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# CARIES-AFFECTED DENTIN REMINERALIZATION USING NATURAL MARINE-BASED GELS

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### *ABSTRACT*

This study evaluated the mineral content of remineralized caries-affected dentin (CAD) using nano coral calcium versus nano coral calcium with chitosan and their effect on microshear bond strength (μSBS) to composite resin in relation to sound dentin. 50 teeth were randomly divided in which 20 dentin specimens were used for EDX evaluation; another 20 were qualitatively analyzed using FESEM. For the remaining 10, each tooth was cut into four quadrants obtaining 40 dentin specimens for μSBS evaluation. All dentin surfaces were subjected to four different surface treatments; group 1: sound dentin, group 2: CAD, group 3: demineralized then nano coral calcium, group 4: demineralized then nano coral calcium with chitosan. EDX showed increase of Ca and P (wt%), Ca/P ratio in two remineralizing materials when compared to CAD, meanwhile close to that of sound dentin. Furthermore, μSBS showed the highest value with nano coral calcium followed by sound dentin, then nano coral calcium with chitosan and the lowest was CAD. FESEM confirmed these results. Nano coral calcium and nano coral calcium with chitosan showed promising results regarding the remineralization of CAD improving its μSBS to resin composite. Coral reefs, natural calcium sources, have positive impact in solving CAD clinical problem.

**KEYWORDS:** Bond strength, caries-affected dentin, chitosan, coral calcium, remineralization

## INTRODUCTION

Caries-affected dentin (CAD) produces lower bond strength and poor quality of the hybrid layer than normal dentin. The exposure of the adhesive interface of caries-affected dentin to oral environment will compromise the longevity of the composite restoration which might be due to hydrolysis of the resin and collagen fibrils. This is attributed to lower mineral content, a deeper demineralized zone in CAD, changes in morphological and other chemical characteristics of mineralized tissues [1,2].

Ocean provides a diverse array of natural products that capture scientists' great attention due to their significant and extremely potent biological activities.

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In addition to being rich sources for pharmaceutical drugs and marine natural products [3]. Coral calcium, created from fossilized animal remnants and coral reefs, is a calcium carbonate natural material with many other minerals and provides a natural source of calcium [4]. Moreover, its benefits range from correcting calcium deficiencies and strengthening tooth enamel to preventing against bone loss and osteoporosis [5]. Chitosan is a safe compound to use with many positive properties for applications in oral surgery and restorative dentistry, including remineralization that hardens the tooth's tissues, and its antimicrobial effect in caries prevention [6].

Since CAD is uninfected, partially demineralized and physiologically remineralizable, therefore should be preserved during clinical treatment. Thus, the improvement of bonding potential to CAD through the implementation of nano-sized marine-derived minerals may be safer as they have a natural origin. Subsequently, this could lead to reinforcement of tooth-composite restoration complex, protecting from secondary caries and tooth fracture [1].

The aim of this study is to answer the following participant, intervention, comparator, and outcome (PICO) questions: Does remineralized caries-affected dentin using nano coral calcium without and with chitosan have similar mineral content as sound dentin? Is the microshear bond strength of the remineralized CAD similar to that of sound dentin?

## MATERIAL AND METHODS

### Teeth Selection

A total of 50 caries free permanent third molars were collected under the protocol approved by the local Ethics Committee (REC.Dent.MSA 1/2022). Teeth were stored in thymol solution of 0.025% and used within 3 months after extraction. Any teeth with cracks, caries or restoration were excluded from the study.

### Specimens Grouping and Preparation

All 50 teeth were sectioned 2 mm below the cemento-enamel junction (CEJ) separating the roots. The mesial wall was then removed, followed by removing the occlusal surface, 0.5 mm beyond the dentino-enamel junction (DEJ) using Isomet 4000 micro-saw double sided (Isomet 4000, Buehler, USA) under copious amount of water to expose flat dentin surface. 20 specimens of which, were stored in distilled water for Energy Dispersive X-ray Spectroscopy (EDX) evaluation. Meanwhile, another 20 specimens that were qualitatively evaluated using the field emission scanning electron microscopy (FESEM), were cut 2 mm beyond the flat dentin surface to obtain dentin discs (2 mm thick) and stored in distilled water till usage.

The remaining 10 dentin specimens that were used for the microshear bond strength testing ($\mu$SBS), each tooth was sectioned into four quadrants perpendicular to the occlusal surface of the tooth, in a Mesio-Distal direction and Bucco-Lingual direction. In which, a pencil and a graph paper were used to mark the middle part in both directions. It was then cut using the microtome under copious amount of water. Finally, the four quadrants were separated using cutting disc, giving four quadrants of each tooth, obtaining 40 dentin specimens, they were then mounted on self-curing acrylic resin blocks. All four quadrants of each tooth were stored together in distilled water till usage.

The dentin surface was subjected to four different surface treatments:

Group 1: Sound dentin (Positive control)

Group 2: CAD (Artificially demineralized dentin, Negative control)

Group 3: Demineralized then nano coral calcium (Remineralized CAD)

Group 4: Demineralized then nano coral calcium with chitosan (Remineralized CAD)

**Artificial Dentin Caries Induction (CAD)**

Artificial dentin carious lesion was introduced in the specimens using demineralizing solution contained 2.2 mM Calcium chloride, 2.2 mM Monosodium phosphate, 0.05 M acetic acid, the pH was adjusted with 1 M Potassium hydroxide to 4.4. Specimens were placed in the demineralizing solution for 72 hours and changed every 24 hours.

**Preparation Method and Characterizations of the Remineralizing materials**

***Nano coral calcium:***

The dried coral calcium powder was milled by ball mill machine (planetary-ball-mill-pm-400) for 10 h, speed 350 rpm and 3 min intervals. The coral calcium nano powder (spherical-like shape and of average size less than 50 nm) was dispersed in distilled water by 20% w/v with stirring and then gradually adding poly ethylene oxide (MW = 60,000) 4% w/v to the suspension to produce nano coral calcium in gel form. TEM was performed on JEOL JEM-2100 high resolution transmission electron microscope at an accelerating voltage of 200 kV showing coral calcium nanoparticles (Figure 1).

***Nano coral calcium with chitosan:***

Chitosan nanoparticles (spherical-like shape and of average size 40 nm) were prepared according to the ionotropic gelation process [7]. Blank nanoparticles were obtained upon the addition of a tripolyphosphate (TPP) aqueous solution to a chitosan solution, then the formed slurry was washed several times and dried by lypholization process. The coral calcium nano powder 20% w/w was dispersed in distilled water that contained nano chitosan 2% w/v by 20% w/v with stirring and then gradually adding poly ethylene oxide (MW = 60,000) 4% w/v to the suspension to produce chitosan nanoparticles – nano coral calcium in gel form.

**Application of the Remineralizing materials**

Nano coral calcium and Nano coral calcium with chitosan were applied to demineralized dentin specimens in groups 3 and 4 respectively, after storage in distilled water for 24 hours to remove acid remnants. The remineralizing gel was applied in 2-3 coats on the hydrated flat dentin specimens using microbrush, which was displaced every 5 specimens. Agitation was done for 20-30 seconds, then left undisturbed for 3 minutes and rinsed gently with water spray for 2-3 seconds till removal of all the remnants. Specimens were then stored in distilled water till usage.

**Energy dispersive X-ray spectroscopy (EDX) Evaluation**

20 dentin specimens were used for the EDX evaluation (EDAX GENESIS, USA), were divided according to the two remineralizing materials (nano

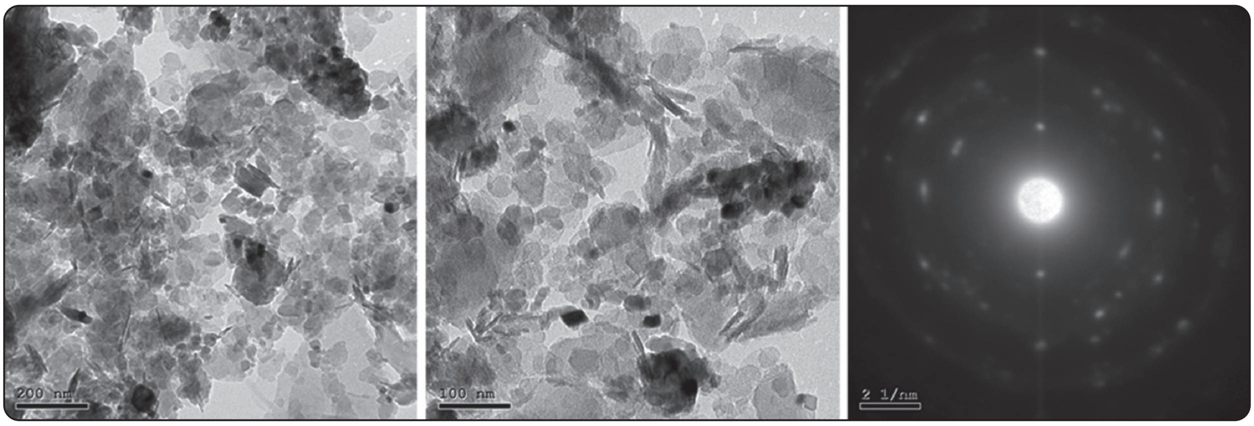


Fig. (1) TEM images showing coral calcium nanoparticles coral calcium and nano coral calcium with chitosan) having n=10 for each material. Each specimen, was evaluated three times, as sound dentin specimens were firstly evaluated, followed by the induction of artificial dentin caries (CAD) and, finally after the application of the remineralizing materials. Quantitative analysis was done to evaluate the Ca and P composition.

## Field Emission Scanning Electron Microscopy Evaluation

20 dentin specimens were divided into four groups according to the surface treatment where (n= 5) and they were imaged using high resolution scanning electron microscopy (SEM Quanta FEG 250 with field emission gun, FEI company, Netherlands) with a beam of 20 kV to observe the surface morphology and evaluate the occlusion status and integrity of the dentinal tubules.

## Microshear Bond Strength Evaluation

### *Bonding procedure*

For standardization, the same tooth was divided into 4 groups according to the surface treatment used. All 40 dentin specimens were bonded using the universal adhesive in self-etch mode (ALL-BOND UNIVERSAL- BISCO) (Table 1), which was applied according to the manufacturer instructions. It was applied in 2 consecutive coats using microbrush, which was displaced every 5 specimens. Agitation was done for 10-15 seconds per coat, then air thinned for 10 seconds using oil-free air syringe, as to allow the solvent evaporation and light cured using RTA mini-S light cure unit, 1000 mW/cm$^2$-1200 mW/cm$^2$ (Guilin Woodpecker Medical Instrument) for 10 seconds.

### *Resin composite application*

A catheter with an internal diameter of 0.9 mm was used, as well as, a lancet and endometer to obtain micro-cylinders of 0.9 mm internal diameter and 2 mm height. Prior light curing of the bonding agent, the micro-cylinders were placed on the dentin surface and then light cured. Nanohybrid composite (Table 1) was packed in the micro-cylinders using periodontal probe and then light cured for 20 seconds. Composite micro-cylinders were then obtained, which were 0.9 mm in diameter and 2 mm height. Specimens were then stored in distilled water for 24 hours to allow the maturation of the bond then the catheter was removed using a scalpel before evaluation.

TABLE (1) Materials used in this study

| Product | Ingredients | Manufacturer |
|---|---|---|
| ALL-BOND UNIVERSAL (Universal Adhesive) | Hydroxyethyl methacrylate, Bis-GMA, Phosphate esters (10-MDP), Ethanol, Water, Initiator, pH 3.2 | BISCO, Schaumburg, USA |
| Filtek Z350 XT Universal (Nanohybrid resin composite) | Matrix: Bis-GMA, UDMA, Bis-EMA, PEGDMA and TEGDMA resins. Fillers: combination of non-agglomerated/non-aggregated 20 nm silica filler, non-agglomerated/non-aggregated 4 to 11 nm zirconia filler, and aggregated zirconia/silica cluster filler (comprised of 20 nm silica and 4 to 11 nm zirconia particles). The inorganic filler loading is about 78.5 wt%. | 3M ESPE, Saint Paul, USA |
| Coral Calcium | Calcium (as Coral Calcium 1100 mg) 400 mg, Magnesium (as magnesium Oxide 200 mg, Vitamin D (as Cholecalciferol D-3 200 IU, Cellulose, Titanium Dioxide and gelatine, derived from fossilized coral. | General Nutrition Corporation Pittsburgh, PA 15222 |

### *Microshear bond strength*

After 24 hours each acrylic block containing the dentin specimen and the bonded composite micro-cylinder was mounted in the universal testing machine (Instron model 3345, England). Forces were applied to the bonded composite interface at a cross head speed of 1mm/min until fracture. Data was calculated and recorded using computer software (BlueHill universal Instron, England).

### Statistical analysis

Data was entered and statistically analyzed on the Statistical Package of Social Science Software program, version 25 (IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). Data was presented using mean and standard deviation for quantitative variables. Comparison between groups was conducted using Mann Whitney test for unpaired data, while comparison of paired data was performed through Friedman test followed by post-hoc Wilcoxon test. *P*-values less than or equal to 0.05 were considered statistically significant.

## RESULTS

### EDX

The change in Ca (wt%), P (wt%), Ca/P ratio of sound dentin, nano coral calcium and nano coral calcium with chitosan were significantly higher than CAD. The Ca (wt%) and P (wt%) of nano coral calcium and nano coral calcium with chitosan were close to sound dentin. In comparison to sound dentin, Ca/P ratio was significantly higher in nano coral calcium, and non-significantly in nano coral calcium with chitosan as shown in Table 2 and Figure 2. Both materials showed degree of improvement and increase of minerals. The percent of change for Ca (wt%) and P (wt%) in nano coral calcium with chitosan were non-significantly higher than nano coral calcium. The % of change of Ca/P ratio of both ma-

TABLE (2) Changes of minerals (Ca and P wt%, Ca/P ratio) within each group separately

| Group | Mineral content | Sound | CAD | Remineralized | *P*-value |
|---|---|---|---|---|---|
| Nano coral calcium | Ca | 27.72 ± 1.87 | 15.3 ± 2.44 | 25.85 ± 2.48 | A vs B=0.000<br>A vs C=0.109<br>B vs C=0.000 |
| | P | 13.06 ± 0.85 | 7.42 ± 1.07 | 11.91 ± 1.08 | A vs B=0.000<br>A vs C=0.036<br>B vs C=0.000 |
| | Ca/P | 2.12 ± 0.01 | 2.06 ± 0.04 | 2.17 ± 0.03 | A vs B=0.001<br>A vs C=0.001<br>B vs C=0.000 |
| Nano coral calcium with chitosan | Ca | 27.85 ± 2.33 | 14.64 ± 3.9 | 24.58 ± 3.36 | A vs B=0.000<br>A vs C=0.020<br>B vs C=0.000 |
| | P | 13.09 ± 1.04 | 7.1 ± 1.69 | 11.44 ± 1.52 | A vs B=0.000<br>A vs C=0.009<br>B vs C=0.000 |
| | Ca/P | 2.13 ± 0.03 | 2.05 ± 0.07 | 2.15 ± 0.04 | A vs B=0.009<br>A vs C=0.207<br>B vs C=0.001 |

***A: Sound, B: CAD, C: Remineralized, vs: versus***

terials were minimal, which is an important indicator of remineralization process is accompanied by constant increase in the ratio of calcium to phosphorus as shown in Table 3 and Figure 3.

### Microshear bond strength test (μSBS)

Nano coral calcium showed the highest mean μSBS followed by sound dentin then nano coral calcium with chitosan and the least was CAD as shown in Table 4 and Figure 4. In comparison to CAD, the mean μSBS of sound dentin, nano coral calcium and nano coral calcium with chitosan were significantly higher ($p$=0.002, 0.002, 0.011 respectively). There were non-significant differences for the two materials in comparison to sound dentin ($p$=1.000). The mean μSBS between two materials was non-significant ($p$=1.000).

### Field Emission Scanning electron microscopy evaluation (FESEM)

FESEM images of representative samples of sound dentin, CAD, nano coral calcium and nano coral calcium with chitosan at 10,000x magnification, with additional magnifications for two remineralizing materials at 5000x, 30,000x are presented in Figure 5. Sound dentin group showed complete closure of dentinal tubules and presence of minerals (Figure 5 A). CAD group showed a frank and wide opening of the dentinal tubules (Figure 5 B), which confirmed the demineralization of dentin sample. In remineralizing materials' groups, mineral deposition on the dentin surface was observed. Regarding the nano coral calcium group, obvious closure of the tubules by mineral depositions was observed, with different degrees of tubule occlusion being registered (Figure 5 C,D) and confirmed at higher magnification 30,000x (Figure 5 E) . In nano coral calcium with chitosan, most of the tubules were partially occluded meanwhile, other tubules showed mineral deposition intra and peritubular (Figure 5 F,G) which was obvious at higher magnification 30,000x (Figure 5 H).

TABLE (3) Comparison between the two remineralizing materials regarding the percent of change (Ca, P and Ca/P ratio)

| % change | Nano coral calcium | Nano coral calcium with chitosan | *P*-value |
|---|---|---|---|
| **Ca** | 71.53 ± 22.94 | 74.3 ± 32 | 0.826 |
| **P** | 62.68 ± 20.09 | 66.13 ± 29.88 | 0.765 |
| **Ca/P** | 5.34 ± 1.75 | 4.83 ± 3.29 | 0.670 |

TABLE (4) Means and standard deviations of Microshear bond strength test in the four groups (MPa)

| Group | Mean (±SD) |
|---|---|
| **Sound dentin** | 14.48 ± 3.24 |
| **CAD** | 7.39 ± 3.3 |
| **Nano coral calcium** | 14.84 ± 5.69 |
| **Nano coral calcium with chitosan** | 11.88 ± 5.24 |

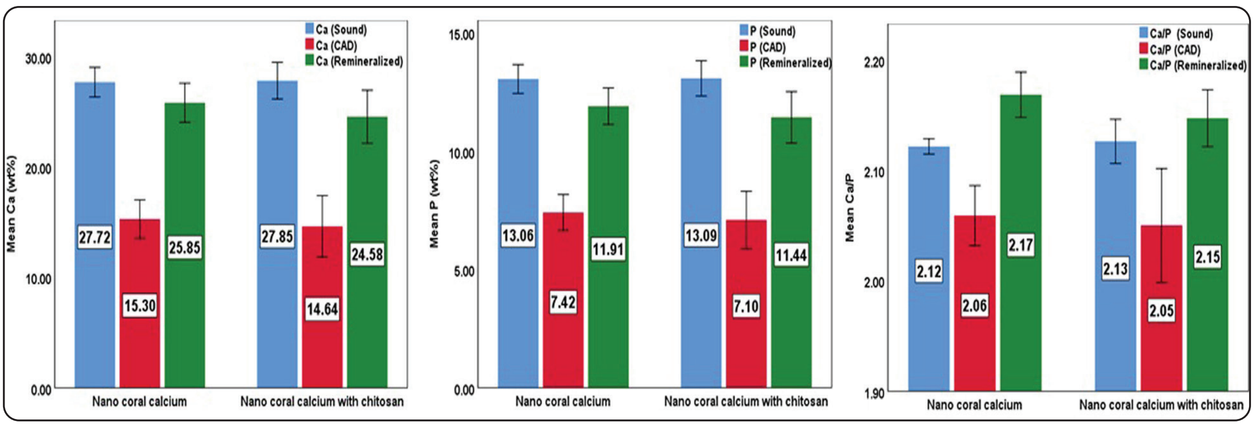


Fig. (2) EDX results showing mineral change of sound dentin, CAD, remineralizing materials

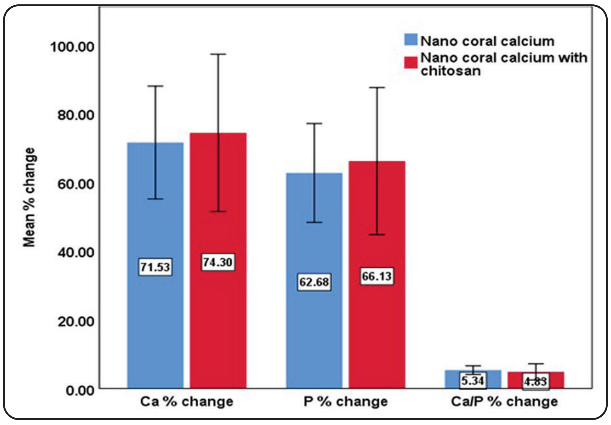


Fig. (3) Mean % change of Ca, P and Ca/P ratio in the two remineralizing materials

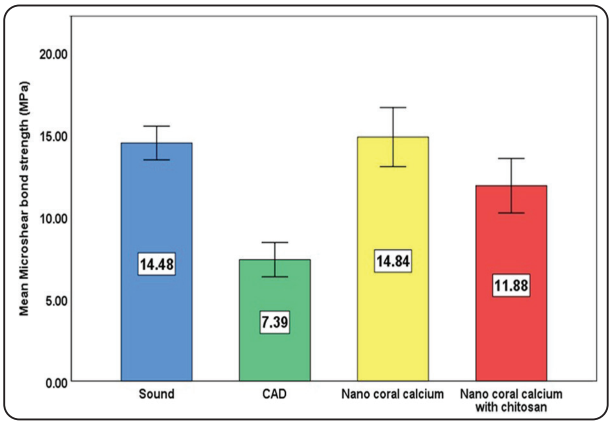


Fig. (4) Mean Microshear bond strength of sound dentin, CAD and remineralizing materials

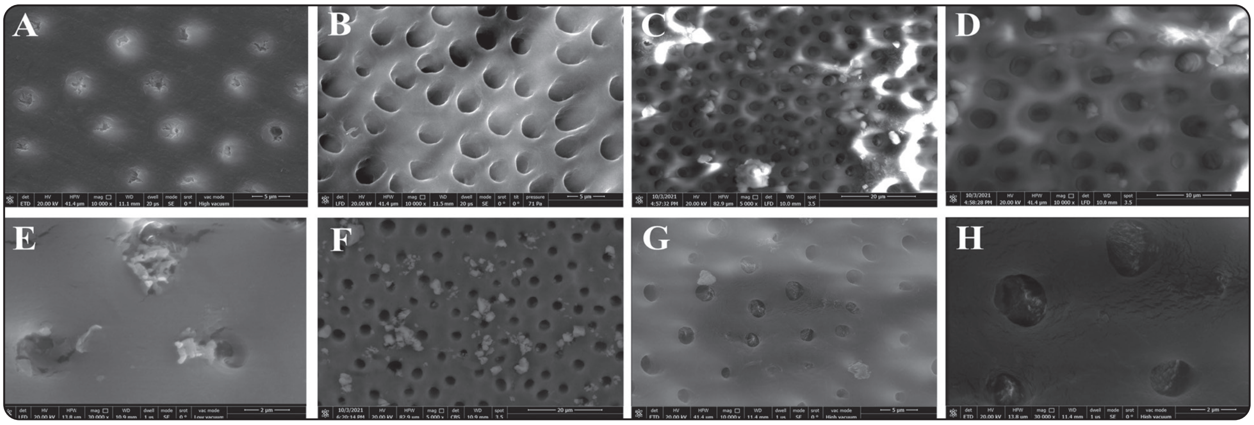


Fig. (5) FESEM photomicrograph at 10,000x magnification of (A) Sound dentin, (B) CAD, (D) nano coral calcium and (G) nano coral calcium with chitosan with additional magnifications for remineralizing materials at 5000x (C: nano coral calcium, F: nano coral calcium with chitosan); 30,000x (E: nano coral calcium, H: nano coral calcium with chitosan)

## DISCUSSION

Performance and durability of resin composite bonding is highly dependent on the structural and morphological changes of the dental substrate. Dentin is a more complex structure than enamel, therefore reaching a long-term bonding durability is highly dependent on the bonding mechanism, as well as the procedure that is carried out. Although there are multiple efforts to minimize and simplify the difficulties facing clinicians, still remains the weakest point in the bonding, is dealing with the dentin substrate [2]. Meanwhile, the sound dentin shows a higher bond strength than the caries-affected dentin, but nowadays with the more conservative and minimally invasive concept focuses on the limitation of the caries removal, maybe it is still debatable, but in most of the cases, caries is excavated leaving behind caries-affected dentin which creates a situation of dealing with different types of dentin in bonding procedure when using resin composite restorations, leaving the clinicians to rely on their own clinical judgement and decisions in choosing the best approach to deal with this situation [8]. Therefore, the caries-affected dentin is known to be a frequent substrate in bonding procedure, which possess morphological and ultrastructural changes as loss of mineral content, high porosity of inter-tubular dentin, dissolution of apatite crystals, which may negatively affect the bond performance and durability [2].

The biomimetic remineralization mimics the natural biomineralization by replacing the demineralized collagen matrix water content with apatite crystallites. Caries-affected dentin contains about 14-53% water when compared to sound dentin, therefore by replacing water with minerals at the dentin-resin interface, this would increase the mechanical properties and inhibit water-related hydrolysis. Many researchers demonstrated that the use of remineralizing agents on the caries-affected dentin may recover its mechanical properties as NaF, CPP-ACP, as well as nanohydroxyapatite [1,9]. Nanoparticles are considered to be much smaller in size than micro-meter scale, which leads to changing in the properties of any material as hardness, chemical reactivity, active surface area and biological activity. Therefore, in the present study coral calcium was used in the form of nanoparticles, as coral calcium when analyzed it was found that the coral is composed of high $CaCO_3$ (Aragonite) compound, $MgSiO_3$, FeSi and other minerals [4,10]. Its main component helps in remineralization of bone as in osteoporosis as well as teeth, as studied by Abdelnabi et al. [5] in the remineralization of enamel, when compared with nanohydroxyapatite, it showed equivalent amount of mineral deposition. In the present study, nano coral calcium was evaluated for the remineralization of the caries-affected dentin, as well as nano coral calcium with chitosan. Chitosan has been recently evolved as a biomaterial with biocompatibility and complete biodegradability which enhance remineralization, as it is rich in calcium and phosphate, which has an affinity to calcium ions due to its abundance to the carboxyl groups in it, as was described by Santoso et al. [11] and Xu et al. [12].

Elemental analysis of the dentin specimens in each group was conducted using EDX for evaluation of Ca, P and Ca/P ratio which are an indication for the demineralization and/or remineralization. Demineralization of dentin specimens was done by storing them in the demineralizing solution as described by Lo et al. [13]. Furthermore, Isolan et al. [2] concluded from their meta-analysis review that regardless of the induction methods of the carious dentin types the results were the same in all analyses [14, 15]. Meanwhile, Zhao et al. [16] emphasized from their study that the natural caries-affected dentin is more complex than the induced CAD in its microstructure, as the dentinal tubules in the natural caries-affected dentin are occluded by acid resistant minerals, which may reduce the permeability of the dentin, affecting the efficacy of the remineralization. In the present study, Ca (wt%), P (wt%) and Ca/P ratio were significantly decreased in the CAD group when compared with the sound dentin, indicating demineralization. The nano coral calcium showed increase in the Ca (wt%) and P (wt%) which were close to that of the sound dentin, with insignificant difference for Ca and significant difference for P. However, the Ca/P ratio of the nano coral calcium was significantly higher than that of the sound dentin. The nano coral calcium with chitosan showed significant difference of the Ca (wt%) and P (wt%) when compared with sound dentin, meanwhile the Ca/P ratio was higher than the sound dentin, but with insignificant difference. These results were in agreement with Xu et al. [12] who suggested that coating and cross-linking P-Chitosan on the surface of dentin showed better deposition and adsorption of calcium and phosphate ions, explaining this by that there will be more negatively charged surface of the collagen and lower interfacial free energy, which enhanced the deposition of calcium and phosphate on the partially demineralized dentin, although in the current study the nano coral calcium and the nano coral calcium with chitosan showed the same results in remineralization, that the presence of chitosan did not make a difference in the calcium and phosphorous weight percent. On comparing both remineralizing materials with the CAD, they showed higher Ca (wt%), P (wt%) and Ca/P ratio with significant difference. The elemental composition results confirmed that dentin remineralization using nano coral calcium yielded equivalent results with the nano coral calcium with chitosan.

Regarding the nano coral calcium, dentinal tubules showed different degrees of occlusion when observed under the magnification of 5000x and 10,000x, while on higher magnification of 30,000x it showed deposition of the nanoparticles of the material inside the dentinal tubules. On observing the nano coral calcium with chitosan under FESEM, it showed deposition on the surface of the dentin specimens at magnification of 5000x, meanwhile on higher magnification of 10,000x and 30,000x it showed partial closure of some of the dentinal tubules as well as mineral deposition inside the dentinal tubules and the peritubular dentin. These qualitative analyses are in correlation with the EDX results in degree of improvement in mineral increase.

There is tremendous change in the adhesive system to improve its performance and durability of the material, as well as the bond with different types of dentine. The complex interfacial layer of the dentin-adhesive is still one of the major problems of adhesion strategy. Universal adhesive All-Bond was used in this study, as it is more user friendly, in which the self-etch mode allows simultaneous demineralization and infiltration of the resin monomer into the dentin surface, which prevent incomplete infiltration of the adhesive within the collagen network. Furthermore, it contains 10-MDP which is a functional monomer that ionically bond with calcium in hydroxyapatite providing efficient chemical bond with dentin [17]. The Nanohybrid resin composite was used in the current study, as it shows low polymerization shrinkage, durability, ease of handling and superior esthetic performance with nano-sized filler particles and higher filler loading [18]. Microshear bond strength is a test which uses specimens with smaller dimensions, which allows testing in smaller areas, preparing multiple specimens from the same tooth, which allows standardization, therefore in the present study the same tooth was cut into four quadrants allowing the four groups to be applied on the same tooth (sound dentin, CAD, remineralized with nano coral calcium and remineralized with nano coral calcium with chitosan).

Regarding the microshear bond strength results, revealed that the nano coral calcium had the highest $\mu$SBS followed by sound dentin, then the nano coral calcium with chitosan and the lowest $\mu$SBS mean value was the CAD group. On comparing sound dentin with nano coral calcium and nano coral calcium with chitosan, there was no significant difference. Also, the two remineralizing materials showed insignificant difference in the $\mu$SBS results. Furthermore, there was significant difference between the CAD group and the other 3 groups. These results might be due to the replacement of excess water present in the CAD as studied by Barbosa-Martins et al. [9], with minerals deposited from the nano coral calcium and nano coral calcium with chitosan, which as well increases the mechanical properties. These results were agreed upon with the EDX evaluation of the present study and observed under the FESEM, showing deposition of minerals which might act as receptors for chemical bonding with the 10-MDP present in the ALL Bond universal adhesive enhancing the bond strength of resin composite which was in accordance with Zhou et al. [17]. These results were in agreement with Rabee et al. [19], Alagha [20] who found that the remineralization of the CAD improved the shear bond strength of dentin, meanwhile they used fluorohydroxyapatite and nanohydroxyapatite, also Kamozaki et al. [21] study was in agreement with the present study, but with using CPP-ACP as a remineralizing material. Cao et al. [22]; Rahiotis and Vougiouklakis [23] assumed that the calcium and phosphate ions from the remineralizing materials diffuse in the porous lesion and deposit in the partially demineralized crystals, building the hydroxyapatite crystals, as well as Yoshida et al. [24] results were in agreement with the results of the current study. Meanwhile, Ghallab et al. [25] results were in contradiction to the results of the present study, as they accepted that the remineralized caries-affected dentin showed lower bond strength than the caries-affected dentin, explaining their results that the CPP-ACPF obliterated the dentinal tubules preventing the bond complete penetration. Meanwhile in the current study the remineralizing materials used were nano-sized which allowed better penetration inside the dentinal tubules without complete obliteration as confirmed with the FESEM results.

Results in the present study that showed CAD with lowest $\mu$SBS mean value of all groups, were in agreement with Nakajima et al. [1,26], who demonstrated that the dentin type has a significant effect on the bond strength due to the chemical and morphological characteristics of the CAD. They explained from their study that the hybrid layer is thicker, but with wide region of non-encapsulated collagen, they concluded that the bond strength of the CAD was lower than sound dentin using self-etch adhesive system. In which, their study indicated that there were exposed collagen in the bottom of the thick hybrid layer formed with the CAD, as well as that most dentinal tubules were occluded with acid-resistant mineral crystals which interfered with monomer infiltration and resin tag formation. They explained that the zone found is more porous and weaker, which might lead to organic matrix degradation by time.

Therefore, within the limitation of the present study in lack of the dynamic biological complex, results emphasized that the treatment of the caries-affected dentin with marine-based remineralizing materials showed promising effect in the remineralization. Also, having a positive effect on the microshear bond strength with universal adhesive with the self-etch mode. As well as, the protocol of the application of the remineralizing materials used in the present study could be considered applicable in the clinical use. Further in vivo studies to evaluate their remineralization potential and bond performance are recommended.

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