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RESEARCH ARTICLE

MICROTENSILE BOND STRENGTH OF TTEMA/TEGDMA REMINERALIZING ADHESIVE TO EARLY CARIOUS ENAMEL LESIONS

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ABSTRACT

Objectives: Resin adhesives could be used as a carrier of nano-hydroxy apatite particles added to treat early enamel caries. The aim of this study was to evaluate micro tensile bond strength of a remineralizing enamel infiltrant to early carious enamel lesion. **Methods:** An artificial caries model was used to induce early partially-cavitated enamel caries-like lesion in maxillary centrals. Nano hydroxyapatite particles (nHA) were applied in two forms to a low shrinkage low viscosity resin adhesive (TTEMA/TEGDMA): particles were directly added to resin adhesive or silanated before addition. Unmodified adhesive was used as control. Micro-tensile bond strength (MTBS) was performed to evaluate bond strength to demineralized enamel after application of the remineralizing resin adhesive (n=20, =0.05). Scanning electron microscopy (SEM) was performed to assess mode of failure and to study infiltrant-enamel interface. **Results:** Direct addition of nano-particles resulted in significant reduction (F=32, P<0.001) in MTBS (19 MPa ± 3.8) compared to addition of silanated particles (24 MPa ± 3.2) which presented comparable values to the unmodified enamel infiltrant (26 MPa ± 4.1). SEM image analysis revealed adhesive failure observed for directly added particles while the other two groups demonstrated cohesive failure observed in the resin adhesive. On the other hand, direct addition of nHA resulted in partial remineralization of enamel (42% recovery in calcium content). **Conclusion:** TTEMA/TEGDMA enamel infiltrant could be used as a carrier for nano hydroxyapatite particles without compromising bond strength to early carious enamel lesions.

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INTRODUCTION

Remineralization of early carious enamel (ECE) and dentine focused on using different forms of calcium ions as a source of ion exchange across the carious surface (Abou Neel *et al.*, 2016). Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) is one of the most successful forms which gained lots of attention in remineralization efforts especially in white spot lesions of enamel. Calcium in an amorphous form is easily transported across porous carious tooth structure and can readily participate in re-crystallization of ion deficient hypomineralized enamel (Manoharan *et al.*, 2018). Different forms of Casein phosphopeptide-amorphous calcium phosphate are currently available in the market. However, application of CPP-ACP has a negative effect on bond strength to treated enamel (Shyam *et al.*, 2017). Another approach in treating ECE is sealing the porous enamel lesion using low viscosity resins known as enamel infiltrants (Arora *et al.*, 2019).

Infiltration of non cavitated enamel lesion using different forms of infiltrants have significantly reduced white spot appearance and protected enamel against further demineralization (Eckstein *et al.*, 2015; Kumar *et al.*, 2017). Increasing penetration depth of enamel infiltrants indicated higher efficiency in conservatively treating these lesions (Kielbassa, 2017). Several attempts focused on loading enamel infiltrants with different forms of remineralizing agents and studied their penetration depth. Addition of fillers of different sizes and concentrations had little effect on the penetration depth of modified enamel infiltrants (Askar, 2015). Synthetic nano hydroxyapatite (nHA) is considered one of the most biocompatible and bioactive materials, and has gained wide acceptance in medicine and dentistry in recent years. It mimics hydroxyapatite crystals in morphology and structure and arrested mineral loss in vitro. Recently several reports have shown that synthetic nano-hydroxyapatite has some potential in repair and in remineralization of early carious lesions in both enamel and dentin (Juntavee, 2018; Philip, 2019). When added to a tooth paste, synthetic nano hydroxyapatite increased enamel hardness indicating successful ion exchange (Aziznezhad, 2017).

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Several trials focused on addition of nHA to enamel infiltrants in order to improve its anti-cariogenic properties (Andrade Neto, 2016; Bajaj, 2016). If white spots are part of a larger lesion, restoration of cavitated teeth would require a liable bond strength to resin composite. Thus the effect of enamel infiltrant on bond strength was a point worth investigating (Lopez Lopez, 2019). The effect to demineralization/remineralization of bond strength to enamel was previously evaluated (Farias de Lacerda, 2016; Memarpour, 2019). Infiltration of demineralized enamel prior to establishing enamel bond improved bond strength and durability (Gelani, 2014; Mohamed *et al.*, 2018). However, in order to improve penetration of the infiltrant, surface pretreatments as application of hydrochloric or phosphoric acids are used causing substantial further loss of an already weakened enamel (Yim, 2014; Zhou, 2017).

Using a low viscosity resin as Tris[4-(2'-hydroxy-3'-methacryloyloxypropoxy)phenyl] methane diluted with triethyleneglycol dimethacrylate (TTEMA/TEGDMA) could enhance penetration of ECE without the need to rely on a separate etching procedure (Colak, 2016). Loading this infiltrant with a remineralizing medium could enhance ion exchange across resin-enamel interface. The aim of this study was to evaluate remineralization potential of a low viscosity enamel infiltrant enriched with nano hydroxy apatite particles directly added to the resin adhesive or silanated before their addition to improve hydrolytic stability (Pratap, 2019), and its effect on microtensile bond strength to resin composite. The null hypothesis of this study was that there will be no difference in MTBS sin between the unsilanated and the silanated groups.

MATERIALS AND METHODS

60 extracted teeth were collected and demineralized in-vitro to produce early enamel caries-like lesions. Produced lesions were characterized by scanning electron microscopy and cross-sectional hardness testing; Nano-Hydroxyapatite particles were partially hydrolyzed and added to a low viscosity resin before and after silanation to create three groups for the study: group I using nonsilanated nHA (direct addition), group II using silanated nHA particles and unmodified adhesive (group III) with $n=20$ for each group ($N=60$). Subsequently composite blocks were bonded to the enamel surface and microtensile bond strength was evaluated.

Production of caries-like lesions: 60 extracted caries free human maxillary central incisors were selected (20 teeth for each group tested $n=20$). Teeth were sectioned at cemento-enamel junction level using diamond disc mounted on a precision cutter (Micracut 1.5, Metkon, Turkey). The cut crowns were covered with 2 layers of acid resistant varnish leaving only 4x4 mm facial window. Demineralizing solution was prepared according to Ten Cate (ten Cate, 2015). Briefly the solution was composed of 50 mM acetic acid + 2.2 mM Ca (NO₃)₂.2H₂O + 2.2 mM KH₂PO₄ + 0.1 ppm NaF. The pH of the solution was adjusted to 4.2 using small amounts of NaOH. The acidity of the solution was measured daily by pH meter (Jenway 3020 pH meter, UK) and was kept between 4.2 and 4.25 by adding refreshing amounts of HCL 30% solution. Teeth were immersed in the demineralizing solution until the center part of the window demonstrated a visibly porous enamel lesion.

Cross-sectional microhardness test: 60 specimens were randomly selected and were embedded in acrylic resin to protect the specimens from any surface damage. The specimens were longitudinally sectioned using a diamond disc mounted in a precision cutter (Micracut 1.5, Metkon, Turkey) under water cooling. Each section was gradually polished using silicon carbide paper ascending grits, 800, 1000, 1200, and 2000 to remove any surface damage during sectioning then each block was ultrasonically cleaned in 90% ethanol. Cross-section micro-hardness was performed using a Vickers indentation point (Wilbert Wilson, Germany) using 200 g weight applied for 30 seconds. Measurements were taken at from the surface of enamel to a maximum depth of 500 μ m at 25 μ m steps. Hardness values of sound enamel were measured and used as a control. This test was repeated after 3 months of water storage to measure the remineralization potential of each group tested.

Assessment of morphological changes and calcium loss: All Carious enamel specimens obtained were gold-sputter coated (Fine coat jfc-1100E, Jeol, Munich, Germany) and examined using scanning electron microscope (JSM-5300, Jeol, Munich, Germany). Calcium and phosphorus contents were evaluated from the surface of carious enamel to a maximum depth of 300 μ m at 25 μ m steps using Energy-dispersive X-ray spectroscopy (EDX, JSM-5300) unit and average spot size of 1 μ m.

Preparation of nano-hydroxyapatite infiltrant (nHA): Nano hydroxyapatite particles were prepared by sol gel chemical reaction. Particle size and distribution were evaluated using particle size analyzer (Mastersizer 3000E, Grovewood, UK) and particles size in range of 40-60 nanometers was selected. The surface of the artificially created white spot was etched with a mixture of 2% chlorhexidine and 10% sodium hypochlorite for 2 min. The teeth were finally washed and dried. Low viscosity TTEMA/TEGDMA resin infiltration was prepared by first preparing Tris[4-(2'-hydroxy-3'-methacryloyloxypropoxy)phenyl] methane (TTEMA) by mixing triphenylolmethane triglycidyl ether (TTE) with methacrylic acid (MA) in the presence of 4-(dimethylamino)pyridine. 50% TEGDMA was added to the mixture with 0.1% photo initiator (Ferro, 2019). The particles were partially hydrolyzed using 10% phosphoric acid and 10%wt nHA powder was either directly mixed with the prepared infiltrant or coated with a silane coupling agent (Silane coupling agent, 3M ESPE, St Paul, MN, USA) before addition to the resin infiltrant. A thin coat of was applied using a microbrush and light polymerized using 2500 mW LED device (Bluephase, Ivoclar vivadent, Liechtenstein). A layer 3 mm thick of resin composite (Filteck z350, 3M ESPE, USA) was added and polymerized on the demineralized facial surface. To evaluate calcium uptake, some specimens were stored under demineralized water for 3 months before calcium content was evaluated as previously discussed.

Microtensile bond strength test (MTBS): Microbars (1x1x5 mm) were prepared by cutting bonded specimens using precision cutting machine (Isomet 1000, Buehler, Lake Bluff, Ill, USA). At least 25 microbars were obtained from each specimen. The microbars were examined under stereomicroscope (SZ,Olympus;Tokyo,Japan) and only structurally intact, crack-free bars were selected. All specimens were loaded to failure using universal testing machine (Instron 6022, Instron Limited, High Wycombe, UK) at constant crosshead speed of 0.5 mm/min.

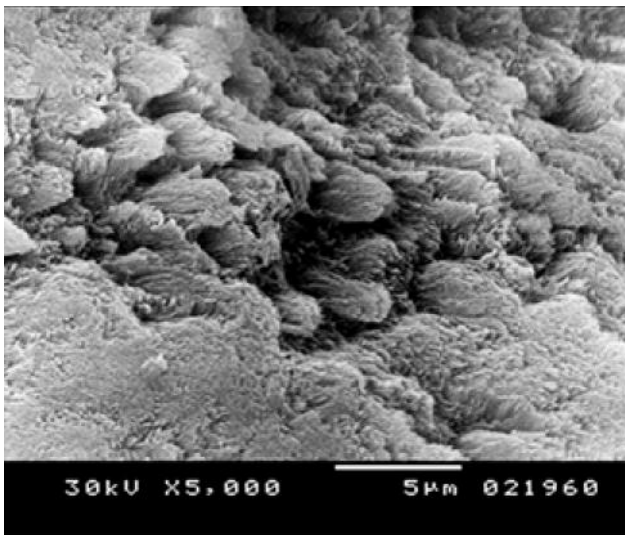


Figure 1. A. SEM image, 5000x, and showing demineralization of artificially induced white spot demonstrating partial loss of surface minerals

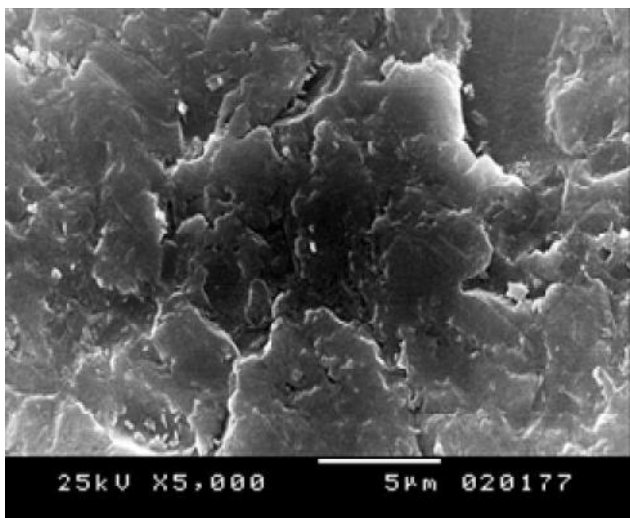


Figure 1. B. SEM image, 5000x, and showing all enamel porosities sealed after application of the infiltrants

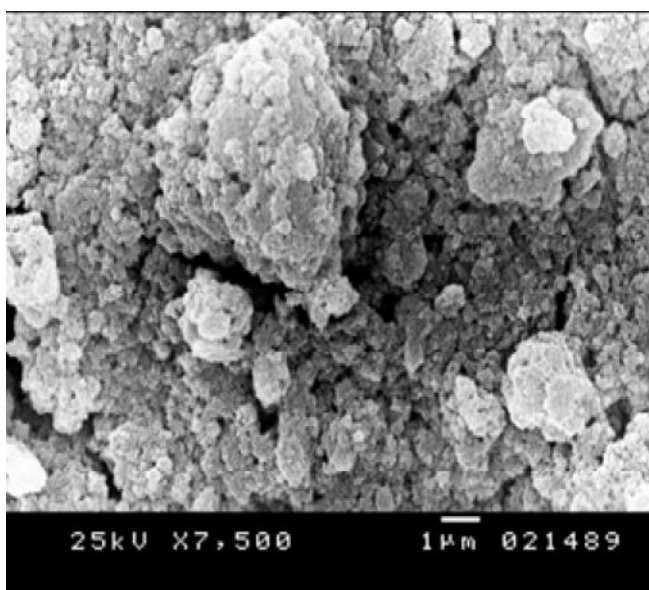


Figure 2. A. SEM image, 10000x, demonstrating surface appearance of enamel after

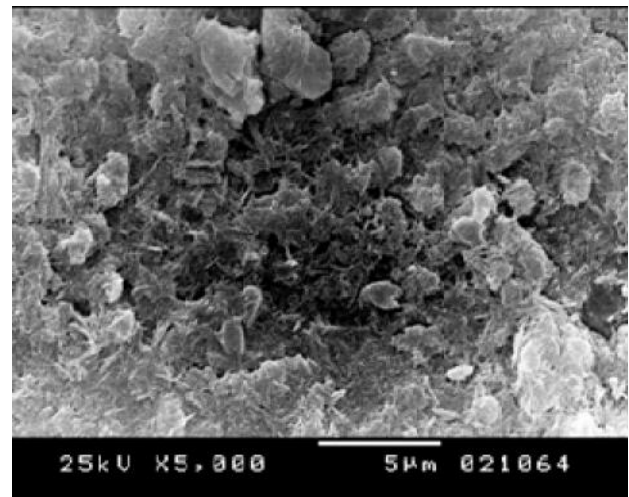


Figure 2. B , 2-C. SEM image, 5000x, showing dispersion of unsilanized hydroxyapatite enriched infiltrant

Fractured microbars were examined under stereo-microscope to detect failure mode, which was classified as adhesive, cohesive, or mixed failure (Yang, 2018).

Scanning electron microscopy (SEM): Intact sections were collected during sectioning procedure as described previously. The sections were polished using silicon carbide paper (800, 1000, and 1200 grit). The sections were ultrasonically cleaned in demineralized water for 15 min and dried at 60°C for 60 min. Sections were gold sputter coated and prepared for SEM examination (XL 30, Phillips, Eindhoven, the Netherlands) (Manoharan, 2018).

Statistical analysis: Levene's test of equality of error variances was performed to test the null hypothesis that error variance in MTBS was similar in tested groups. One way analysis of variance (ANOVA) was selected to analyze the data with 1 within-group factor (method of application of nHA). Bonferroni post hoc test was selected for pair-wise comparisons ($\alpha = .05$, $n=20$). Data were analyzed using computer software (SPSS 14.0; SPSS, Inc, Chicago, Ill).

RESULTS

Cross sectional hardness values showed significant differences between demineralized enamel lesions and sound enamel ($F=35$, $P<.008$). Mean lesion depth was $377.1 \pm 47.5 \mu\text{m}$ after which hardness values reached a constant plateau equivalent to sound enamel. Mean hardness value of demineralized enamel was 202.6 ± 19 HVN compared to 380.3 ± 13 HVN of sound enamel. Scanning electron microscopy revealed characteristic inter-rod dissolution and exposure of enamel rod peripheries resulting in increasing apparent surface roughness, Figure 1-A. After application of the infiltrants, all enamel porosities were sealed, Figure 1-B. EDX demonstrated that demineralized enamel had a significant reduction of calcium and phosphorous content compared to sound enamel ($F=33$, $P<.005$). There was a marked reduction of 54% wt of both calcium and phosphorous content within the observed lesion. Calcium and phosphorous content reached normal values beyond the observable mean lesion depth. After 3 months of water storage, 42% recovery of calcium content was observed for the directly added nano-particles group. This was associated with increase of enamel microhardness to 255 VHN.

Silanated particles failed to produce similar results as calcium content was not changed after water storage. There were significant statistical differences ($F=32$, $P<0.001$) in MTBS values between the tested groups. Direct addition of hydroxyapatite particles reduced MTBS values ($19 \text{ MPa} \pm 3.8$) compared to non-modified adhesive ($26 \text{ MPa} \pm 4.1$). On the contrary application of silanated nano particles did not influence MTBS values ($24 \text{ MPa} \pm 3.2$) compared to the unmodified adhesive. SEM of fractured microbars obtained from different groups showed that direct addition of nHA resulted in agglomeration of the particles on the surface of demineralized enamel which resulted in adhesive failure of this test group, Figure 2-A. On the other hand, using silanated particles did not result in creation of structural defects on the surface of demineralized enamel and remained properly distributed in the resin adhesive. This group was associated with entirely cohesive failure located in resin cement, Figure 2-B and C.

DISCUSSION

The selected demineralization model used in this study produced surface features similar to those observed in early enamel carious lesions as the characteristic selective demineralization pattern observed in white spots observed clinically, Figure 1-A, where the inter-rod enamel region was the most affected [26]. Cross section micro-hardness test revealed a mean lesion depth of $377.1 \pm 47.5 \mu\text{m}$ similar to that reported by Vongsavan *et al* 2016 who studied microhardness of bovine enamel with artificial caries-like lesions. and Mohammadi *et al* 2018 studied resistance of primary teeth enamel to demineralization (Vongsavan, 2016; Mohammadi, 2018). This may be attributed to the lower ($\text{pH}<3.4$) used in this study compared to the previously mentioned studies. Demineralized enamel lesions lost almost 46.9% of its hardness compared to sound enamel with mean lesion hardness of 202.6HV, these results coincide with those reported in the study by Eskelsen *et al.* (2018) where the enamel hardness significantly decreased after treatment causing micromorphology alterations (Eskelsen, 2018).

Regarding bond strength, direct addition of unsilanized nHA to low viscosity enamel infiltrant (group I) resulted in reductions in MTBS values and in clustering of the added particles on top of demineralized enamel. Although nHA could be directly adsorbed on demineralized enamel (Costa, 2014; Neri, 2017). It seems that bond strength was negatively affected due to absence of good contact between resin adhesive and the added particles, as this causes the particles not to be tightly bonded to the matrix causing easy debonding, the opposite occurs when silanated particles are used because they bond tightly to the matrix so bond strength is not affected. Failure pattern in this group tended to be adhesive between resin adhesive and the clustered particles. On the contrary, unsilanated particles resulted in increase in calcium uptake causing improved surface hardness of ECE while silanated particles failed to produce similar effect. Silane coating could have acted as a barrier preventing effective ion exchange with ECE. Further studies are needed to elaborate on this observation. The incorporation of silanated particles (group II) into resin adhesive did not result in deterioration of bond strength to enamel, in contrary, as it increased the bond strength, which caused rejection of the null hypothesis in our study. This comes in agreement with other studies such as that of Costa *et al* (2014) and Neri *et al.* (2017) who completed similar tests and

found out nearly the same results that the MTBS was not affected or slightly increased (Costa, 2014; Neri, 2017). Unfortunately, no results were reported about the effect of the concentration of nHA mixed with resin adhesives on bond strength to ECE. In the present study 10% wt nHA was the highest concentration that did not negatively affect bond strength and maintained good contact with demineralized enamel. Several investigations are furthermore needed to study the effect the ultimate concentration of nano hydroxyapatite needed for remineralization of early enamel lesions without affecting the MTBS of the material used and to study thoroughly the mechanism of ion transfer namely calcium and phosphorous between the particles and the carious enamel surface. TTEMA/TEGDMA is a low viscosity low shrinkage resin designed to reduce polymerization prestresses (Farias de Lacerda, 2016). As an enamel infiltrant, these properties may enhance penetration depth to ECE which deserves further investigations (Altmann, 2017; Yue, 2018). With improved remineralization capacity, this resin may offer a simple clinical procedure to treat ECE without the need of further surface treatments. In light of all the above information the null hypothesis of this study was rejected.

Conclusion

Within the limitations of this study, addition of 10% nano hydroxyapatite to low shrinkage low viscosity resin could be used for treatment of white spot lesions.

Clinical Relevance

It may be advantageous to incorporate non-silanized nanohydroxyapatite into resin adhesive to control caries progression in early carious lesions, then unmodified resin could be applied.

Declarations of Interest: none.

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“All authors gave their final approval and agree to be accountable for all aspects of the work.”

Statement of Ethics

This study was conducted after receiving the approval of the Ethical Committee at the Faculty of Dentistry, Alexandria University, Egypt (November 2018).

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