

Novel incorporation of charged hydroxyapatite nanoparticles into resin adhesive

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ABSTRACT

The direct mixing of Nano-hydroxyapatite with Resin Adhesive often leads to weak molecular interactions, which can compromise the dispersion of Nano-hydroxyapatite within the resin adhesive and cause structural damage to the Nano-hydroxyapatite. Therefore, the present study aimed to identify an approach in which Nano-hydroxyapatite is charged or modified prior to its integration into resin adhesive. In this study, Nano-hydroxyapatite was modified using aminopropyltriethoxysilane (APTS), subsequently, the amine groups present on the surface of Nano-hydroxyapatite were additionally modified using succinic anhydride. This modification process resulted in the production of negatively charged Nano-hydroxyapatite - aminopropyltriethoxysilane (n-HA-APTS-SAH). The charged Nano-hydroxyapatite particles were characterized using FTIR and SEM. Subsequently, the charged Nano-hydroxyapatite particles were incorporated into resin dental adhesive. Comprehensive characterization of the composite material was carried out through SEM, FTIR, EDX mapping, and Micro-Raman Spectroscopy. The results revealed the presence of Nano-hydroxyapatite in the mixture and a homogeneous and well-dispersed state with no observable aggregation of Nano-hydroxyapatite particles within the adhesive. Furthermore, the particles still maintain a spherical shape with their sizes falling within the nanoscale range.

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Introduction

Dental resin composites are widely used in dentistry as restorative materials, primarily due to their aesthetic effects and enhanced mechanical properties (1). Dentin resin adhesives are used to bond hydrophobic resin composites to hydrophilic dentin tissue (2). The strength of the adhesion with dentin depends on the monomer's ability to enter the inter-collagen fiber spaces to form a hybrid layer (3).

Over time, the loss of adhesive bonds is one of the main causes of composite restorative failure. The Nano-gap formation is the result of bond loss which leads to bond failure and secondary caries formation, the addition of inorganic Nano fillers in adhesives decreases the failure of adhesion over time and enhances dentin interaction of adhesive resins and possibly decreases dentin-adhesive degradation(2). The majority of the tooth's inorganic component is made of Hydroxyapatite, and because of its strong remineralizing ability, its utilization for multiple dental applications has increased recently(4). Since tooth structure has direct immediate contact with dental adhesive, adding nano-hydroxyapatite may promote efficient and long-lasting re-mineralization in teeth with demineralized dentin. Also, the adhesive resin with incorporated Nano-HA particles applied to dentin could improve both the mechanical properties of the resin adhesive and the strength of the tooth structure(5).

Nano-hydroxyapatite (Nano-HA) is a non-toxic biocompatible material with specific biological effects that have demonstrated chemical stability in physiological

conditions, Because of its bioactivity and demineralization potential, it can be used as an adhesive filler to improve material properties(6). However, Nano-hydroxyapatite has some inherent features that make it less than ideal for use as a structural material, including brittleness and problems with surface functionalization, Additionally, simple mixing of Nano-hydroxyapatite with a polymer matrix frequently results in weak molecular interactions between the organic and inorganic phases, which may impair processing capacity and destroy Nano-hydroxyapatite structure. Therefore, searching for an approach to functionalize and modify Nano-hydroxyapatite without altering its physicochemical characteristics is of vital importance for its applications in the biomedical field(7). The aggregation of nanoparticles may negatively affect the mechanical characteristic of the nanoparticle-filled system by increasing the size of the filler phase from nanometer to micrometer. (8) . A common silane agent containing amino groups is aminopropyltriethoxysilane (APTS), which can be further functionalized using conventional bio-conjugation methods(9).

The present study aimed to identify an approach in which Nano-hydroxyapatite is charged or modified prior to its integration into resin adhesive. The study further aimed to confirm the presence and homogeneous dispersion of Nano-hydroxyapatite using SEM, FTIR, EDX mapping, and micro-Raman spectroscopy. This is the first study regarding the modification of Nano-hydroxyapatite to generate Nano-hydroxyapatite with surface charge and its subsequent incorporation into the Resin dental adhesive.

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Materials and Methods

Materials and instruments

1-Nano-hydroxyapatite and the other materials, including aminopropyltriethoxysilane (APTS) and succinic anhydride, were obtained from Merck (Germany).

2-Ethanol (99%) and dimethylsulfoxide (DMSO) were obtained from Sigma-Aldrich

3-The Resin adhesive (Palfique Bond) was obtained from Tokuyama Dental Co.

4- Fourier Transform Infrared Spectroscopy (FTIR): Pye Unicam SP3-300S Spectrophotometer (KBr Disc). 5- Ultrasonic Bath (Sonicator): an Ultra Met sonic cleaner from Buehler Ltd. (220/240 V, 50/60 Hz).

6- Centrifuge Machine: The Weswox T-1014-01 Laboratory Centrifuge Machine running at 1000 rpm.

7- Scanning Electron Microscope(SEM): FESEM model MRA 11 SAMX (TESCAN).

8-Raman spectrometer TAKRAM 785-G100 (TEKSAN, IRAN).

Preparation of the charged nano-hydroxyapatite particles (n-HA-APTS-SAH)

Initially, 0.36 g of Nano-hydroxyapatite (n-HA) was dispersed in 400 mL of absolute ethanol. The dispersion underwent ultrasonic irradiation in a water bath at room temperature for 4 hours to attain a homogeneous solution. Subsequently, to obtain n-HA-APTS, 10 mL of aminopropyltriethoxysilane (APTS) was added to the solution while vigorously stirring using a magnetic bar Figure 1. The mixture was quenched with (2 mL) of deionized water to convert the (-OCH₂CH₃) groups of APTS to (-OH) groups. To promote efficient mixing, enhance reaction kinetics, and facilitate the desired transformation of the APTS molecules, the mixture was sonicated for 1 hour. After this period, the mixture was centrifuged at 10,000 rpm to obtain the desired product. To purify the product from unreacted starting materials and by-products, the product was washed four times with water and subsequently washed with methanol (7). After drying the product at room temperature, it was subjected to Fourier transform infrared spectroscopy (FTIR) for characterization.

In the next step, in order to charge the amine groups of n-HA-APTS, carboxylation is performed. The dispersed mixture of 0.3g of n-HA-APTS in 100 mL of dimethylsulfoxide (DMSO) was added to a solution of succinic anhydride in dimethylsulfoxide (2.5g in 25 mL). The mixture was sonicated for 2 hours. After this period, the mixture was centrifuged at 10,000 rpm to separate the product. The separated product was washed four times with water and subsequently washed with methanol to obtain the pure desired product (n-HA-APTS-SAH) Figure 2. Then (FTIR) Fourier transform infrared and (SEM) scanning electron microscope performed for characterization.

Incorporation of n-HA-APTS-SAH into the resin adhesive

The specific amount of n-HA-APTS-SAH (0.5mg) was carefully measured and combined with (1mL) of the Resin dental adhesive (Palfique Universal Bond, Tokuyama, Japan) to develop novel adhesives - n-HA-APTS-SAH. The mixture underwent 30 minutes of sonication.

The mixture (Adhesive - n-HA-APTS-SAH) was subjected to characterization by SEM, FTIR, EDX mapping,

and micro-Raman spectroscopy to confirm and identify functional groups present in both pure substances and the mixture. To confirm the charging effect of Nano-hydroxyapatite on the dispersion within the resin adhesive, another mixture was prepared by combining (0.5mg) of non-charged Nano-hydroxyapatite with (1mL) of the Resin dental adhesive. Subsequently, the mixture underwent 30 minutes of sonication. Then the SEM images of both mixtures were compared to check for the difference in dispersion.

Statistical analysis

Because the data of this research was qualitative and there was no quantitative data, statistical analysis was not used.

Results

Characterization of the charged nano-hydroxyapatite particles

FTIR

The FTIR spectra of Nano-hydroxyapatite, as shown in Figure 3, exhibit two strong absorption bands at 1022 cm⁻¹ and 555 cm⁻¹, which are attributed to PO₄³⁻ and the weak absorption band at 3695 cm⁻¹ corresponding to the hydroxyl group. The FTIR spectra of APTS shows the peak at 3400 cm⁻¹ which is attributed to -NH₂ stretching, while -NH₂ bending vibration bands at 1697 cm⁻¹, the strong band at 1199 cm⁻¹ is corresponding to the Si-O group. In addition to coupled bands at 2931 cm⁻¹ and 2869 cm⁻¹ which are assigned the -CH₂ groups. The FTIR spectrum of the final product (n-HA-APTS) includes all the vibrational peaks

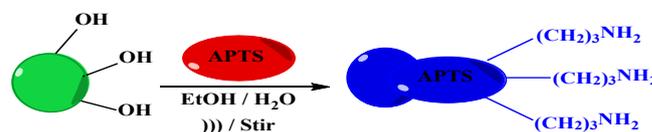


Figure 1. Preparation of n-HA-APTS.

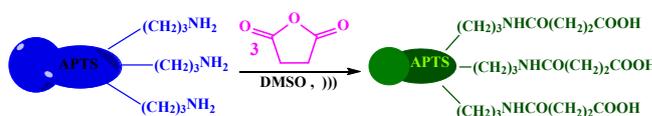


Figure 2. Preparation of Carboxylate n-HA-APTS.

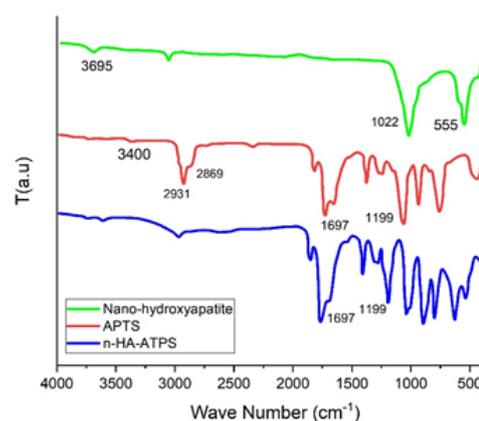


Figure 3. FTIR spectra of Nano-hydroxyapatite, APTS and n-HA-APTS.

described in the FTIR spectrum of Nano-hydroxyapatite and the FTIR spectrum for APTS.

SEM

Figure 4 shows SEM analysis of the Nano-hydroxyapatite particles before any modification revealed spherical particles with an average particle size range are 20.98-39.55 nm. The SEM images exhibited rounded HA nanoparticles without coarse edges, primarily in agglomerated form with a few isolated particles. The nanoparticles did not display any sharp edges.

The morphology of Nano-hydroxyapatite particles doesn't show significant changes upon modification with APTS and subsequent carboxylation of the APTS amines, as compared to the non-modified Nano-hydroxyapatite particles. Also, the particles still maintain a spherical shape and are well dispersed as shown in Figure 5.

Characterization of the novel adhesives- n-HA-APTS-SAH.

FTIR

The FTIR spectra of n-HA-APTS-SAH in Figure 6 shows two significant bands at 3421 cm^{-1} and 1691 cm^{-1} , which are related to the O-H and C=O vibration frequencies of succinic anhydride, respectively. The FTIR spectrum shown in the Resin dental adhesive displays distinct peaks at 3435 cm^{-1} , 1722 cm^{-1} , and 1631 cm^{-1} , which correspond to O-H, C=O, and C=C stretching. Meanwhile, the FTIR analysis of the mixture of n-HA-APTS-SAH and Resin dental adhesive reveals the presence of all the vibration bands.

SEM

SEM of the mixture of charged Nano-hydroxyapatite particles (n-HA-APTS-SAH) with the Resin dental adhesive showed that there was a uniform distribution of spherical particles and very well dispersed within the resin adhesive. Furthermore, no aggregation of charged Nano-hydroxyapatite particles was observed within the resin adhesive as shown in Figure 7 A. The mixture of non-charged Nano-hydroxyapatite particles was found to have very

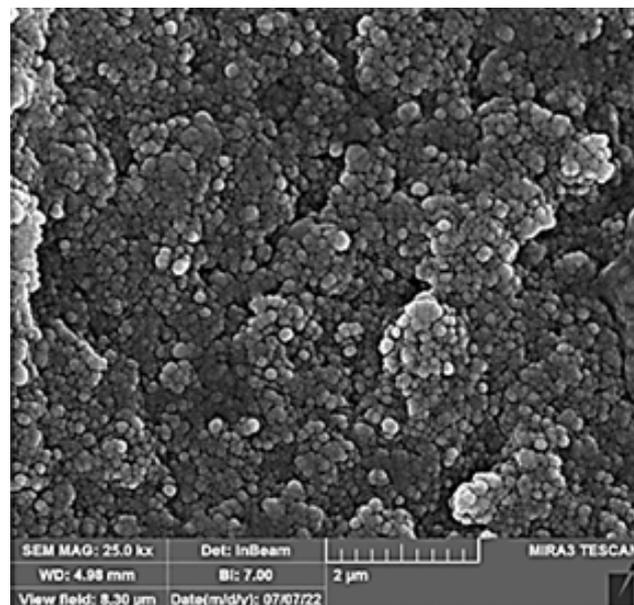


Figure 5. SEM of the charged HA Nanoparticles.

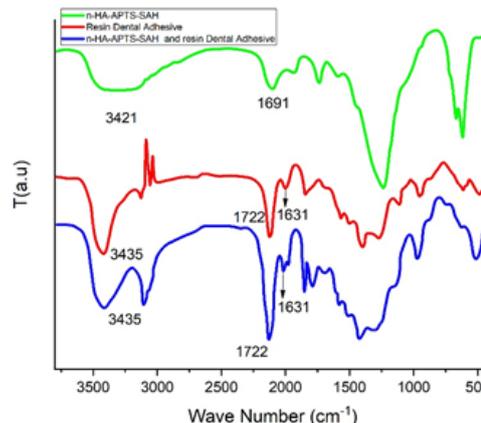


Figure 6. FTIR spectra of n-HA-APTS-SAH, Resin dental adhesive and mixture of n-HA-APTS-SAH with the resin dental adhesive.

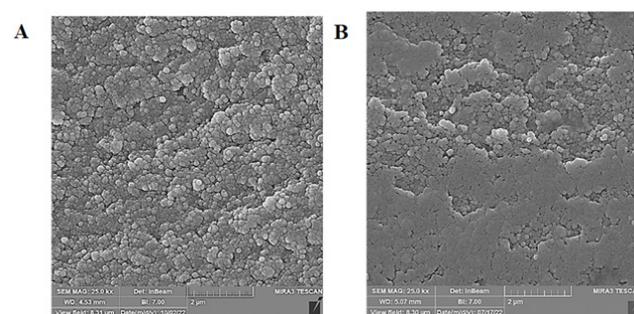


Figure 7. SEM of (A) a mixture of the charged Nano-hydroxyapatite particles (n-HA-APTS-SAH) with the Resin dental adhesive and (B) a mixture of the non-charged Nano-hydroxyapatite particles with the Resin dental adhesive.

poor dispersion, with aggregation and clusters observed within the resin adhesive. As illustrated in Figure 7 B.

EDX

The EDX mapping analysis results demonstrated the presence of oxygen, calcium, and phosphorus in the mixture, indicating the existence of Nano-hydroxyapatite as shown in Figure 8 and Figure 9. The detection of these elements signifies the existence of hydroxyapatite nanopar-

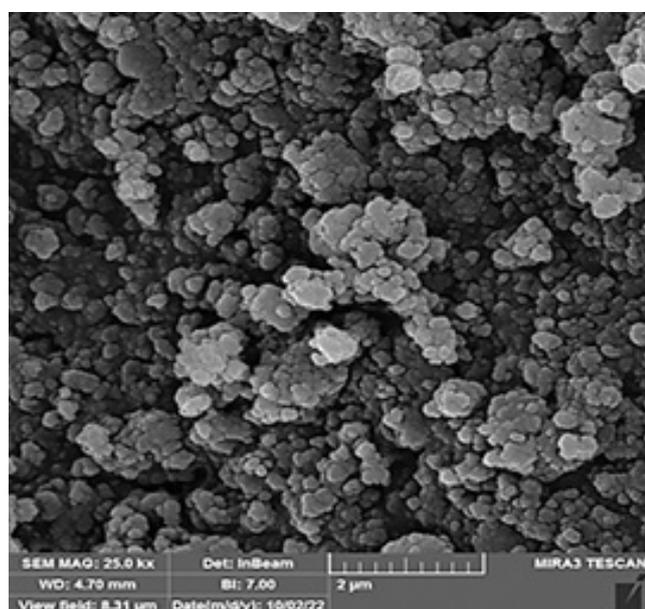


Figure 4. SEM of the non-charged HA Nanoparticles.

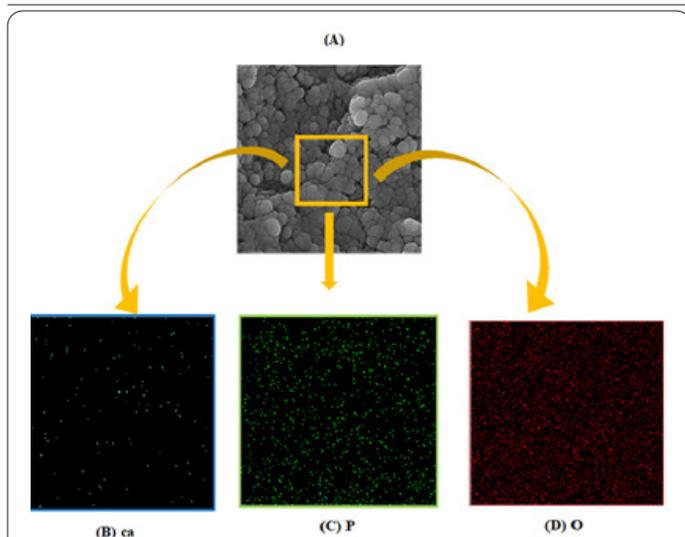


Figure 8. EDX elemental mapping of (A) Adhesives - n-HA-APTS-SAH; (B) presence of calcium; (C) presence of phosphorus; (D) presence of oxygen.

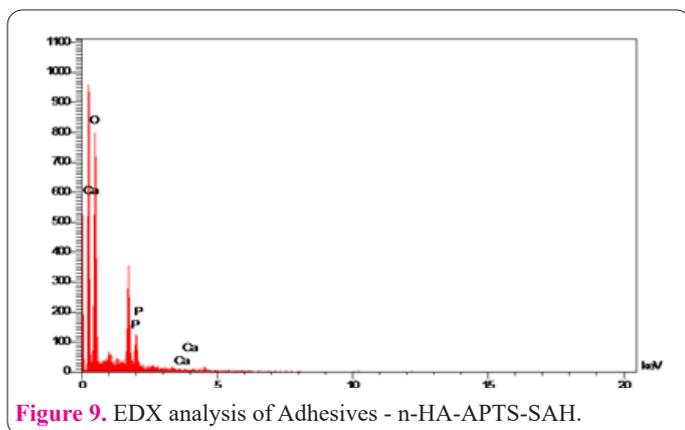


Figure 9. EDX analysis of Adhesives - n-HA-APTS-SAH.

ticles within the mixture of n-HA-APTS-SAH and Resin dental adhesive.

Micro-Raman Spectroscopy

The micro-Raman result of the charged Nano-hydroxyapatite particles revealed characteristic chemical groups, which were identified as phosphate at 960 cm^{-1} and carbonate at 1070 cm^{-1} , as shown in Figure 10 A. while the Micro-Raman result of the resin dental adhesive reveals peaks ranging from 1410 to 1460 cm^{-1} , which correspond to the deformation of $-\text{CH}_3$ and $-\text{CH}_2$ within the resin adhesive as shown in Figure 10 B.

It can be seen from Figure 10 C the inclusion of charged Nano-hydroxyapatite particles in the resin dental adhesive which is evidenced by the strong peaks observed in the spectrum. These peaks correspond to the phosphate (PO_4) at 960 cm^{-1} and carbonate (CO_3) at 1070 cm^{-1} . Additionally, the peaks between 1410 and 1460 cm^{-1} can be attributed to the deformations of the $-\text{CH}_3$ and $-\text{CH}_2$ groups within the resin dental adhesive. Furthermore, a peak observed at 1610 cm^{-1} emphasizes the presence of the adhesive monomer.

Discussion

In this current investigation, Nano-hydroxyapatite was integrated into the resin adhesive, and its presence was subsequently confirmed through characterization using FTIR, SEM, EDX, and Micro-Raman techniques. SEM

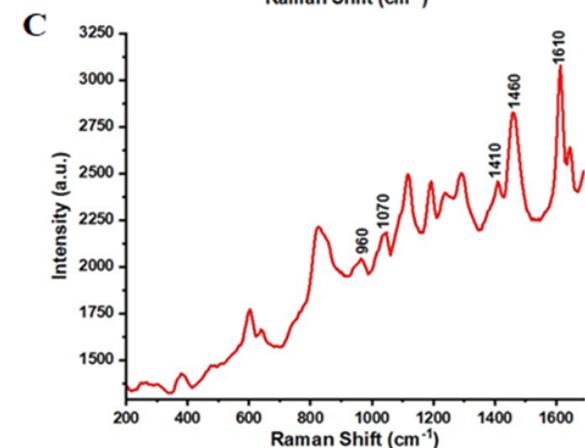
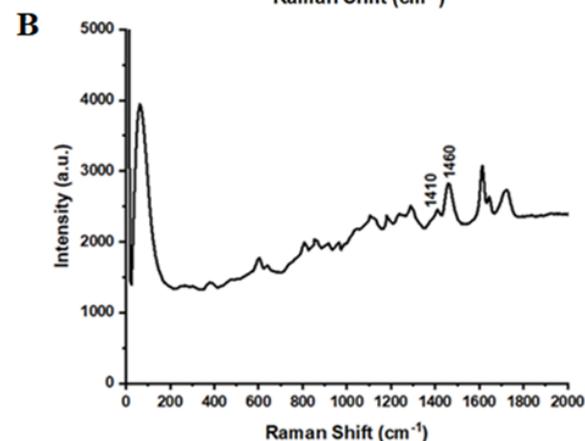
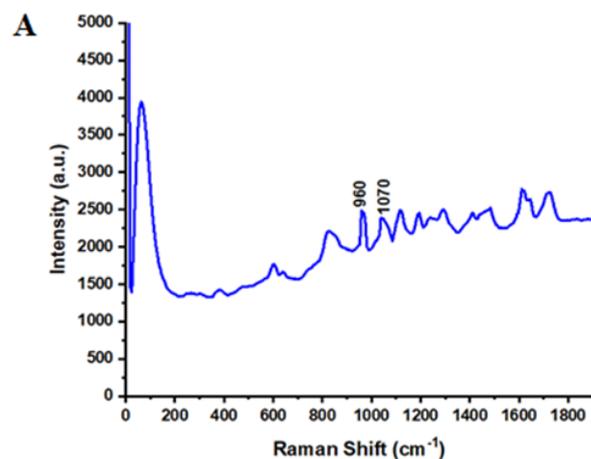


Figure 10. Raman spectra (A) Charged Nano-hydroxyapatite particles. (B) Resin dental Adhesive. (C) The mixture of charged Nano-hydroxyapatite particles with the Resin dental Adhesive.

analysis revealed that the Nano-hydroxyapatite fillers were present at the nanoscale, exhibiting a uniform and homogeneous distribution of hydroxyapatite Nanospheres with a spherical morphology.

Infrared analysis is a widely employed technique, utilized for identifying functional groups present in both pure substances and mixtures, as well as for comparing different compounds. This method relies on the correlation between the vibrational movement of atoms and molecules(10, 11). The FTIR spectra of Nano-hydroxyapatite, as shown in Figure 3, exhibit two strong absorption bands at 1022 cm^{-1} and 555 cm^{-1} , which are attributed to PO_4^{3-} and the weak absorption band at 3695 cm^{-1} corresponding to the hydroxyl group(12). The FTIR spectra of APTS shows the peak at 3400 cm^{-1} which is attributed to $-\text{NH}_2$ stretching, while $-\text{NH}_2$ bending vibration bands at

1697 cm^{-1} , the strong band at 1199 cm^{-1} is corresponding to the Si-O group(13). In the present research, the use of FTIR spectroscopy enables the qualitative verification of the APTS modification performed on the Nano hydroxyapatite surfaces (7).

SEM was utilized to examine the morphology of the Nano-hydroxyapatite particles both before and after charging. The morphology of Nano-hydroxyapatite particles doesn't show significant changes upon modification with APTS and subsequent carboxylation of the APTS amines, as compared to the non-modified Nano-hydroxyapatite particles. Also, the particles still maintain a spherical shape and are well dispersed, indicating that the charging process did not significantly affect the overall morphology or size of the nanoparticles (7).

The FTIR spectra of n-HA-APTS-SAH shows two significant bands at 3421 cm^{-1} and 1691 cm^{-1} , which are related to the O-H and C=O vibration frequencies of succinic anhydride, respectively (14). This indicates the reaction of succinic anhydride with the mixture of n-HA-APTS. The FTIR spectrum shown in the Resin dental adhesive displays distinct peaks at 3435 cm^{-1} , 1722 cm^{-1} , and 1631 cm^{-1} , which correspond to O-H, C=O, and C=C stretching (15). The FTIR analysis of the mixture of n-HA-APTS-SAH and Resin dental adhesive reveals the presence of all the vibration bands. This finding serves as significant evidence for the existence of n-HA-APTS-SAH and the Resin dental adhesive in the mixture (16).

Upon mixing charged Nano-hydroxyapatite particles (n-HA-APTS-SAH) with the Resin dental adhesive, it was observed that there was a uniform distribution of spherical particles and very well dispersed within the resin adhesive. Furthermore, no aggregation of charged Nano-hydroxyapatite particles was observed within the resin adhesive. This outcome can be produced by APTS modifications, which enhance the nanoparticle-polymer interface and enhance filler dispersion in the resin adhesive (14). The mixture of non-charged Nano-hydroxyapatite particles was found to have very poor dispersion, with aggregation and clusters observed within the resin adhesive. This observation is consistent with the finding of Ghyadh et al in 2021 who reported that; modification of the Nano-hydroxyapatite fillers will strengthen Nano-hydroxyapatite binding to the resin matrix and prevent aggregation of the Nano filler. Some manufacturers use functionalized Nano fillers to prevent them from clustering (15). It is suggested that the agglomeration of Nano filler can adversely affect the mechanical properties of the filled material (16). The Nano-HA particles remained spherical after charging and subsequent mixing with the resin adhesive which is According to the literature, The presence of spherical filler particles imparts a lubricating effect on the material, increasing its flowability, while amorphous filler particles can impede flow and raise viscosity(17).

To verify the existence of Nano-hydroxyapatite, EDX was utilized to detect essential elements and chemical groups present in the novel adhesives-n-HA-APTS-SAH. The EDX mapping analysis results demonstrated the presence of oxygen, calcium, and phosphorus in the mixture. Indicating the existence of Nano-hydroxyapatite. The detection of these elements signifies the existence of hydroxyapatite nanoparticles within the mixture of n-HA-APTS-SAH and Resin dental adhesive. The detection of calcium and phosphate confirms that the material

possesses remineralizing properties, which facilitates the formation of a strong bond between the resin and dentin. This indicates that the material has the ability to promote remineralization and enhance the adhesion between the two surfaces (17).

The EDX result verified the existence of essential remineralizing elements, such as calcium (Ca) and phosphorus (P). The presence of these elements indicates the presence of hydroxyapatite (HA) since calcium and phosphorus are vital components of human hard tissues. Furthermore, these elements play a critical role in the remineralization process of dental tissue (2, 5, 18).

Micro-Raman spectroscopy is a diagnostic technique that is utilized to analyze the polarization of molecules through vibrational spectroscopy (19). Unlike absorption spectroscopy, Micro-Raman spectroscopy relies on scattering rather than absorption of light. This characteristic allows specimens of various thicknesses to be visualized without causing any damage or destruction(20). In this study, the charged Nano-hydroxyapatite particles exhibited strong peaks for the phosphate group at 960 cm^{-1} and for the carbonate at 1070 cm^{-1} as shown in Figure 10A. Upon mixing the Charged Nano-hydroxyapatite particles with the Resin adhesive, a distinguishable phosphate peak at 960 cm^{-1} was still observed, although its intensity was reduced as can be seen in Figure 10 C. The possible explanation for this specific observation could be that the intensity of the Raman bands undergoes a transitional change when minerals are substituted with resin. This finding has been previously reported by Van Meerbeek et al. in 1993. The Raman spectroscopy results were consistent with several earlier studies that also reported peak intensities of phosphate groups at 960 cm^{-1} and carbonate at 1070 cm^{-1} , in adhesive containing Nano-hydroxyapatite (2, 5, 21). In contrast, the peak at 1610 cm^{-1} specifically indicates the presence of the adhesive monomer (22). Moreover, the recorded peaks ranging from 1410 to 1460 cm^{-1} were attributed to the deformations of $-\text{CH}_3$ and $-\text{CH}_2$ groups within the mixture Figure10C. These peaks signify the characteristic polymerization activity within the Adhesives - n-HA-APTS-SAH (18, 23).

The results obtained in this study are quite promising, as the incorporation of charged Nano-hydroxyapatite into the resin adhesive demonstrated excellent dispersion within the adhesive material. Additionally, there was no evidence of particle aggregation among the charged Nano-hydroxyapatite particles within the resin adhesive. Consequently, future investigations should focus on exploring the impact of charged Nano-hydroxyapatite on the bonding strength of dental resin adhesives.

Conclusions

Modification of Nano-hydroxyapatite particles through a simple approach before their incorporation into the resin dental adhesive has a significant impact on the dispersion and homogeneity of the particles within the adhesive. The results of this study reveal that charging Nano-hydroxyapatite particles using a reliable method, followed by their incorporation into the adhesive as a filler could have practical applications in clinical dentistry. This approach enhances and improves the mechanical properties of the resin dental adhesive by promoting dispersion and homogeneity within the adhesive. Furthermore, it overcomes the challenges and drawbacks of previous research

that incorporated Nano-hydroxyapatite particles without prior surface modification.

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Ethical approval

The present study was conducted without involving any human or animal subjects. The study protocol received approval from the Ethics Committee of Hawler Medical University.

References

- Bregnocchi A, Zanni E, Uccelletti D, Marra F, Cavallini D, De Angelis F, et al. Graphene-based dental adhesive with anti-biofilm activity. *J. Nanobiotechnology* 2017;15:1-13.
- AlFawaz YF, Almutairi B, Kattan HF, Zafar MS, Farooq I, Naseem M, et al. Dentin bond integrity of hydroxyapatite containing resin adhesive enhanced with graphene oxide nano-particles—An SEM, EDX, micro-Raman, and microtensile bond strength study. *Polym J* 2020;12(12):2978.
- Huang B, Siqueira WL, Cvitkovitch DG, Finer Y. Esterase from a cariogenic bacterium hydrolyzes dental resins. *Acta Biomater* 2018;71:330-8.
- Amaechi BT, AbdulAzees PA, Alshareif DO, Shehata MA, Lima PPdCS, Abdollahi A, et al. Comparative efficacy of a hydroxyapatite and a fluoride toothpaste for prevention and remineralization of dental caries in children. *BDJ open* 2019;5(1):18.
- Al-Hamdan RS, Almutairi B, Kattan HF, Alsuwailem NA, Farooq I, Vohra F, et al. Influence of hydroxyapatite nanospheres in dentin adhesive on the dentin bond integrity and degree of conversion: a scanning electron microscopy (SEM), Raman, Fourier transform-infrared (FTIR), and microtensile study. *Polym J* 2020;12(12):2948.
- Ghyadh A, Ragab H, Osman E. Shear Bond Strength of a Multi-Mode Universal Adhesive Containing Hydroxy-Apatite Nanoparticles to Dentin (In vitro study). *Egypt. Dent. J* 2021;67(3):2749-58.
- Wang S, Wen S, Shen M, Guo R, Cao X, Wang J, et al. Aminopropyltriethoxysilane-mediated surface functionalization of hydroxyapatite nanoparticles: synthesis, characterization, and in vitro toxicity assay. *Int J Nanomed* 2011:3449-59.
- Wagner A, Belli R, Stötzel C, Hilpert A, Müller FA, Lohbauer U. Biomimetically-and hydrothermally-grown HAp nanoparticles as reinforcing fillers for dental adhesives. *J Adhes Dent* 2013;15(5).
- Tang T, Fan H, Ai S, Han R, Qiu Y. Hemoglobin (Hb) immobilized on amino-modified magnetic nanoparticles for the catalytic removal of bisphenol A. *Chemosphere* 2011;83(3):255-64.
- Salem SS, Hammad EN, Mohamed AA, El-Dougoud W. A comprehensive review of nanomaterials: Types, synthesis, characterization, and applications. *Biointerface Res Appl Chem* 2022;13(1):41.
- Delgado AH. Characterization of dental bonding systems and commercial versus novel self-adhesive restoratives: UCL (University College London); 2021.
- Zanca C, Patella B, Capuana E, Lopresti F, Brucato V, Carfi Pavia F, et al. Behavior of Calcium Phosphate–Chitosan–Collagen Composite Coating on AISI 304 for Orthopedic Applications. *Polym J* 2022;14(23):5108.
- Fang Y, Lakey PS, Riahi S, McDonald AT, Shrestha M, Tobias DJ, et al. A molecular picture of surface interactions of organic compounds on prevalent indoor surfaces: limonene adsorption on SiO₂. *Chem. Sci* 2019;10(10):2906-14.
- Kavrik F, Kucukyilmaz E. The effect of different ratios of nano-sized hydroxyapatite fillers on the micro-tensile bond strength of an adhesive resin. *MRT* 2019;82(5):538-43.
- Van Landuyt KL, Snauwaert J, De Munck J, Peumans M, Yoshida Y, Poitevin A, et al. Systematic review of the chemical composition of contemporary dental adhesives. *Biomater* 2007;28(26):3757-85.
- Deng C, Weng J, Duan K, Yao N, Yang X, Zhou S, et al. Preparation and mechanical property of poly (ϵ -caprolactone)–matrix composites containing nano-apatite fillers modified by silane coupling agents. *J. Mater. Sci* 2010;21:3059-64.
- Aljamhan AS, Alrefeai MH, Alhabdan A, Alhuseini SA, Farooq I, Vohra F, et al. Influence of er-cr-ysgg laser and photodynamic therapy on the dentin bond integrity of nano-hydroxyapatite containing resin dentin adhesive: sem-edx, micro-raman, micro-tensile, and ftir evaluation. *Polym J* 2021;13(12):1903.
- Al-Hamdan RS, Almutairi B, Kattan HF, Alresayes S, Abduljabbar T, Vohra F. Assessment of hydroxyapatite nanospheres incorporated dentin adhesive. A SEM/EDX, micro-Raman, microtensile and micro-indentation study. *Coat* 2020;10(12):1181.
- Rangan S, Schulze HG, Vardaki MZ, Blades MW, Piret JM, Turner RF. Applications of Raman spectroscopy in the development of cell therapies: State of the art and future perspectives. *Anst* 2020;145(6):2070-105.
- Miyazaki M, Onose H, Iida N, Kazama H. Determination of residual double bonds in resin–dentin interface by Raman spectroscopy. *Dent. Mater. J* 2003;19(3):245-51.
- Nosenko V, Strutynska N, Vorona I, Zatonovsky I, Dzhanagan V, Lemishko S, et al. Structure of biocompatible coatings produced from hydroxyapatite nanoparticles by detonation spraying. *Nanoscale Res. Lett* 2015;10:1-7.
- Ishimaru Y, Oshima Y, Imai Y, Iimura T, Takanezawa S, Hino K, et al. Raman spectroscopic analysis to detect reduced bone quality after sciatic neurectomy in mice. *mol* 2018;23(12):3081.
- Esmonde-White KA, Le Clair SV, Roessler BJ, Morris MD. Effect of conformation and drop properties on surface-enhanced Raman spectroscopy of dried biopolymer drops. *Appl. Spectrosc* 2008;62(5):503-11.