Pesquisa Brasileira em Odontopediatria e Clínica Integrada 2024; 24:e220123
https://doi.org/10.1590/pboci.2024.018
ISSN 1519-0501 / eISSN 1983-4632

ORIGINAL ARTICLE

Assessment of Efficiency of Bioactive Glass, Self-Assembling Peptide, and Ozone Remineralising Agents on Artificial Carious Lesion

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Academic Editor: Wilton Wilney Nascimento Padilha

Received: July 20, 2022 / **Review:** June 08, 2023 / **Accepted:** June 27, 2023

How to cite: Alessa N, Ali Shah S, Bhardwaj R, Syed Ismail PM, Mahabob MN, Babaji P, et al. Assessment of efficiency of bioactive glass, self-assembling peptide, and ozone remineralizing agents on the artificial carious lesion. Pesqui Bras Odontopediatria Clín Integr. 2024; 24:e220123. https://doi.org/10.1590/pboci.2024.018

ABSTRACT

Objective: To assess the efficacy of bioactive glass, self-assembling peptide, and ozone-remineralizing agents on the artificial carious lesion. **Material and Methods:** On the extracted 60 premolar teeth, an artificial carious lesion/demineralization was created. Later, the remineralization of demineralized teeth was done with respective remineralizing agents (Group A: Calcium sodium phosphosilicate (bioactive glass), Group B: Self-assembling peptide, Group C: Ozone remineralizing agents and Group D (Control): De ionized water. The degree of demineralization and remineralization were evaluated using the Vickers Hardness Number. **Results:** There was a decrease in microhardness from baseline to demineralization in all the groups, and this reduction was found to be statistically considerable. After the remineralization of demineralized samples with respective remineralizing agents, there was an increase in microhardness of 312.38, 276.67, and 254.42 in groups A, B, and C, respectively. In contrast, in Group D, there were no changes. **Conclusion:** Bioactive glass and self-assembling peptides had higher remineralizing capacities, which can be used to treat early carious lesions.

Keywords: Dental Caries; Efficiency; Dental Enamel; Ozone.

Introduction

Continuous demineralization and remineralization occur at the interface between the tooth surface and biofilm after acid action [1-3]. If the demineralization phase continues for an extended period, excessive loss of minerals from tooth enamel may occur, which results in the loss of enamel structure with cavitations and dental caries [1].

Dental caries, in their initial phase of formation, can be remineralized, and this remineralization can be assisted by agents such as fluoride in the form of mouth rinse or dentifrice. The initial clinical sign of dental caries appears as a white spot [4,5].

Due to the drawbacks of fluoride, such as skeletal fluorosis and dental fluorosis [2], numerous non-fluoridated remineralizing agents were studied such as Casein phosphopeptide amorphous calcium phosphate (CPP-ACFP), CCP-ACFP, biomimetic remineralization materials, theobromine, bioactive glass (BAG-Novamin), Xylitol, Ozone, Sensistatetc, tricalcium phosphte (TCP), self-assembling peptide, and Nano-hydroxyapatite (nHAp) [2-4,6-12]. Various remineralizing agents are used for the noninvasive management of initial carious lesions. The remineralization efficacy of multiple agents can be evaluated through enamel surface microhardness (SMH), energy-dispersive spectroscopy (EDS), and laser fluorescence method [6,12].

Bioactive glass (BAG) is commercially available as Novamin (e.g., Bioglass) that has been crushed into a fine particulate size lesser than 20 μ. Bioactive glass can act as a biomimetic mineralizer similar to the body's mineralizing behavior and also influence cell signals in a way that helps restore tissue function and structure [13,14].

Self-assembling peptide (SAP) P11-4 was developed to regenerate enamel by forming matrix-mediated mineralization. These short-chain peptides have the belongings to assemble into a 3-dimensional structure resembling the extracellular matrix, thereby encouraging regeneration [7,10].

Ozone is the layer present on the outer core of the earth. It is an effective oxidizing agent. Ozone is capable of altering acidogenic and aciduric microorganisms to normal commensalism. The mechanism of Heal Ozone's action is related to ozone's potent antimicrobial properties and its ability to oxidize proteins associated with caries [15].

Studies related to bioactive glass, Self-Assembling Peptide, and ozone remineralizing agents on artificial carious lesions are very scarce; hence, this study was done to estimate the remineralizing effectiveness of BAG, Self-Assembling Peptide and ozone remineralizing agents on the artificial carious lesion.

Material and Methods

Study Design and Sampling

The present *in vitro* study included freshly extracted sixty premolar teeth for orthodontic reasons free from any pathology. The sample size of sixty premolar teeth was estimated using the power calculation $\alpha = 0.05$ and $\beta = 0.20$, with 80% being the power of the study. The study was done from March 2021 to September 2021. Samples were cleaned from stain calculus and soft tissue remnants. Later, a one-millimeter cut was created one millimeter beneath the CEJ junction, and the teeth were positioned in the acrylic slab with an exposed crown portion. A 5 mm × 5 mm window was formed on the buccal areas of all specimens. The crown portion was coated with a nail varnish, excluding the window. The enamel specimens were then stored in de-ionized water before testing.

Preparation of Artificial Enamel Lesion

In the present study, artificial carious lesion/demineralization was created by keeping teeth in calcium chloride (2.0 mmol/L) and trisodium phosphate [2.0 mmol/L] in a buffer solution of acetate (75 mmol/L) for five days at a 4.6 pH.

Remineralisation Process

Sixty demineralized enamel samples were then categorized into four groups with fifteen samples in each group: GA: Calcium sodium phosphosilicate (bioactive glass), GB: Self-assembling peptide, GC: Ozone remineralizing agents, and GD (Control): De ionized water. The remineralization of demineralized teeth was done with respective remineralizing agents three times a day for 12 days for 4 min. The ozone-generating HealOzone Unit (Cur Ozone; USA) was used for ozone application. The device allowed the application of high-concentration gaseous ozone at 2100 ppm with a flow rate of 615ccs/min to the demineralized tooth surface under controlled conditions. A self-assembling peptide consisting of 50 µl distilled water was used. It was applied and left uninterrupted on the tooth surface for 4 min to allow diffusion [8]. The control group was treated with de-ionized water only. Later, the samples were preserved in artificial saliva.

Microhardness Testing

The degree of demineralization and remineralization was evaluated with Vickers microhardness values at baseline, after demineralization, and after remineralization. The specimens were stabilized and tested with a tester. The specimens were subjected to a load of 50g with a dwell time of fifteen seconds to record the indentations. Two indentations were made to avoid any discrepancy in values, and an average value of both was obtained and tabulated. The values were recorded in terms of Vickers Hardness Number. The indentations formed were viewed carefully on the display monitor.

Data Analysis

The values obtained were tabulated and statistically evaluated with SPSS version 21.0 by Tukey HSD and ANOVA test. The p-value was set below 0.05.

Results

For all four sample types, microhardness was tested at baseline, after demineralization and remineralization. There was a decrease in enamel microhardness values after demineralization, with 31% demineralization in Group A, 28 % in Group B, 23 % in Group C, and 20 % in Group D, respectively. There was a decrease in microhardness from baseline to demineralization in all the groups, and this reduction was found to be statistically considerable (Table 1).

Table 1. Mean microhardness values at baseline and post-demineralization.

Groups	Baseline Value	Mean Post Demineralization	Percentage Demineralization	p-value
GA	328.56	238.32	31%	0.001
GB	319.36	231.65	28%	
GC	323.42	234.76	23%	
GD	346.23	238.18	20%	

After the remineralization of demineralized samples with respective remineralizing agents, there was an increase in microhardness of 312.38, 276.67, and 254.42 in groups A, B, and C, respectively. In contrast, in Group D, there were no changes. This indicated a higher remineralization percentage with Group A, followed by Groups B and C. There were 0% changes in Group D, a control group. The variation in microhardness values from demineralization to remineralization in all three tested groups appears to be statistically highly considerable (Table 2).

Table 2. Mean microhardness values at post-remineralization.

Group	Mean Post Demineralization	Mean Post Remineralization	Percentage of Remineralization
GA	238.32	312.38	36.1%
GB	231.65	276.67	27.5%
GC	234.76	254.42	17.1%
GD	238.18	238.28	0.0%

On intra-group comparison for remineralization potential, Group A to Group C and D was significant (0.001), and Group B with Group A was insignificant. There was statistically considerable variation among groups B with D, D with A, and D with B and C (Table 3). The highest remineralization was found in the present study in group A, followed by groups B and C. The inter-group comparison was significant.

Table 3. Comparison of percentage of remineralization potential between groups.

Groups		Mean Difference	p-value
GA (Bioactive Glass)	GB	-3.373	0.06
	GC	-21.318	0.001
	GD	-66.623	<0.001
GB (Self-Assembling Peptide)	GA	3.523	0.081
	GC	-14.325	0.001
	GD	-47.216	<0.001
GC (Ozone)	GA	21.326	0.002
	GB	13.523	0.05
	GD	-23.457	0.01
GD (Control)	GA	67.623	0.001
	GB	45.425	0.001
	GC	23.476	0.002

Discussion

The present concept identifies caries as a dynamic process that can be remineralized [4,16]. Demineralization can be reversed if the pH is neutralized and enough phosphate and calcium ions are available in the oral condition [17].

The present study evaluates the efficacy of 3 experimental groups: self-assembling peptides, bioactive glass, ozone remineralizing agents, and a control group on artificial carious lesions. We found a higher remineralization percentage with the self-assembling peptide and bioactive glass group, followed by ozone. Joshi et al. [13] concluded that bioactive glass, f-TCP, and nHAp indicated significant remineralization. Patil et al. [18] observed higher remineralization with tested products (CPP-ACPF, CPP-ACP, and tricalcium phosphate). Gangwar et al. [6] concluded that bioactive glass remineralizes artificially induced carious portions in deciduous teeth. The results are similar to our findings for the bioactive glass group.

Bioactive glass (Bioglass®) acts as a biomimetic mineralizer matching the body's mineralizing traits and influencing cell signals, thus restoring the tissue structure and function [6]. Bioactive glass can act as a

biomimetic mineralizer matching the body's mineralizing traits, while it also influences cell signals in a way that benefits tissue structure and function restoration. In contact with an aqueous environment, it discharges bio-available calcium, phosphate, and sodium ions, contributing to remineralization [13].

Self-assembling peptides (P11-4) are designed to undergo an immediate hierarchical self-assembly structure, forming three-dimensional fibrillar scaffolds. These assembled scaffolds encourage natural hard tissue remineralization through saliva by attracting calcium ions and reduce demineralization by resisting acid attacks. In addition, it enhances de novo hydroxyapatite precipitation [16]. Hence, the present study found better results with P11-4 than other tested groups. However, using bioactive glass (Novamin Technology) and self-assembling peptides in remineralizing enamel is promising.

The limitation of the study was the smaller sample size, and the study was *in vitro* evaluation. Further clinical research with a larger sample is needed.

Conclusion

Bioactive glass and self-assembling peptides had higher remineralizing capacity, which can be used clinically to treat early carious lesions.

Authors' Contributions

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PMSI	https://orcid.org/0000-0002-9661-8280	Writing - Review and Editing, Supervision and Project Administration.
MNM	https://orcid.org/0000-0001-8211-2193	Software and Validation.
PB	https://orcid.org/0000-0003-0605-0095	Conceptualization, Methodology, Investigation, and Writing - Review and Editing.
VVR	https://orcid.org/0000-0002-1056-2037	Writing - Original Draft and Visualization.

All authors declare that they contributed to a critical review of intellectual content and approval of the final version to be published.

Acknowledgments

Authors would like to thank the college of Dentistry Research Centre and Deanship of Scientific Research at King Saud University for funding this research project.

Financial Support

None.

Conflict of Interest

The authors declare no conflicts of interest.

Data Availability

The data used to support the findings of this study can be made available upon request to the corresponding author.

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