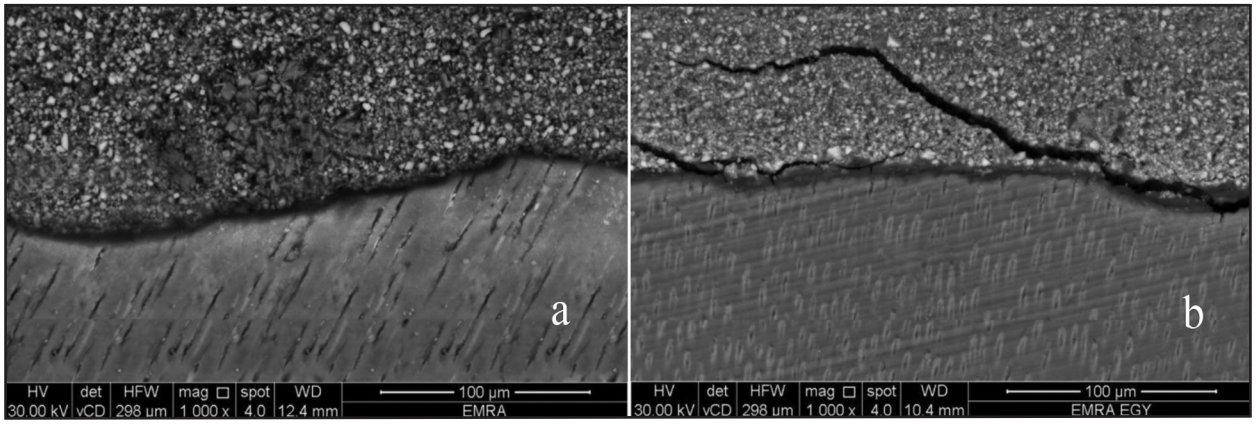

Figure (7): FESEM photomicrographs at dentin _ Nano-HAp RMGIC restoration interfaces after 24 hours aging period (a) Sound dentin, (b) Caries-affected dentin.

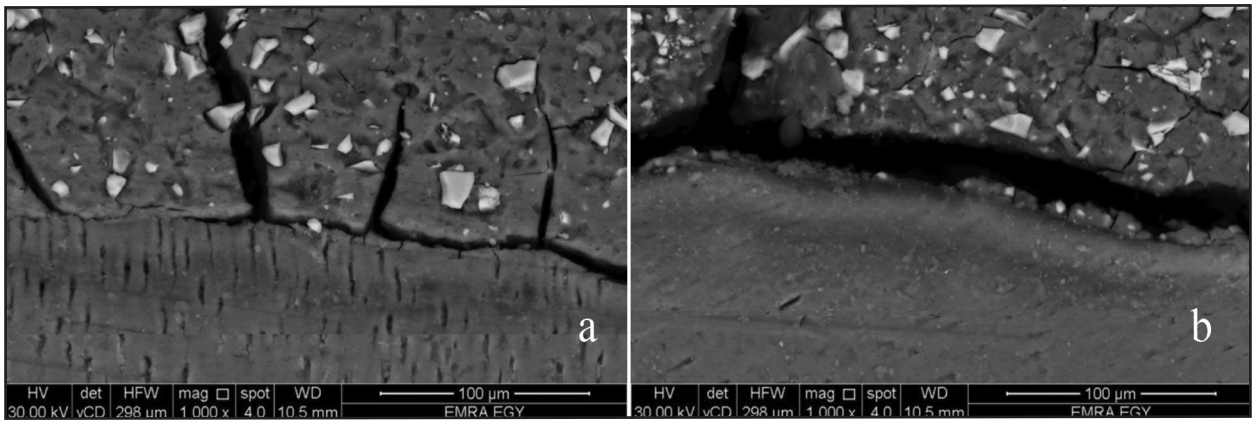

Figure (8): FESEM photomicrographs at dentin _ CGIC restoration interfaces after 9 months aging period (a) Sound dentin, (b) Caries -affected dentin.

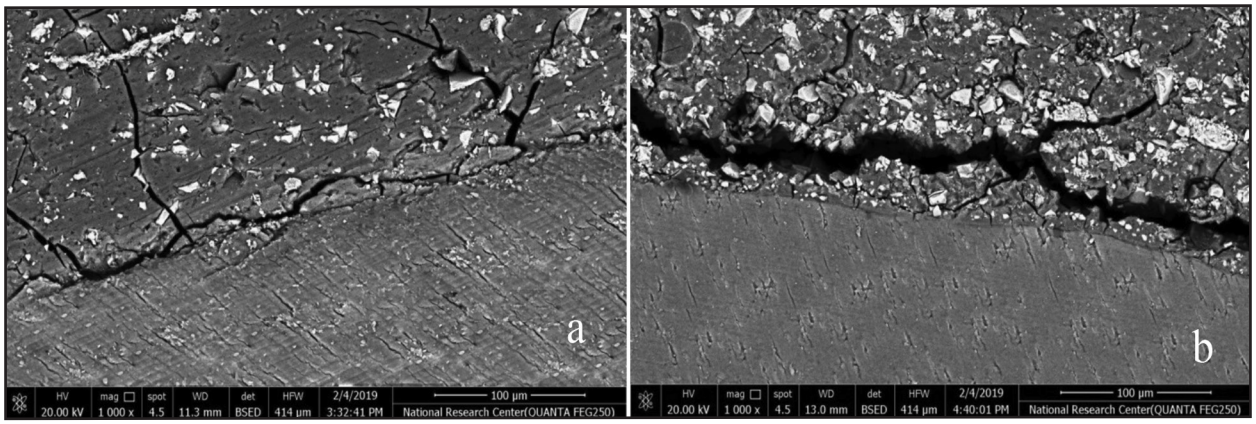

Figure (9): FESEM photomicrographs at dentin _ Nano-HAp GIC restoration interfaces after 9 months aging period (a) Sound dentin, (b) Caries-affected dentin.

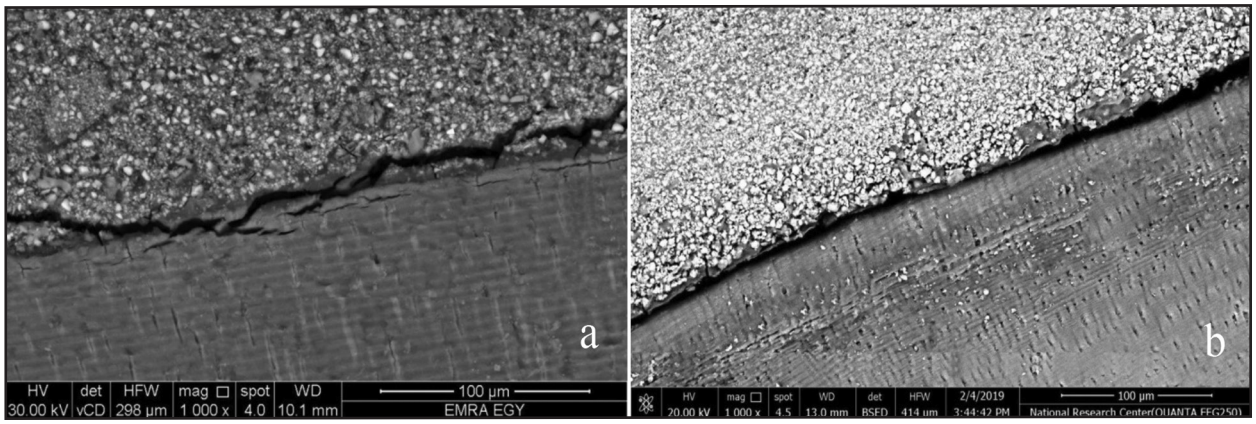

Figure (10): FESEM photomicrographs at dentin _ RMGIC restoration interfaces after 9 months aging period (a) Sound dentin, (b) Caries-affected dentin.

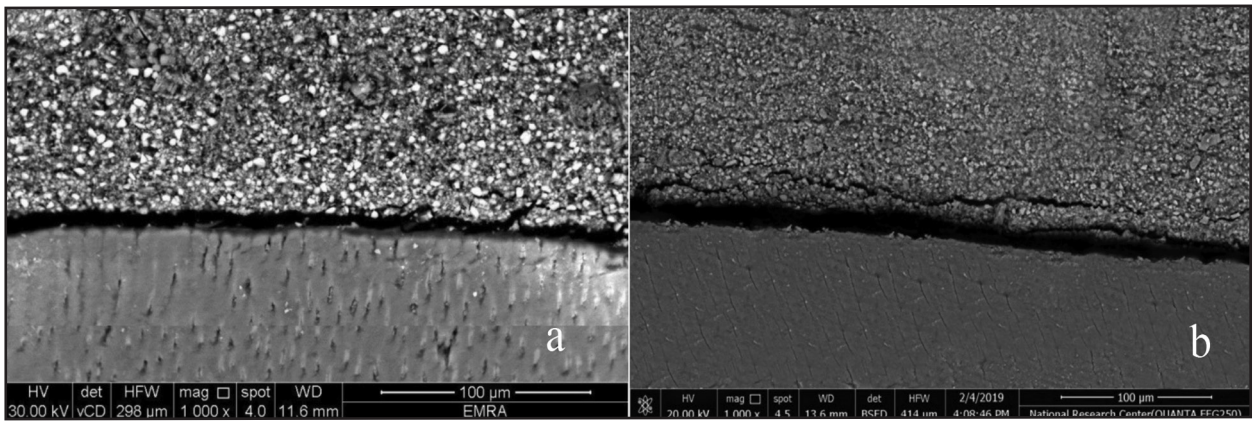

Figure (11): FESEM photomicrographs at dentin _ Nano-HAp RMGIC restoration interfaces after 9 months aging period (a) Sound dentin, (b) Caries-affected dentin.

DISCUSSION

In spite of all advances and modifications evolved for improvement of the poor mechanical properties associated with glass ionomer restorative materials, a significant improvement has not yet been approached. Nanoparticles are recently used as strengthens fillers in dental restorative materials, since they have a noticeable effect on their physical and mechanical properties [17]. However; the information about the effect of these nanoparticles on the ultra-morphological characterization of theses modified glass ionomer cement and resin-modified glass ionomer cement to dentin is rare.

FESEM is considered an advisable method to analyse fine morphological details and provide clues about the bond quality at the interface. In addition; it is a more suitable tool for evaluation of sensitive material as GICs rather than scanning electron microscopy, as it eliminates the need for vacuuming and gold sputter steps which may affect the cement properties [9,18].

The current study was carried out to analyse the interfacial morphological characteristics of CGIC and RMGIC modified with 5 wt% Nano-Hap on sound and caries-affected dentin at two different aging periods either; after 24 hours or 9 months.

The selection for Nano-hydroxyapatite particles "Nano-HAp" as an additive material for both CGIC and RMGIC in current study was based upon its proven biocompatibility, bioactivity, low solubility in water, non-toxicity and for being the main constituent of the mineral part of bone and teeth [19].

Also, nano-scale of hydroxyapatite particles has a high degree of crystallinity and colloidal stability in compared to micro-HAp due to decreased particle size, that lead to increase surface area available for reaction [6].

Nano-Hydroxyapatite particles with controlled size, crystallinity and shape were synthesized through typical "wet-chemical precipitation" method for the present study. This method is one of the most widespread used approaches due to their simplicity, availability and use of relatively inexpensive row materials [20]. Moreover; the powder generated by this method is characterized by being highly pure, with fine homogenous particles [21].

Furthermore; five percent by weight (5% w/w) of CGIC or RMGI powder was replaced by Nano- HAp in this study. The use of such percent was advised and recommended by previous studies [22-24]. As addition of 5 wt% Nano-HAp was claimed to result in a significant improvement in the physical and mechanical properties for the tested glass ionomer cements. Moreover; they found that addition of percentage higher than 5wt%, lead to agglomeration of nanoparticles in the cement matrix which act as weak points, thus result in adverse effect on the bond strength of cement mixture [23].

The result of SEM and TEM photomicrographs for the prepared Nano-HAp (Fig. 1& 3) showed well defined uniform shape nano rod HAp with a length varying from (20-80) nm and diameter varying from (10-25) nm. This considers the optimal form for the nano-hydroxyapatite crystals that provide high physical and chemical properties [24]. However, agglomeration of some Nano-HAp could be observed in SEM and TEM photomicrograph. This observation is commonly detected with Nano-HAp prepared with the wet-chemical participation method, which occur due to the high particle energy associated with small crystal size [25].

The results of FESEM characterization for this studies showed that the interfaces between GIC and RMGIC/dentin is greatly affected by dentin substrate either sound or caries-affected dentin and aging periods either 24 hours or 9 months aging periods. While there was no effect observed from addition of Nano-hydroxyapatite particles to these tested cements on their ultra-morphological characterization under FESEM.

FESEM photomicrographs showed multiple microcracks in all GIC groups extending along the interface and propagate upward through the cement matrix, (Fig. 4 & 8). This may indicate that the interfacial strength of the bond is actually higher than the inherent strength of the materials [9]. Also, many pores could be detected within CGIC matrix, this pores act as a point of stress concentration leading to crack propagation and failure of the dental material. Moreover; these pores can accumulate oral fluid and biofilms with subsequent degradation of the material [26]. On the other hand FESEM photomicrographs for RMGIC showed homogenous restoration matrix with no crack and fewer pores, which may indicate stronger cement matrix. These results were in accordance with other studies[9,18-26].

The ultra-morphological evaluation of dentin-restoration interface in this study showed significant increase in microcracks all over the restoration matrix with microgaps formation at dentin - restoration interface for all caries-affected dentin groups (Fig. 4b to 11b). This may refer to poor adhesion of the tested glass ionomer cements to caries-affected dentin, which may contributed to chemical and morphological alteration that occur in the demineralized caries-affected dentin such as; loss of its organic content, increased porosity of intertubular dentin, dissolution of apatite crystals and degradation of collagen fibrils might negatively impact the performance of the GIC bonding to caries-affected dentin.

On the other hand; ultra-morphological evaluation demonstrated fewer microcracks and gap free junction with intact interface in the sound dentin groups, which indicates good adhesion of the tested

cements to sound dentin, (Fig. 4a to 11a)**.** These findings are in accordance with SEM observation obtained by another previous study [16].

The effect of aging on the tested GIC and RMGIC either control or experimental could be related to deterioration of the surface integrity and enhanced crack propagation within the cements matrices, which act as a weak point with subsequent decrease in the mechanical properties of glass ionomer cements [16]. In addition, aging process has been speculated to cause hydrolytic degradation of collagen fibrils, thereby compromising restorative-dentin bonding interface [28].

CONCLUSION

Under the limitation of this study, it can be concluded that the interfaces between GIC and RMGIC/dentin is greatly affected by dentin substrate (either sound or caries-affected dentin) and aging periods (either 24 hours or 9 months). The unique properties of glass ionomer cements were greatly diminished with bonding to caries-affected dentin and gradually declined with aging process. While there is no additional effect was observed from addition of Nano-hydroxyapatite particles to these tested cements regarding their ultra-morphological characteristics features under FESEM.

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