Journal of Carcinogenesis
Covering Genesis to Therapeutics

Journal of Carcinogenesis, ISSN: 1477-3163
20yy; Vol 24: Issue 1s, https://carcinogenesis.com/

Biomimetic Remineralization of Dentin Using Aspartic Acid Pre-conditioning with Different Remineralizing Agents: An FTIR Analysis

Ibrahim El Dossoky Basha[1], Walaa Mohamed Ahmed Alsamoully[2], Hamed Ibrahim Mohamed Ibrahim[3], Eslam Hassan Gabr[4], Mohamed Ahmed Basha[5], Osama Nasr Elden Eltobgy[6], Ashraf Marawan El Azzazy[7], Galal Eldeen Mosaad Sadek[8], Ahmed Elbakry Khder[9]

[1] Assistant Professor of Operative Dentistry Department, Faculty of Oral and Dental Medicine, Al–Azhar University (Boys-Cairo). E-mail: ibrahim33.ib@gmail.com

[2] Assistant professor of Operative Dentistry Department, Faculty of Oral and Dental Medicine, Al–Azhar University (Boys-Cairo). E-mail: Walaasamolly@gmail.com.

[3] Associate professor of Operative Dentistry Department, Faculty of Oral and Dental Medicine, Al–Azhar University (Boys-Cairo). E-mail: HamedIbrahim.209@azhar.edu.eg

[4] Lecturer of Operative Dentistry Department, Faculty of Dental Medicine (Cairo-Boys), Al-Azhar University, Cairo, Egypt. E-mail: EslamGaber.209@azhar.edu.eg

[5] Lecturer of Operative Dentistry Department, Faculty of Dental Medicine (Cairo-Boys), Al-Azhar University, Cairo, Egypt. E-mail: mohamedbasha.209@azhar.edu.eg

[6] Lecturer of Operative Dentistry Department, Faculty of Dental Medicine (Cairo-Boys), Al-Azhar University, Cairo, Egypt. E-mail: OsamyEltobgy.209@azhar.edu.eg

[7] Lecturer, Department of Dental Biomaterials, Faculty of Dental Medicine Al-Azhar University (Assiut), Assiut Branch, Egypt. E-mail: drashrafmarawan@gmail.com

[8] Assistant Professor of Dental Biomaterials Department, Faculty of Oral and Dental Medicine, Al–Azhar University (Boys- Cairo). E-mail: galalsadek@hotmail.com

[9] Lecturer of Operative Dentistry Department, Faculty of Dental Medicine (Cairo-Boys), Al-Azhar University, Cairo, Egypt. E-mail: ahmedelbkry15@gmail.com

ABSTRACT

Introduction: Effective dentin remineralization necessitates functional nucleation sites, often compromised during demineralization from non-collagenous protein degradation. Aspartic acid (Asp) can mimic these proteins and may facilitate intrafibrillar mineral deposition.

Objective: This in-vitro study evaluated the efficacy of Asp pre-conditioning on dentin remineralization using Chitosan-loaded Nano-hydroxyapatite (Cs-nHA), Bio-Active Glass (BAG), and Silver Diamine Fluoride (SDF).

Materials and methods: Sixty human dentin specimens were pH-cycled to produce standardized lesions and divided into two main groups (n=30): Asp-conditioned and non-conditioned groups. Each main group was subdivided by remineralizing agent treatment (n=10): chitosan-loaded nano-hydroxyapatite (Cs-nHA), bioactive glass (BAG), or 38% silver diamine fluoride (SDF). Treated specimens were stored in artificial saliva for 2 or 6 weeks. The remineralization potential was quantified via Fourier Transform Infrared Spectroscopy (FTIR) before and after treatment by calculating the Mineral-to-Matrix (M:M) ratio.

Results: All remineralizing treatments demonstrated significant improvement in M:M ratios compared to the untreated demineralized dentin baseline. Asp pre-conditioning produced significantly higher M:M ratios compared to non-conditioned controls across all groups. Cs-nHA achieved the highest M:M ratios, outperforming SDF and BAG, while SDF and BAG did not differ significantly from each other. All groups showed greater M:M ratios at 6 weeks versus 2 weeks.

Conclusions: Preconditioning dentin surfaces with Aspartic acid markedly enhances the dentin remineralization potential of the tested remineralizing agents. The application of various remineralizing agents improves dentin remineralization,

with Cs-nHA yielding the best results among SDF and BAG, while there was no significant difference in efficacy between them. Additionally, extending the storage time of the remineralizing agents further promotes dentin remineralization.

Keywords: Aspartic acid, Bioactive Agents, Silver Diamine Fluoride, Biomimetic dentin remineralization, FTIR spectroscopy.

How to Cite: Ibrahim El Dossoky Basha, Walaa Mohamed Ahmed Alsamoully, Hamed Ibrahim Mohamed Ibrahim, Eslam Hassan Gabr, Mohamed Ahmed Basha, Osama Nasr Elden Eltobgy, Ashraf Marawan El Azzazy, Galal Eldeen Mosaad Sadek, Ahmed Elbakry Khder (2025) Biomimetic Remineralization of Dentin Using Aspartic Acid Pre-conditioning with Different Remineralizing Agents: An FTIR Analysis, Vol.24, No.1s, 116-125

1. INTRODUCTION

Dental caries is a noncommunicable, multifactorial disease caused by the interaction of dietary sugars, biofilm activity, and host factors, resulting in progressive demineralization of dental hard tissues [(1)]. The traditional management of demineralized dentin and hypersensitivity has focused on two main strategies: nerve desensitization (e.g., potassium salts) or the physical occlusion of dentinal tubules using various agents [(2)]. However, simple mechanical occlusion is often temporary, as the deposited materials can be dislodged by dietary acids or mechanical wear [(3)]. Consequently, modern restorative dentistry has shifted toward a "biomimetic" approach. The goal is no longer to simply plug the tubules but to induce functional remineralization, the regrowth of mineral crystals within the collagen fibrils (intrafibrillar mineralization) and around them (extrafibrillar mineralization) to restore the mechanical properties of the dentin [(4)].

To achieve this, various advanced calcium-phosphate-based systems have been developed. Among the most promising is Nano-hydroxyapatite (nHA). Because natural tooth mineral exists as nano-sized crystals, synthetic nHA exhibits superior bioactivity and biocompatibility compared to micro-sized particles. It acts as a filler to repair microscopic defects and serves as a direct template for crystal growth [(5)]. To enhance the efficacy of nHA, biopolymers like Chitosan have been introduced as carriers (Cs-nHA). Chitosan is a natural, biodegradable polysaccharide derived from chitin [(6)].

Another significant advancement is the development of Bio-Active Glass (BAG). Traditional bioactive glass (45S5) has long been used for bone regeneration, but its application in dentin remineralization has been limited by particle size. The ultrafine bioactive glass particles offer a vastly increased surface area, leading to a rapid release of calcium, sodium, and phosphate ions upon contact with saliva [(7)]. Furthermore, silver diamine fluoride (SDF) has gained immense popularity for arresting caries and treating hypersensitivity. SDF works through a dual mechanism: the silver ions act as potent antimicrobial agents and react with dentin proteins to form a protective layer of silver proteinates (which blocks tubules), while the fluoride ions promote the formation of fluorapatite [(8)]. Fluorapatite is critical in the oral environment as it has a lower critical pH than hydroxyapatite, making the remineralized dentin more resistant to future acid attacks [(9)].

Despite the availability of these potent remineralizing agents, a major challenge remains: the lack of nucleation sites on demineralized dentin collagen. In natural dentinogenesis, non-collagenous proteins (NCPs), such as phosphophoryn, play a crucial role in regulating mineral deposition. These proteins are rich in acidic amino acids, particularly Aspartic Acid and Serine, which bind to collagen and sequester calcium ions to initiate crystallization [(10)]. In demineralized dentin, these natural NCPs are often lost or denatured. Therefore, to achieve true biomimetic remineralization, it is necessary to introduce an analog that mimics the function of these proteins. Thus, the use of NCP analogs in conjunction with remineralizing biomaterials enhanced the remineralization process [(11)].

DL-Aspartic acid has been identified as a promising biomimetic analog. It functions based on the Polymer-Induced Liquid-Precursor process. By pre-conditioning the dentin with Aspartic acid, we hypothesize that we can create artificial nucleation sites. Aspartic acid is known to promote dentin remineralization by acting as an analog to non-collagenous proteins, which induce the formation of artificial nucleation sites for apatite crystals [(12)].

To accurately evaluate the quality of remineralization, Fourier Transform Infrared Spectroscopy (FTIR) is a valuable analytical tool. FTIR provides quantitative chemical information about the molecular structure of the tissue. It allows for the calculation of the Mineral-to-Matrix ratio (specifically the ratio of Phosphate bands to Amide I collagen bands), which is a direct indicator of the degree of mineralization within the organic matrix [(13)].

Therefore, the aim of the current in-vitro research was to assess the influence of a conditioning solution of DL-aspartic amino acid on the enhancement of dentin remineralization using three remineralization agents: Cs-nHA, BAG, and SDF. The remineralization potential was assessed quantitatively using FTIR. The null hypotheses tested were that pre-conditioning with Aspartic acid would have no significant effect on dentin remineralization, and that there would be no significant difference in remineralization efficacy among the three tested materials across the storage periods.

2. MATERIALS AND METHODS

Sample size calculation:
Based on data from a previous study evaluating remineralization potential [14], a power analysis was conducted. To detect a significant difference in mineral content with a power of 95% and a significance level (α) of 0.05 (two-tailed), a sample size of 60 specimens was determined to be sufficient. This allows for 5 specimens per division, ensuring statistical validity even with potential sample loss.

Teeth collection and preparation:
A total of sixty sound human posterior teeth, extracted for periodontal or orthodontic reasons, were collected for this in-vitro research. The teeth were thoroughly cleaned of debris and soft tissue utilizing a hand scaler and stored in a 0.1% thymol solution to avoid bacterial growth. Teeth exhibiting cracks, caries, or developmental defects were excluded after visual inspection under 2.5x magnification.

From these teeth, sixty dentin blocks were prepared. Utilizing a slow-speed diamond saw (Isomet, Buehler, Lake Bluff, IL, USA) under water coolant, dentin discs with dimensions of 3 × 3 × 3 mm were sectioned from the mid-coronal dentin. The dentin disc surfaces were polished sequentially with 600, 800, and 1200-grit silicon carbide papers to create a standardized, flat surface and to produce a uniform smear layer. The samples were then ultrasonically cleaned in deionized water for 10 minutes to remove polishing residues.

Creation of artificial demineralized dentin:
Marquezan et al. [15] adapted a pH-c method to induce artificial dentin caries. For eight hours, each sample was submerged in one milliliter of a demineralizing solution that included 2.2 mM $CaCl_2$, 2.2 mM NaH_2PO_4, and 50 mM acetic acid that had been calibrated to a pH of 4.8. Samples were then placed in 1 ml of a remineralizing solution comprising 1.5 mM $CaCl_2$, 0.9 mM NaH_2PO_4, and 0.15 M KCl adjusted to a pH of 7.0 for 16 hours. Sigma-Aldrich, Saint Louis, MO, USA, provided chemical reagents (≥99.0% purity) for solution production. The solutions were changed every day, while every sample went through cycles for 14 days. The pH-c test was conducted without stirring at an ambient temperature [16].

Grouping of specimens:
The sixty demineralized dentin discs were randomly allocated into two main groups (n=30) according to the surface pre-conditioning protocol: Group I (Conditioned): Treated with Aspartic acid, and Group II (Non-conditioned): No pre-treatment. Three subgroups (n=10) were created from each major group based on the remineralizing agent applied: Subgroup A: Chitosan-loaded Nano-hydroxyapatite (Cs-nHA). Subgroup B: Bio-Active Glass (BAG). Subgroup C: Silver Diamine Fluoride (SDF). Finally, each subgroup was split into two divisions (n=5) based on the storage duration in artificial saliva: 2 weeks and 6 weeks.

Denin surface pretreatment using aspartic acid:
For Group I, the dentin surfaces were conditioned. Specimens were immersed in 10 ml of 10 mmol/L DL-Aspartic acid solution (Sigma-Aldrich, St. Louis, MO, USA) and placed in a shaker at 37°C for 30 minutes. Afterward, the samples were rinsed with distilled water to remove unbound aspartic acid before the application of remineralizing agents [17].

Preparation and application of remineralizing agents:
Group A: Chitosan-loaded Nano-hydroxyapatite (Cs-nHA)
A 10% w/w Chitosan-loaded paste was prepared by incorporating Chitosan powder (low molecular weight, Sigma-Aldrich, St. Louis, MO, USA) into a commercially available Nano-hydroxyapatite paste (Remin Pro, VOCO GmbH, Cuxhaven, Germany). To achieve the 10% concentration by weight, 0.5 g of Chitosan powder was first weighed separately. Subsequently, 4.5 g of Remin Pro paste was dispensed onto a tared glass slab using a precision electronic balance (Shimadzu, Japan). The powder was then manually mixed into the paste using a stainless-steel spatula via the geometric dilution method to ensure a homogeneous dispersion of the chitosan particles without significantly altering the consistency. The mixture was prepared fresh prior to application to ensure stability [18].

Application protocol: The paste was applied to the dentin surface via a micro-brush and rubbed gently for 2 minutes. This protocol was conducted twice daily, separated by a 12-hour interval, for six consecutive weeks. Excess paste was left undisturbed for an additional minute before being gently rinsed with deionized water [18].

Group B: Bio-Active Glass (BAG)
The commercial product used was BioMin F (BioMin Technologies Ltd, London, UK). To simulate clinical conditions, a slurry was prepared by mixing the paste with distilled water in a 1:3 ratio by weight (1 g of paste to 3 ml of distilled water) [19]. The mixture was vortexed for 30 seconds to ensure homogeneity.

Application protocol: The prepared slurry was applied to the dentin surface utilizing a soft-bristle toothbrush (Meridol; GABA, Lörrach, Germany). The specimens were brushed for 2 minutes twice daily, separated by a 12-hour interval, for six consecutive weeks [20]. Following brushing, the slurry was allowed to remain in contact with the surface for an additional 1 minute (total exposure time of 3 minutes) to facilitate ion exchange and precipitation before being gently rinsed with deionized water for 10 seconds [21].

Group C: Silver Diamine Fluoride (SDF)
The commercial agent used Advantage Arrest silver diamond fluoride (38% SDF, Elevate Oral Care, West Palm Beach, FL, USA).

Application protocol: The dentin surface was dried with a gentle stream of air. A single drop of SDF was applied directly to the lesion using a micro-applicator and permitted it to soak for 1 minute. After that, the surface was washed using distilled water for 30 seconds to remove excess silver ions [22]. This application was performed once at the beginning of the cycle and repeated weekly for the duration of the six-week study.

Storage of samples:
The Artificial Saliva (A.S.) was prepared in the laboratory with the following composition by weight %(wt): 0.08% Sodium chloride (NaCl), 0.12% Potassium chloride (KCl), 0.01% Magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$), 0.03% Potassium dihydrogen phosphate (KH_2PO_4), 0.01% Calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$), 0.10% Sodium carboxymethyl cellulose (CMC-Na), and 99.6% deionized water. The pH of the solution was adjusted to 7.0 using Sodium hydroxide (NaOH) [14].

After each remineralization session, the specimens were gently rinsed and immediately immersed in sealed jars containing 50 mL of fresh A.S. The jars were stored in an incubator at 37°C. To prevent solution saturation and mimic oral fluid turnover, the artificial saliva was renewed daily throughout the experimental period until the next treatment cycle [23].

Assessment: Fourier Transform Infrared Spectroscopy (FTIR):
Chemical representation of the dentin surfaces was performed at baseline, post-demineralization, and after the 2-week and 6-week remineralization periods. In total, 60 samples were employed for the analysis using an Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR-FTIR) system (Bruker VERTEX 80, Germany) equipped with a platinum diamond ATR accessory.

Within a spectral range of 600 to 4000 cm^{-1}, the spectra were captured in absorption modality during 124 scan accumulations at a resolution of 2 cm^{-1}. The absorption bands in the ATR-FTIR were linked to several molecular groups connected with the molecular makeup of the organic matrix elements (Amide I, II, III) and dentin mineral (v1, v3 PO_4^{3-} phosphate band).

To analyze the data, Curve fitting software (Peakfit v4.12, Systat Software, San Jose, CA, USA) was used to solve overlapping peaks and quantify their combined regions. The peak predictions across every spectral area were resolved using the subsequent derivative approach. A mixed Gaussian-Lorentzian function was employed to modify the peak profile after the degree of smoothing was set to 10% (Savitzky-Golay method). To explain the compositional qualities of dentin, the subsequent parameter was computed:

Mineral-to-Matrix Ratio (M:M): This parameter represents the proportion of minerals to the organic matrix. It was calculated as the proportion of the integrated area of the primary phosphate band (PO_4^{3-} stretch: ~1030 cm^{-1}) to the Amide I band (type I collagen: ~1640 cm^{-1}). A higher M:M ratio indicates a higher degree of mineralization and mineral density relative to collagen, whereas a lower ratio indicates a demineralized state [13,16].

Statistical analysis:
The SPSS software (Version 25.0) was used for statistical analysis, and the results were displayed as mean ± standard deviation. After verifying normality via the Shapiro-Wilk test, a Three-way ANOVA was employed to assess overall interactions. Specific group comparisons were conducted using One-way ANOVA followed by Tukey's HSD post-hoc test for materials. Furthermore, the independent t-test was employed to compare the means between two independent groups (e.g., Conditioned vs. Non-conditioned). Statistical significance was set at $p < 0.05$.

3. RESULTS

A Three-way ANOVA was conducted to assess the influences of conditioning, material kind, and storage period on the Mineral/Matrix ratio. The analysis revealed statistically significant interactions between all three variables ($p < 0.05$). Consequently, the data were separated to analyze the specific effect of each variable independently using t-tests and One-

way ANOVA.

Comparison of treated groups versus untreated demineralized dentin (baseline):
To evaluate the overall efficacy of the remineralizing treatments, the Mineral/Matrix ratios of all treated specimens were compared to untreated demineralized dentin (baseline caries lesions). Independent t-test analysis revealed statistically significant differences between the baseline demineralized dentin and all treatment groups at both time intervals ($p < 0.05$). Moreover, the test revealed that the untreated demineralized dentin exhibited the lowest Mineral/Matrix ratio (2.45 ± 0.32). Following treatment, all experimental groups, regardless of pre-conditioning status or storage duration (2 or 6 weeks), demonstrated a statistically significant increase in mineral content compared to the baseline ($p < 0.001$) (Table 1).

Comparative efficacy of different remineralizing materials:
A one-way ANOVA followed by Tukey's Honestly Significant Difference (HSD) post-hoc test was conducted to compare the efficacy of the three remineralizing agents. The analysis revealed a statistically significant overall difference among the tested materials ($p < 0.05$). In both 2 and 6 weeks, the Cs-nHA (Group A) recorded the highest mean mineral/matrix ratio significantly. This was followed by SDF (Group C). The lowest recorded mean mineral/matrix ratio was recorded with the BAG (Group B).

Post-hoc pairwise comparisons using the Tukey HSD test revealed that the mean Mineral/Matrix ratio of the Cs-nHA group was significantly higher than both the SDF and BAG groups ($p < 0.05$). However, there was no statistically significant difference found between the SDF and BAG groups ($p > 0.05$), indicating comparable remineralization potential between these two agents (Table 1).

Table 1: Comparison of Mineral/Matrix ratio between untreated demineralized dentin (Baseline) and treated groups at 2 and 6 weeks.

Treatment Group	Baseline (Mean ± SD)	2-Week (Mean ± SD)	6-Week (Mean ± SD)	P-value (vs. Baseline)
Cs-nHA (Group A)				
With Asp	2.45 ± 0.32	9.67 ± 1.13[a]	12.44 ± 0.50[a]	<0.001*
Without Asp	2.45 ± 0.32	7.89 ± 0.81[a]	9.91 ± 0.59[a]	<0.001*
SDF (Group C)				
With Asp	2.45 ± 0.32	5.19 ± 0.85[b]	7.12 ± 0.49[b]	<0.001*
Without Asp	2.45 ± 0.32	3.78 ± 0.54[b]	5.85 ± 0.47[b]	<0.001*
BAG (Group B)				
With Asp	2.45 ± 0.32	5.17 ± 0.84[b]	6.93 ± 0.38[b]	<0.001*
Without Asp	2.45 ± 0.32	3.57 ± 0.51[b]	5.81 ± 0.13[b]	<0.001*

** Indicates statistically significant difference compared to Baseline (Independent t-test, $p < 0.05$).*
[a],[b] Different superscript letters indicate statistically significant differences among remineralizing materials within each time point (One-way ANOVA with Tukey's HSD post-hoc test, $p < 0.05$). Same letters indicate no significant difference.

Effect of aspartic acid pre-conditioning on mineral/matrix ratio:
The impact of pre-conditioning was evaluated by comparing dentin specimens treated with Aspartic acid (Asp) versus non-conditioned specimens. An independent t-test study showed that there was a statistically significant variation among the two main groups at both time intervals ($p < 0.05$).

Regardless of the remineralizing agent used or the storage duration, specimens pre-conditioned with Asp consistently displayed significantly greater mean Mineral/Matrix ratios in comparison to the non-conditioned group. Specifically, the Cs-nHA group showed the most pronounced increase in mineralization following Asp conditioning, followed by the SDF and Bio BAG groups (Tables 2 and 3).

Table 2: Comparison of the mean Mineral/Matrix ratio for dentin with and without Asp pre-conditioning after 2 weeks.

Remineralizing Agent	With Asp (Mean ± SD)	Without Asp (Mean ± SD)	t-value	p-value
Cs-nHA (Group A)	9.67 ± 1.13	7.89 ± 0.81	2.85	0.021*

SDF (Group C)	5.19 ± 0.85	3.78 ± 0.54	3.10	0.015*
BAG (Group B)	5.17 ± 0.84	3.57 ± 0.51	3.61	0.006*

** Significant at p < 0.05.*

Table 3: Comparison of the mean Mineral/Matrix ratio for dentin with and without Asp pre-conditioning after 6 weeks.

Remineralizing Agent	With Asp (Mean ± SD)	Without Asp (Mean ± SD)	t-value	p-value
Cs-nHA (Group A)	12.44 ± 0.50	9.91 ± 0.59	7.30	< 0.001*
SDF (Group C)	7.12 ± 0.49	5.85 ± 0.47	4.12	0.003*
BAG (Group B)	6.93 ± 0.38	5.81 ± 0.13	6.26	< 0.001*

** Significant at p < 0.05.*

Effect of storage time on remineralization (2 vs. 6 weeks):

Independent t-test analysis revealed a significant difference between the tested groups with and without Asp pre-conditioning for different remineralizing materials after 2 and 6 weeks ($p < 0.05$).

The results demonstrated that dentin specimens remineralized with and without Asp pre-conditioning across all remineralizing materials recorded significantly higher mean Mineral/Matrix ratios after 6 weeks compared to 2 weeks ($p < 0.05$) (Tables 4 and 5).

Table 4: Comparison of the Mineral/Matrix ratio for dentin pre-conditioned with Asp after 2 and 6 weeks.

Remineralizing Agent	2-Week (Mean ± SD)	6-Week (Mean ± SD)	t-value	p-value
Cs-nHA (Group A)	9.67 ± 1.13	12.44 ± 0.50	4.99	0.001*
SDF (Group C)	5.19 ± 0.85	7.12 ± 0.49	4.36	0.002*
BAG (Group B)	5.17 ± 0.84	6.93 ± 0.38	4.25	0.003*

** Significant at p < 0.05*

Table 5: Comparison of the Mineral/Matrix ratio for dentin without pre-conditioning after 2 and 6 weeks.

Remineralizing Agent	2-Week (Mean ± SD)	6-Week (Mean ± SD)	t-value	p-value
Cs-nHA (Group A)	7.89 ± 0.81	9.91 ± 0.59	4.48	0.002*
SDF (Group C)	3.78 ± 0.54	5.85 ± 0.47	6.42	< 0.001*
BAG (Group B)	3.57 ± 0.51	5.81 ± 0.13	9.48	< 0.001*

** Significant at p < 0.05.*

4. DISCUSSION

Remineralization is the natural process of restoring minerals lost during demineralization. Conventional approaches primarily promote surface-level repair and have limited penetration into the dentin matrix. As dentin is more porous and collagen-rich compared to enamel, successful repair requires deeper, intrafibrillar mineral deposition [24]. Two main theories explain this process. The classical (ion-based) theory relies on preexisting HA crystals as templates, whereas the nonclassical pathway emphasizes amorphous calcium phosphate (ACP) nano-precursors infiltrating collagen fibrils to enable intrafibrillar remineralization without seed crystals [25]. This shift has driven interest in biomimetic agents that mimic noncollagenous proteins (NCPs), which naturally regulate mineral nucleation and growth through acidic functional groups [26]. Amino acids such as aspartic acid, abundant in NCPs, have emerged as promising biomimetic tools due to their ability to modulate ACP transformation into organized HA crystals and promote mechanical recovery [12].

Fourier Transform Infrared (FTIR) spectroscopy is a popular, non-invasive analytical method for evaluating the chemical composition of dentin, particularly in the context of remineralization studies. FTIR provides distinct signatures for both organic (primarily collagen) and inorganic (mainly hydroxyapatite) components of dentin, allowing for the quantification of changes in mineral content relative to the organic matrix [27].

Furthermore, the experimental design employed artificially demineralized dentin lesions created through a pH-cycling methodology, followed by remineralization in artificial saliva. The oral environment is not static; rather, it is characterized by dynamic pH fluctuations resulting from dietary intake and bacterial metabolism. By subjecting the dentin specimens to alternating periods of demineralization and remineralization for 14 days, this study effectively mimicked the natural cariogenic challenges that teeth undergo [28]. This approach is supported by a previous review [29], which identified the pH-cycling model as the most prevalent method (48%) for inducing dentin demineralization and evaluating the response of restorative materials to caries.

Therefore, this study evaluated the influence of a conditioning solution of DL-aspartic amino acid on the enhancement of dentin remineralization using three remineralization agents: Chitosan-loaded Nano-hydroxyapatite, Bio-Active Glass, and Silver Diamine Fluoride. The evaluation was conducted using FTIR spectroscopy.

The results of this study demonstrated that dentin specimens pre-conditioned with Aspartic acid consistently showed significantly greater Mineral/Matrix ratios in comparison to the non-conditioned group, regardless of the remineralizing agent used. This result agrees with Zhao et al.[12], who found that pre-conditioning dentin with aspartic acid promoted remineralization of demineralized dentin. They stated that Aspartic acid facilitates the crystallization kinetics of amorphous calcium phosphate (ACP) to nano-apatite, leading to both internal and external mineralization of collagen fibers.

Indeed, Aspartic acid functions as a successful analog to natural non-collagenous proteins (NCPs), which are inherently rich in acidic amino acids, including aspartic acid residues [12]. In natural dentinogenesis, these NCPs possess two essential functions: they bind strongly to collagen fibrils and sequester calcium ions to initiate crystallization. When dentin becomes demineralized through carious or non-carious processes, these natural NCPs are often lost or denatured, leaving the collagen matrix depleted of the nucleation sites necessary for mineral deposition. The pre-conditioning protocol addresses this fundamental limitation [11].

Moreover, Aspartic acid is highly negatively charged, enabling it to bind to specific amino acid sequences on collagen fibrils and attract calcium ions from the remineralizing solution through electrostatic interactions [12]. This mechanism operates through the Polymer-Induced Liquid-Precursor process, wherein aspartic acid stabilizes ACP precursors and facilitates their transformation into stable apatite crystals [30]. Previous research has demonstrated that aspartic acid can accelerate the crystallization kinetics of ACP-stabilized precursors into hydroxyapatite [12]. Furthermore, Aspartic acid induces the formation of artificial nucleation sites, a process termed heterogeneous nucleation, that mimics the natural mineralization front created by NCPs [12]. The presence of aspartic acid promotes intrafibrillar mineralization, wherein mineral crystals are deposited within the gap zones of collagen fibrils, leading to structural restoration analogous to natural dentin [12].

Among the tested materials, the Cs-nHA group recorded the highest mineral/matrix ratio significantly at all time points and was significantly superior to both SDF and BAG. This may be due to the nanoparticles of Cs-nHA having a higher surface to volume ratio, which are more effective than the bigger particles (SDF and BAG) and lead to more dentinal tubules' infiltration [31]. Also, this superior performance may be due to the chitosan's unique ability to stabilize nHA, making it a promising candidate for developing biomimetic remineralizing agents. This is due to the strong electrostatic interactions and hydrogen bonding between the positively charged amino groups of chitosan and the negatively charged phosphate groups of nHA. These interactions create a protective sheath around nHA crystals, inhibiting their aggregation and dissolution, and promoting their growth and adhesion to tooth surfaces [32]. Additionally, chitosan acts as a bio-adhesive scaffold, prolonging the retention of remineralizing agents at the lesion site and preventing the agglomeration of nanoparticles, thereby facilitating deeper penetration into the dentinal tubules [33]. This result is in line with that of Geevarghese et al.[34], who found that the addition of chitosan to nHA drastically enhanced the remineralization potential of the demineralized dentin.

Apart from this, by continually drawing substantial quantities of calcium and phosphate ions from the surrounding media (saliva) to the tooth tissue, nHA works as a template in the remineralization processes when it penetrates dentinal tubules, encouraging crystal integrity and growth [35]. Indeed, nHA closely resembles the morphology of the original apatite crystals in the dental tissue in the form of needle-like crystals, and this will enable the formation of biomimetic apatite coating on the dentin surfaces [36].

The present research's findings showed that both SDF and BAG significantly improved the mineral content compared to

demineralized dentin. Regarding SDF, the solution's fluoride ions enhance the remineralizing capability. Fluoride precipitates calcium fluoride and generates a stronger acid-resistant fluorapatite crystal in a mixture of calcium and phosphate, increasing the mineral's hardness and density [9]. Additionally, it has been demonstrated that SDF increases the degradation resistance of the collagenous matrix, which serves as a scaffold for the fresh crystal deposition, by blocking matrix metalloproteinases and creating a silver-protein conjugation [8]. This finding in line with Abdellatif et al.[37], who found that SDF has the potential to remineralize dentin caries-like lesions.

Nonetheless, the intermediate ranking of SDF may be due to the formation of an insoluble silver phosphate layer that blocks tubules, potentially rendering the external dentin layer impermeable to additional mineral gain [37]. Also, it may be due to the fact that SDF requires weekly reapplication compared to twice-daily application of Cs-nHA, potentially limiting overall mineral deposition kinetics.

Regarding BAG, it operates via rapid ion exchange upon saliva contact, releasing calcium, sodium, and phosphate ions. This leads to a localized pH increase, resulting in a calcium phosphate layer that crystallizes into hydroxycarbonate apatite (HCA), resembling natural tooth mineral [7]. The present findings are consistent with those of Wu et al. [38], who stated that BAG possessed a promising remineralization effect on carious dentin.

However, the lower mineral-to-matrix ratios observed with BAG in this study may be due to BAG forming a protective biomimetic coating, which may prevent further mineral gain [39]. Additionally, the absence of a significant difference between SDF and BAG indicates comparable remineralization potential between these two agents. Both form apatite-like minerals (fluorapatite and hydroxycarbonate apatite, respectively) through distinct ionic pathways, achieving similar overall mineral precipitation despite mechanistic differences [40].

In the present study, remineralized dentin specimens recorded significantly higher mean mineral-to-matrix ratios after 6 weeks than those recorded after 2 weeks, across all experimental groups. This may be related to increasing time contact with the corresponding remineralizing agent, leading to the formation of an outer mineral-rich layer in dentin, which increases the mineral percentage and is more resistant to acid attacks [41].

Furthermore, at the 2-week interval, the mineral deposits formed by remineralizing agents may still be in a precursor phase, amorphous calcium phosphate (ACP) or octacalcium phosphate, which are less dense and have different spectral signatures than fully crystallized apatite. Over the course of 6 weeks, these precursors undergo phase transformation and maturation [42]. Through a process known as Ostwald ripening, smaller, less stable crystals dissolve and redeposit onto larger, thermodynamically stable crystals [43].

Based on our results, the null hypotheses were rejected, as significant differences were observed regarding pre-conditioning, material type, and storage time. This finding has important clinical implications: it demonstrates that extended application and storage periods are necessary to achieve optimal remineralization. Single applications or short-term treatments, even if successful at initial mineralization, do not fully exploit the material's remineralizing potential. The sustained 6-week regimen, with twice-daily Cs-nHA application or appropriately timed SDF and BAG applications maintained throughout, represents a necessary commitment to achieve biomimetically complete dentin restoration.

The limitations of this study include its in-vitro nature, which cannot fully replicate the biological complexity of the oral cavity. Additionally, the artificial demineralized dentin lesions do not accurately reflect the intricate biochemical environment found in clinical carious lesions, such as bacterial biofilms and proteolytic degradation products. Furthermore, the experimental duration was limited to six weeks. Future research should focus on conducting long-term in situ trials using biological caries models to validate the durability of the remineralized layer in dynamic oral conditions.

5. CONCLUSIONS

Preconditioning dentin surfaces with Aspartic acid markedly enhances the dentin remineralization potential of the tested remineralizing agents. The application of various remineralizing agents improves dentin remineralization, with Cs-nHA yielding the best results among SDF and BAG, while there was no discernible variation in effectiveness among them. Additionally, extending the storage time of the remineralizing agents further promotes dentin remineralization.

REFERENCES

[1] Fejerskov O. Concepts of dental caries and their consequences for understanding the disease. Community Dent Oral Epidemiol. 1997;25(1):5–12.
[2] Dionysopoulos D, Gerasimidou O, Beltes C. Dentin Hypersensitivity: Etiology, Diagnosis and Contemporary Therapeutic Approaches—A Review in Literature. Appl Sci. 2023;13(21):12002.
[3] Wiglusz K, Dobrzynski M, Gutbier M, Wiglusz RJ. Nanofluorapatite Hydrogels in the Treatment of Dentin

Hypersensitivity: A Study of Physiochemical Properties and Fluoride Release. Gels. 2023;9(4):340.
[4] Luo X, Niu J, Su G, Zhou L, Zhang X, Liu Y, et al. Research progress of biomimetic materials in oral medicine. J Biol Eng. 2023;17(1):56.
[5] Pushpalatha C, Gayathri VS, Sowmya SV, Augustine D, Alamoudi A, Zidane B, et al. Nanohydroxyapatite in dentistry: a comprehensive review. Saudi Dent J. 2023;35(6):741–52.
[6] Bushra A, Subhani A, Islam N. A comprehensive review on biological and environmental applications of chitosan-hydroxyapatite biocomposites. Compos Part C Open Access. 2023;12:100364.
[7] Raszewski Z, Chojnacka K, Mikulewicz M. Investigating bioactive-glass-infused gels for enamel remineralization: an in vitro study. J Funct Biomater. 2024;15(5):124.
[8] Alowid AM, Hebbal M, Almufarji FS, Almutairi GG, Alkait SS, Jodalli P. Antimicrobial effect of silver diamine fluoride (SDF) in arresting dentine caries of permanent teeth: A Systematic review. F1000Res. 2024;13:144.
[9] Epple M, Enax J, Meyer F. Prevention of Caries and Dental Erosion by Fluorides—A Critical Discussion Based on Physico-Chemical Data and Principles. Dent J (Basel). 2022;10(1):5.
[10] Niu LN, Zhang W, Pashley DH, Breschi L, Mao J, Chen JH, et al. Biomimetic remineralization of dentin. Dent Mater. 2014;30(1):77–96.
[11] Nysel DEN, Fathy SM, Osman MF. Biomimetic remineralization of artificial dentinal carious lesions using two different bioactive capping materials. Mansoura J Dent. 2019;6(2):34–43. *(Note: Corrected author name and issue number).*
[12] Zhao L, Sun J, Zhang C, Chen C, Chen Y, Zheng B, et al. Effect of aspartic acid on the crystallization kinetics of ACP and dentin remineralization. J Mech Behav Biomed Mater. 2021;115:104239.
[13] Lopes C de CA, Limirio PHJO, Novais VR, Dechichi P. Fourier transform infrared spectroscopy (FTIR) application chemical characterization of enamel, dentin and bone. Appl Spectrosc Rev. 2018;53(9):747–69.
[14] Aras A, Celenk S, Dogan M, Bardakci E. Comparative evaluation of combined remineralization agents on demineralized tooth surface. Niger J Clin Pract. 2019;22(11):1546–52.
[15] Marquezan M, Corrêa FNP, Sanabe ME, Rodrigues Filho LE, Hebling J, Guedes-Pinto AC, et al. Artificial methods of dentine caries induction: a hardness and morphological comparative study. Arch Oral Biol. 2009;54(12):1111–7.
[16] Cifuentes-Jiménez CC, Bolaños-Carmona MV, Enrich-Essvein T, González-López S, Álvarez-Lloret P. Evaluation of the remineralizing capacity of silver diamine fluoride on demineralized dentin under pH-cycling conditions. J Appl Oral Sci. 2023;31:e20220410.
[17] Zhang Y, Wang Z, Jiang T, Wang Y. Biomimetic regulation of dentine remineralization by amino acid in vitro. Dent Mater. 2019;35(2):298–309.
[18] Rangappa KG, Garain R, Ahmad ZH, Jain K, Arroju R, Boregowda V. Remineralization potential of a combination of chitosan with nanohydroxyapatite and a self-assembling peptide with nanhydroxyapatite: an in vitro study. J Contemp Dent Pract. 2022;23(12):1255–9.
[19] Sadiasa A, Dong-Woo J, Deb Nath S, Seo HS, Yang HM, Lee BT. Addition of hydroxyapatite to toothpaste and its effect to dentin remineralization. Korean J Mater Res. 2013;23(3):168–76.
[20] Ganss C, Marten J, Hara AT, Schlueter N. Toothpastes and enamel erosion/abrasion – impact of active ingredients and the particulate fraction. J Dent. 2016;54:62–7.
[21] Tschoppe P, Zandim DL, Martus P, Kielbassa AM. Enamel and dentine remineralization by nano-hydroxyapatite toothpastes. J Dent. 2011;39(6):430–7.
[22] Yılmaz N, Ocak M, Ökte Z. Remineralization of primary molar dentine with silver diamine fluoride and sodium fluoride: an in vitro study. Cumhur Dent J. 2020;23(4):340–7.
[23] Berg C, Unosson E, Engqvist H, Xia W. Comparative study of technologies for tubule occlusion and treatment of dentin hypersensitivity. J Funct Biomater. 2021;12(2):25.
[24] Yang Q, Zheng W, Zhao Y, Shi Y, Wang Y, Sun H, et al. Advancing dentin remineralization: Exploring amorphous calcium phosphate and its stabilizers in biomimetic approaches. Dent Mater. 2024;40(8):1282–95.
[25] He L, Hao Y, Zhen L, Liu H, Shao M, Xu X, et al. Biomineralization of dentin. J Struct Biol. 2019;207(2):115–22.
[26] Jee SS, Thula TT, Gower LB. Development of bone-like composites via the polymer-induced liquid-precursor (PILP) process. Part 1: Influence of polymer molecular weight. Acta Biomater. 2010;6(9):3676–86.
[27] Moretti P, Rosi F, Miliani C, Daugherty M, van den Berg KJ, Cartechini L. Non-invasive reflection FT-IR spectroscopy for on-site detection of cleaning system residues on polychrome surfaces. Microchem J. 2020;157:104943.
[28] Zuluaga-Morales JS, Bolaños-Carmona MV, Álvarez-Lloret P. pH-cycling, Dynamic Chemical Model In Dentine Caries Creation. A Systematic Review And Meta-Analysis. Int J Med Dent. 2022;26(4):585–608.
[29] Fu Y, Ekambaram M, Li KC, Zhang Y, Cooper PR, Mei ML. In Vitro Models Used in Cariology Mineralisation Research—A Review of the Literature. Dent J (Basel). 2024;12(10):314.
[30] Veis A, Dorvee JR. Biomineralization mechanisms: A new paradigm for crystal nucleation in organic matrices. Calcif Tissue Int. 2013;93(4):307–15.

[31] Vano M, Derchi G, Barone A, Pinna R, Usai P, Covani U. Reducing dentine hypersensitivity with nano-hydroxyapatite toothpaste: a double-blind randomized controlled trial. Clin Oral Investig. 2018;22(1):313–20.
[32] Rizeq BR, Younes NN, Rasool K, Nasrallah GK. Synthesis, bioapplications, and toxicity evaluation of chitosan-based nanoparticles. Int J Mol Sci. 2019;20(22):5776.
[33] Nimbeni SB, Nimbeni BS, Divakar DD. Role of chitosan in remineralization of enamel and dentin: A systematic review. Int J Clin Pediatr Dent. 2021;14(4):562–8.
[34] Geevarghese S, Mathew S, Mathew R. Evaluation of the effect of a remineralising paste containing strontium doped nanohydroxyapatite with Non-Collagenous protein analogue - Chitosan on the characteristics of partially demineralized dentin -An invitro study. Mater Today Proc. 2020;46:5958–63.
[35] Anil A, Ibraheem WI, Meshni AA, Preethanath RS, Anil S. Nano-Hydroxyapatite (nHAp) in the Remineralization of Early Dental Caries: A Scoping Review. Int J Environ Res Public Health. 2022;19(9):5630.
[36] Gopinath NM, John J, Nagappan N, Prabhu S, Kumar ES. Evaluation of Dentifrice Containing Nano-hydroxyapatite for Dentinal Hypersensitivity: A Randomized Controlled Trial. J Int Oral Health. 2015;7(8):118–22.
[37] Abdellatif AA, El-Tekeya MM, Talat DA, Nagui DA. In-Vitro Remineralizing Effect of Silver Diamine Fluoride on Dentin Caries in Primary Teeth. Alexandria Dent J. 2022;47(1):170–7.
[38] Wu Q, Mei ML, Wu X, Shi S, Xu Y, Chu CH, et al. Remineralising effect of 45S5 bioactive glass on artificial caries in dentine. BMC Oral Health. 2020;20(1):49.
[39] Elkassas D, Arafa A. The innovative applications of therapeutic nanostructures in dentistry. Nanomedicine. 2017;13(4):1543–62.
[40] Meshki R, Ghahramani N, Kouchak M, Taravati S. Comparison of the Effect of Bioglass, Chitosan, and SDF Compounds on Remineralization of Primary Caries Lesions in Primary Teeth: An in vitro Study. J Dent. 2024;25(3):229–35.
[41] Chen C, Mao C, Sun J, Chen Y, Wang W, Pan H, et al. Glutaraldehyde-induced remineralization improves the mechanical properties and biostability of dentin collagen. Mater Sci Eng C. 2016;67:657–65.
[42] Nudelman F, Lausch AJ, Sommerdijk NAJM, Sone ED. In vitro models of collagen biomineralization. J Struct Biol. 2013;183(2):258–69.
[43] Uskoković V, Uskoković DP. Nanosized hydroxyapatite and other calcium phosphates: Chemistry of formation and application as drug and gene delivery agents. J Biomed Mater Res B Appl Biomater. 2011;96(1):152–91.