Research Article

The Effect of carbon quantum dots on the microhardness and the morphological characteristics of human dental enamel: in vitro study

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Abstract: Background: Nanoparticles have been widely used in dentistry due to their numerous physical and biological benefits. Carbon quantum dots (CQD) are used in many biological applications due to their small size and low cytotoxicity. It is expected to improve the surface properties of tooth enamel against acid dissolution when applied at low concentrations. The objective: This study evaluated the effect of CQD solution on the microhardness and surface characteristics of sound and demineralized enamel surfaces. Material and Methods: The solution was prepared with laser ablation and then examined with UV light (325 mm). The distribution, size, and dimensions of particles were analyzed using field emission scanning electron microscopes and transmission electron microscopes. Forty solid teeth (the first upper premolar) were prepared and divided into four groups (n=10). Group 1 (control group), Group 2 (Sound + CQD), Group 3 (Sound + CQD + Demineralization) and Group 3 (Sound+ Demineralization + CQD). The sample was immersed in the test solution for 4 minutes at 37 °C. The enamel microhardness was tested using Vickers microhardness testers, and the morphological characteristics were observed under a scanning electron microscope. Results: The CQD particles appear to be sphere-shaped with diameters of 2-10 nm and blue cyan light. After application of CQDs, the average microhardness value increased significantly ($p<0.001$) compared to the control group. Demineralized enamel (group 4) recorded a higher microhardness ($p=0.006$) than sound enamel (group 3) after CQD application. The treated surface was smooth and homogeneous, with a better crystal structure and closed pores than the untreated surface. Conclusion: The addition of CQDs had a profound effect on the sound surface hardness and demineralized enamel with enhanced morphological properties, which is considered an effective method for the prevention of dental disease.

Keywords: carbon quantum dots, enamel microhardness, FE-SEM, TEM.

Introduction

Enamel is the outer layer of the exposed tooth. It is a strong, thin, and translucent coating of calcified substance that envelopes and protects dentin (1). In addition, enamel is the most mineralized tissue in the human body. The unique mechanical properties of tooth enamel are based on the different shapes and structures of enamel crystals with higher microhardness to increase resistance to fracture and acid degradation (2). Dental caries develops as a result of an imbalance between the demineralization and remineralization process (3), when the rate of demineralization exceeds the rate of remineralization, calcium and phosphate ions diffuse out of the enamel, resulting in a chalky white spot that, if not managed, can develop cavitation(4). Pathological variables can change the balance in the direction of dental caries and disease progression, while protective factors like salivary components, fluoride, together with calcium and phosphate, can enhance the remineralization of demineralised lesions (5).

Nanotechnology, defined as the deliberate manipulation of matter on the molecular and atomic scales, that has been used in various domains of medical and dental utilization (6–9). It uses individual atoms or molecules (nanoparticles) to construct a structure, offering innovative approaches for preventing and treating dental caries [10-12]. It focuses on the process of remineralization in the early stages of dental caries [13]. Carbon Quantum Dots (CQDs), which is a type of nanomaterial that has been introduced into the field of biological studies since 1998 [14]. These zero-dimensional (0D, 10 nm) carbonaceous nanoparticles possess several distinctive characteristics, making them particularly advantageous for various applications due to their biocompatibility, chemical inert, and low cost [15]. Seibert et al. [24] demonstrated that carbon quantum dots (CQDs) exhibited superior mechanical and thermal conductivity qualities compared to graphene nanoplatelets (GNPs) because of their smaller size and better dispersibility. The interfacial bonding with other matrices can enhance load transfer, thereby improving the performance of nanocomposites [25]. They have been used successfully in various biomedical directions with limited dental applications, especially if they can enhance the surface properties of tooth enamel against acid dissolution.

Microhardness is considered an effective tool to obtain indirect information about mineral changes in dental hard tissues [20]. The Vickers microhardness tester is widely implemented in dentistry due to its ability to assess the degree of mineralization and reflect the better crystalline structure in hard dental tissues [21]. The values varied according to the level of mineralization, the alteration of the enamel prisms and tufts in different regions of the tooth enamel, the presence or absence of structural defects [18], the bioenvironmental conditions, the fluoridation of drinking water, the age of the tooth, and the diverse dietary habits in different societies [19].

Research on demineralization and remineralization is directed towards the understanding the intricate processes that lead to the depletion or absorption of mineral ions that affect the speed of ion diffusion and apatite crystal formation. The period of cariogenic challenge and potential healing in the oral cavity is often limited, which differs from most in vitro models. After consuming fermentable carbohydrates, there is a noticeable drop in the pH level of dental plaque, which is then steadily restored to the normal physiological pH level [22].

The overarching objective of this study was to develop an innovative approach to improve the surface properties of tooth enamel to prevent caries development and progression, as it was not evaluated yet. The proposed null hypothesis was that there are no significant differences in the surface microhardness values of sound and demineralised enamel after the application of CQDs.

Materials and Methods

A. Carbon Quantum Dot (CQD) Preparation Procedure

CQDs were prepared using picosecond Nd: YAG pulsed laser (PicoSure LTD, China) ablation of the graphite target (Goodfellow 99.995% purity) immersed in a glass container filled with 2 ml of deionized water. The procedure was followed by Sun et al., 2006 [26]. The setting parameters of the laser are; 1064-532 nm wavelength, 600 mJ (1.2 J/cm^2) pulse energy, one nanosecond pulse duration, 0.2 cm beam diameter, 1000 pulses at 3 Hz repetition rate. The water depth to the graphite to the target was adjusted at 1 cm. The obtained carbon cluster suspension was transferred to another tube (5 mm diameter x 50mm height). The suspension carbon nanostructures were re-irradiated (1000 laser pulses) with 532 nm wavelength (800 mJ laser energy) from the same laser to achieve the quantum size. The colloidal carbon solution obtained was centrifuged at 15,000 rpm for 30 min to separate the uniform sized quantum dots and to remove the large particles and the materials of the by-products to obtain nanoscale carbon particles in aggregates of various sizes [27].

B. Identification and Characterisations of Carbon Quantum Dots

First, the prepared solution was evaluated optically by photoillumination (PL) to identify the fluorescence of the suspension when exposed to ultraviolet light (365 nm). Photoluminescence (PL) was calculated by using an PL/ Lamp (Edinburgh, Germany Instruments, Ltd.).

The Filed Emission Scanning Electron Microscopy (FE-SEM) [28] (AxiaTM ChemiSEMTM Scanning Electron Microscope- Thermo Fisher Scientific Inc. America) was used to examine the distribution and homogenicity. The High-Resolution Transmission Electron Microscope (HR-TEM) [29] was used to identify the size, shape, and configuration of CQDs particles.

C. Teeth preparation and grouping

Forty-three caries-free first premolars extracted for orthodontic purposes were collected using an ethics protocol approved by the health research committee (Ref. No. 564 on April 17, 2022). The selected teeth were thoroughly cleaned with a handpiece and a rubber cup containing nonfluoridated pumice and deionized water, and then examined under a magnifying lens and lighting for the absence of any cracks or defects. The teeth were stored in deionized water with 0.1% thymol crystals to prevent bacterial growth in a refrigerator at 4 °C until beng used (30). A circular window of 6 mm in diameter was exposed on the buccal surface of each tooth, while the rest of the surface was covered with acid-resistant nail varnish. This circular window of each tooth was flattened and polished with 400, 800, 1000, and 1200 grit abrasive paper (China), respectively, to generate a flat surface suitable for microhardness testing [31]. The samples were divided randomly into four groups as follows:

- Group 1: Control sound teeth in deionised water.
- Group 2: Sound teeth treated with CQD suspension solution.
- Group 3: Sound teeth treated with CQD suspension solution and then subjected to demineralisation.
- Group 4: Sound teeth subjected to demineralisation and then treated with CQDs suspension solution.

D. Application of the prepared CQDs suspension

Each sample was immersed individually in a plastic container containing 20 ml of CQD suspension for 4 minutes at 37 ° C, followed by 2 minutes rinsing with deionized water. The treated samples were re-stored in deionised water in an incubator at 37 °C the following day. This procedure was repeated for 7 consecutive days [23].

E. pH Cycling Procedure

The pH cycling procedure was performed using demineralising and remineralizing solutions to activate enamel surface caries lesions following Featherstone et al., 1999 [23]. In the third group, the buccal surfaces were subjected to a pH cycling. In which the teeth were placed individually in 20 ml of demineralising solution (0.075 Mol/L acetic acid, 1.0 mMol/L calcium chloride and 2.0 mMol/L potassium phosphate). The pH was adjusted to 4.3 using a pH metre at 37°C.for 6 hours, followed by 2 min rinising in deionized water. This was followed by dipping 20 ml of remineralizing solution (150 mMol/L potassium chloride, 1.5 mMol/L calcium nitrate and 0.9 mMol/L potassium phosphate). The pH was adjusted to 7, at 37 ° C. for 17 hours at 37 ° C and two minutes of rinsing with deionised water. This process was done once a day for ten days. All samples were examined under a light microscope (×16 magnification power) to assess surface alterations following the acid challenge procedure.

F. The Microhardness Measurements:

The microhardness is measured with a Vickers digital microhardness tester (model HVS-5, LARYEE Technology Co., Ltd., China) loaded with 100 gram for 15 seconds. The Vickers hardness value was the average of three indents on each surface. The degree of remineralization was calculated as a percentage of surface microhardness recovery (%SMHR). Statistical analyses were carried out using the T test and the P value.

Results

Identification of carbon quantum dots:

A. Optical Characterisation and Photoillumination (PL):

The optical representation of CQDs dispersed in water is illustrated in Figure 1 (A,B) under normal and ultraviolet light, respectively. The dispersion of CQDs in water emits cyano-blue fluorescence when exposed to ultraviolet light (365 nm). The final concentration was adjusted to 25 μg/mL.

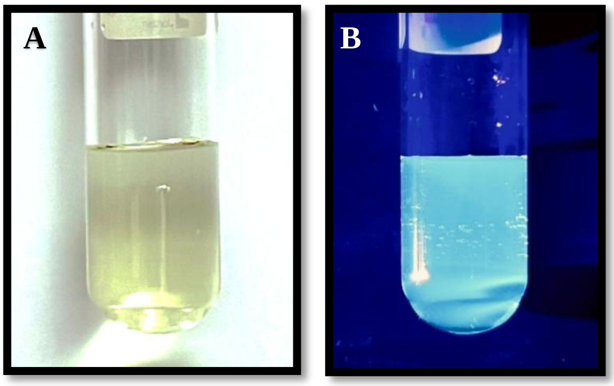

Figure 1: Optical images of C-QD dispersion in water. A. C-QDs under normal daylight exposure, B. C-QDs under the UV exposure (365 nm).

B. Filed Emission Scanning Electron Microscopy (FE-SEM)

The prepared quantum solution showed nonagglomerated particles evenly distributed with very small sizes per each particle. Two different resolutions were used to characterize the quantum dots.

C. Transmission Electron Microscope (TEM):

The size and shape of the CQDs were further identified under transmission electron microscopes (TEM) at low and high resolution , as seen in Figs. 2 A and B, respectively. The CQD particles appeared uniformly in size, with the formation of a hexagonal pattern indicating that they were synthesized in a monodisperse manner. (as seen in Fig. 2 B).

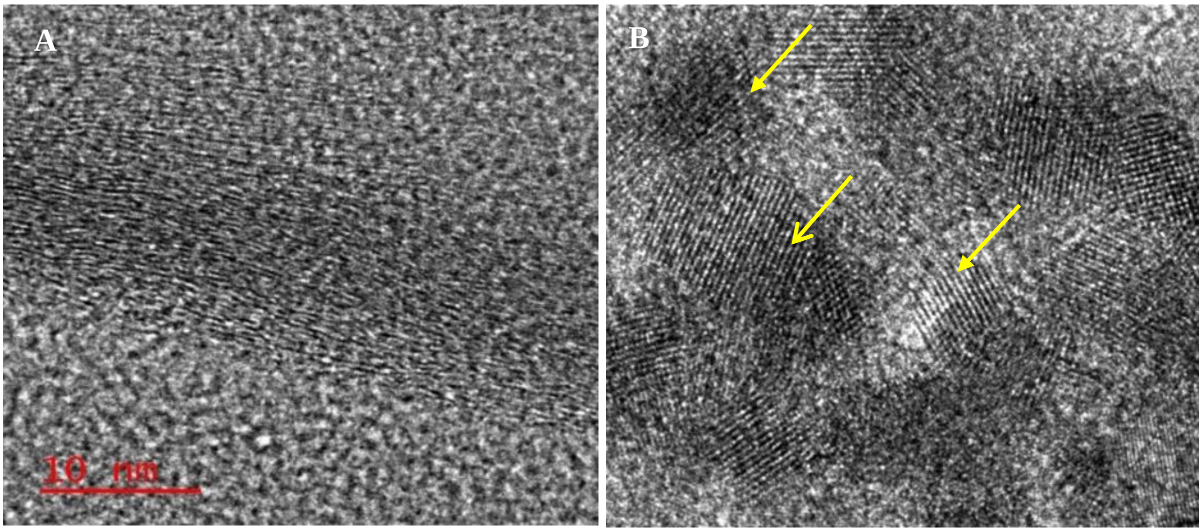

Figure 2: TEM images of CQDs A. at low resolution (10 nm). B. At high-resolution (2 nm) crystalline lattices can be seen.

D. Microhardness

Data were normally distributed when examined by the Shapiro-Wilk test as the p value was > 0.05l.

The one-way ANOVA test (Table 1) showed statistically significant differences in the groups and stages (p=0.00) with higher SMH in the (Sound + CQD) followed by (Sound +DW) and (Sound + CQDs) while lower in the (Sound +CQDs +Demin.) and (Sound +Demin.). This was followed by multiple pairwise comparison (Table 2) using Tukey HSD, which revealed significant differences in all results when comparing each stage with the other, except when comparing (Sound +DW) versus (Sound + Min. + CQDs) that showed no significant differences.

Table 1: One-way ANOVA test of surface microhardness among groups and stages

Groups and Stages	N	Mean	Std. Deviation	Std. Error	Minimum	Maximum	F	P value
Sound +DW	10	296.380	26.013	8.226	253.300	328.000	164.203	0.000 Sig.
Sound+ CQDs	10	369.790	43.413	13.728	302.500	426.300		
Sound+ CQDs + Min.	10	175.220	5.405	1.709	167.000	183.100		
Sound+ Demin	10	118.560	4.704	1.487	112.900	126.800		
Sound+ Demin +CQDs	10	278.700	21.096	6.671	238.000	311.600		

Table 2: Comparison of SMH among stages using Tukey HSD

Comparisons		Mean Difference	P value	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Sound+ DW	**Sound +CQDs**	-73.410	0.000	-104.835	-41.985
	Sound + CQDs + Min.	121.160	0.000	89.735	152.585
	Sound + Min.	177.820	0.000	146.395	209.245
	Sound +Demin.+ CQDS	17.680	0.506	-13.745	49.105
Sound+ CQDs	**Sound + CQDs + Min.**	194.570	0.000	163.145	225.995
	Sound + Min.	251.230	0.000	219.805	282.655
	Sound + Min. +CQDS	91.090	0.000	59.665	122.515
Sound+ CQDs+ Demin.	**Sound +CQDS**	-194.570	0.000	-225.995	-163.145
	Sound + Min.	56.660	0.000	25.235	88.085
	Sound + Min. +CQDS	-103.480	0.000	-134.905	-72.055
Sound+ Demin.+ CQDs	**Sound +CQDS**	-251.230	0.000	-282.655	-219.805
	Sound + CQDs + Min.	-56.660	0.000	-88.085	-25.235
	Sound + Min. +CQDS	-160.140	0.000	-191.565	-128.715

Statistical tests between the compared groups are listed in table 3. Comparisons were made between Control and test groups on sound enamel (groups 1 and 2), demineralization stages (group 3 and 4), and Remieralization stages (group 3 and 4). High statistically significant variations were observed between all compared groups ($P < 0.001$).

Table 3: Statistical tests of Microhardness (MH) Comparisons among groups and Stages

Comparisons	T test	P value	
Control and Test groups (on Sound enamel) **Sound- DW (group 1)** **Sound –CQDs (group 2)**	8.004	0.000	Sig.
Demineralization Stages **CQDs-Demin. (group 3)** **Sound- Demin. (group 4)**	3.109	0.006	
Remineralization Stages **Sound-CQDs (group 3)** **Demin.-CQDs (group 4)**	5.711	0.000	

Morphological assessments of enamel surfaces using FE-SEM:

The sound enamel appeared smooth with normal perikymata arranged in parallel lines with few holes, Fig.3 (A), after demineralization, the enamel surface exhibit irregularities in the prismatic stucture with numerous micropores and cavities observed at the surface, Fig.3 (B). The application of CQD on sound enamel produced a smoother and more homogenous surface with a denser structure and closed pores Fig.3 (B) in comparison to untreated sound enamel Fig.3 (A). When the sound-treated surfaces subjected to pH-cycling (group 3), it showed less surface deformity with fewer cavities and irregularities compared to both untreated sound teeth, Fig. 3 (B). However, the application of CQDs on these demineralised surfaces enhanced the exposed surfaces that damaged bydemineralised process by the precipitation of these nanoparticles that occlude all the micropores giving the smoothest appearance with the least surface irregularities.

Discussion

The results of the present study supported the effectiveness of applying the CQDs nanoparticles to enhance the microhardness and the morphological properties of the sound and demineralised enamel surface, and to increase the resistance of the enamel to acid dissolution. The average Vickers microhardness value of enamal (326.7 ± 3) was close to the values previously reported [32, 33]. After demineralisation of both sound and CQDs-treated teeth (with pH cycling), a white spot lesion was initiated, this was associated with a significant reduction in Vicker microhardness of the enamel surfaces in both groups (Group 3 and 4). This is believed to be logic, as the drop in the pH of the surrounding environment ($pH < 5.5$) dissloutes the mineral deposits of the tooth that move out of the surface, creating micropores with changes in the crystalline structure of the exposed surfaces that resulted in lower microhardness values [34, 35]. This was clearly observed under FE-SEM of the demineralised surfaces that showed irregularities with numerous microspaces, microholes, craters and voids (Figure 3).

The application of CQD on sound enamel (Groups 2 and 3) significantly increased the tissue resistance (p value) compared to non-treated enamel surfaces (Group 1). This may support the ability of these tiny quantum dots to absorb in enamel micropores, thus improving the physical mechanical properties of treated surfaces. This is due to the presence of functional groups in CQDs that interact with various proteins on enamel surfaces and are less likely to clump compared to larger carbon compounds[24].

As there are no other studies testing the effect of CQDs of enamel surfce MH, comparison was not possible, but when relating to nanoparticles in general, This results agreed with Al-Bazaz and Ratih et al., as they concluded that the addition of nanopartilces would improve the MH of the enamel surfce significantly [36, 37].

When CQD was applied on deminerlaized enamel surface (group 4), the tissue hardness increased significantly increased in comparison to the sound enamel surface (group 3), (p-value) with higher recovery of the hardness values. This supports the theraputic effect of these tiny dots on damaged tissues that bonded to the defected enamel better than healthy tissue, as they might incorporate inside the irregularities of the demineralised surface, making a stronger bond and filling the micropores and cavities that were created at the surface, leading to a potential remineralisation effect. The FE-SEM micrograph confirmed these speculations, as the adsorption of these nanoparticles can cover the damaged surfaces that are clearly seen in Fig. (3. D) masking the cracks and flaws that are caused by the demineralisation producing smooth homogenous surface with less surface irregularities Fig. (3. E). This supports the ability of these dots to repair flaws and irregularities that cause smmothering of enamel surfaces.

In group 3, the (CQDs + demineralisation) decline in mean values of microhardness was higher when compared to (Sound + demineralization) in group 4. This may be due to the precipitation and incorporation of CQDs being weakly bonded in sound enamel, or because the adsorption of CQDs is not permanent and it can be easily disturbed and removed after demineralisation.

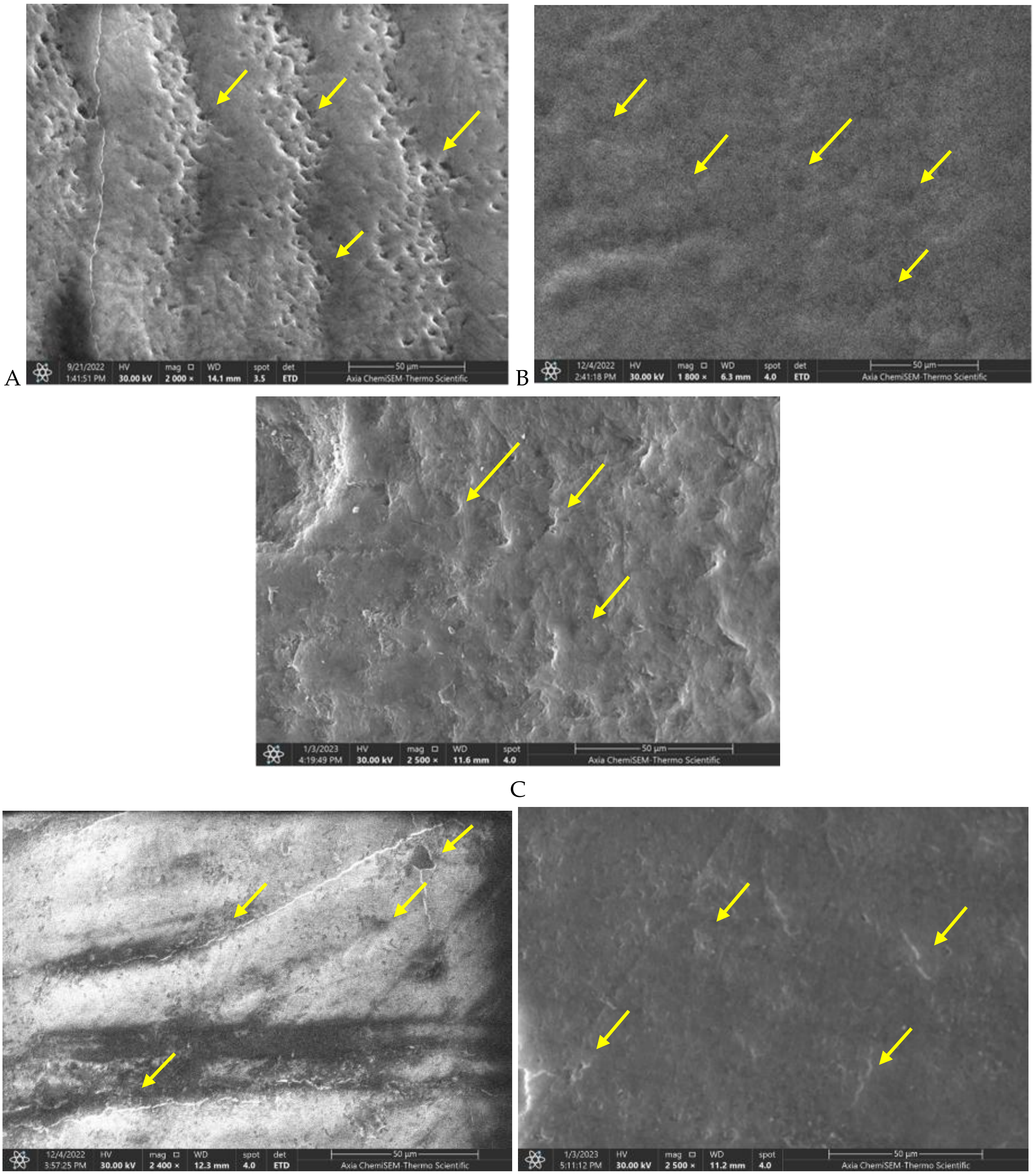

Figure (3): FE-SE micrographs showing a sound enamel surface

A- Sound teeth –**Group 1**: normal enamel pores, B- CQDs treated teeth- **Group 2:** CQDs diffused over the enamel surface with smoother appearance and occluded micropores),

C- CQDs + demineralised –**Group 3:** defected enamel surfce with few irregularities, pores and no cracks. D- demineralised enamel surface (cracks and surface defects) E- demineralized+ CQDs- **Group 4:** CQDs diffused over the demineralised surfcae covering the cracks and major surface defects, giving smoothed appearance with less prominent micropores.

Limitations: To better mimic clinical conditions, a pH cycle model was used. However, in vitro study might not reproduce the results of an in vivo study. Furthermore, the effect of oral factors such as saliva and dental plaque on enamel remineralization could not be considered. In addition, time periods of demineralisation and remineralisation are much faster than those expected to occur in vivo. The lack of studies designed to investigate the effects of the carbon quantum dots suspension solution leads to restriction compared to those of other studies. Comparisons are not always logical because of the possibility that different synthetic sources and procedures of the tested agent are used, leading to a different composition and actions that may produce different results.

Conclusion

The application of CQDs to the surface of the tooth enamel improved the surface microhardness on both sound and demineralised enamel surfaces, with the highest surface microhardness recovery recorded in the demineralised enamel surfaces. The mean values and the FE-SEM showed that the application of CQDs to demineralised enamel surfaces gave the best results by repairing the most found defects, with more homogeneous FE-SEM images and denser mineral content compared to other groups .This treatment could be considered in preventive dental care to prevent dental caries and strengthening the enamel microhardness of demineralised surfaces.

Ethical approval

According to the Helsinki Declaration [38] and its guiding principles, Ethics approval was carried out in the Department of Pediatric and Preventive Dentistry of the College of Dentistry, University of Baghdad, after receiving that approval (Ref. No. 564 on April 17, 2022).

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' contributions

ATM; study conception and design of the study. ISA; data collection. ATM, AKA, and SEE; Methodology. ATM and ISA; statistical analysis and interpretation of results. ISA; original manuscript preparation. ATM, AKA and SEE; review & editing. Supervision;ATM and AKA. All authors reviewed the results and approved the final version of the manuscript to be published.

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تأثير نقاط الكم الكربونية على الصلابة الدقيقة والخصائص السطحية الدقيقة لمينا الأسنان البشرية -دراسة مخبرية

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المستخلص:

الخلفية: تم تطبيق الجسيمات النانوية على نطاق واسع في طب الأسنان بسبب مزاياها الفيزيائية والكيميائية والبيولوجية. تم استخدام جزيئات الكربون النانوية ذات النقاط الكمومية في التطبيقات البيولوجية لبنيتها الصغيرة الحجم ذات السمية المنخفضة. ومن المتوقع أن تعزز الخصائص السطحية لمينا الأسنان ضد الاحماض عند تطبيقه بتركيزات منخفضة .الأهداف: قامت هذه الدراسة بتقييم تأثير محلول نقاط الكم الكربونية على الصلابة الدقيقة والخصائص السطحية لأسطح المينا السليمة والمنزوعة المعادن. المواد والطرق تم تحضير المحلول بطريقة تنازلية باستخدام الاستئصال بالليزر تم فحص المحلول بواسطة الأشعة فوق البنفسجية 325 مم باستخدام الإضاءة الزرقاء السماوية، في حين تم رؤية توزيع الجسيمات وحجمها وأبعادها تحت المجهر الإلكتروني الماسح للانبعاثات الميدانية (FE-SEM) والمجهر الإلكتروني النافذ (TEM) تم تحضير أربعين سناً سليماً الضواحك العلوية الأولى وتقسيمها إلى أربع مجموعات العدد 10 ؛ كألتالي؛ المجموعة 1 المجموعة الضابطة ، المجموعة 2 السليمة نقاط الكم الكربوني)، المجموعة 3 السليمة نقاط الكم الكربوني إزالة المعادن والمجموعة 3 السليمة إزالة المعادن نقاط الكم الكربونية تم غمر العينات في المحلول المحضر لمدة 4 دقائق عند 37 درجة مئوية بعد ذلك، تم اختبار الصلابة الدقيقة باستخدام جهاز اختبار الصلابة الدقيقة فيكرز، في حين تم عرض السمات المورفولوجية تحت المجهر الإلكتروني الماسح بالانبعاث الميداني النتائج ظهرت جزيئات نقاط الكمية الكربونية كروية الشكل بقطر 2 10 نانومتر كانت هناك زيادة (p<0.001) في متوسط قيمة الصلابة الدقيقة للمينا السليمة بعد تطبيق (CQDs) المجموعة 2 مقارنة بالمجموعة السليمة غير المعالجة المجموعة 1 سجلت المينا منزوعة المعادن المجموعة 4 أعلى صلابة مقارنة p 0.006 مع المينا السليمة المجموعة 3 بعد تطبيق CQDs .بدت الأسطح المعالجة ناعمة ومتجانسة مع بنية بلورية أفضل ومسام مغلقة مقارنة بالمجموعة السليمة غير المعالجة والأسطح المنزوعة المعادن عند فحصها تحت FE-SEM. الاستنتاج إن إضافة نقاط الكم الكربونية كان لها تأثيرعلى الصلابة الدقيقة السطحية للمينا السليمة والمينا منزوعة المعادن مع تحسين الخصائص المورفولوجية والتي تعتبر وسيلة فعالة في الوقاية من تسوس الأسنان