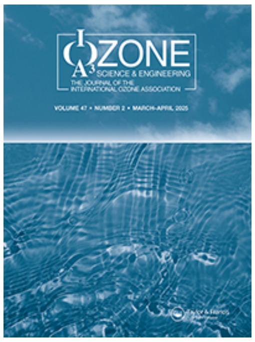

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Evaluation of the Effect of Ozone Gas and Nanohydroxyapatite Gel Application on Remineralization of Initial Enamel Caries: An In Vitro Study

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Evaluation of the Effect of Ozone Gas and Nanohydroxyapatite Gel Application on Remineralization of Initial Enamel Caries: An In Vitro Study

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ABSTRACT

The aim of this study was to evaluate the effect of several remineralization agents on artificial enamel lesions. Sixty orthodontically extracted, caries-free teeth were embedded in acrylic blocks, exposing the buccal surfaces and these surfaces were standardized with SiCi sandpaper (800,1000,1200 grit). Initial laser fluorescence and microhardness values from the samples were measured and recorded. The samples were exposed to a demineralization solution for 72 h to create artificial caries. The teeth were divided into six remineralization groups: Ozone, nano-HAP gel, Ozone + nano-HAP gel, Nano-HAP gel + Ozone, Fluoride varnish, and a negative control group, followed by a 7-day pH cycling process (18-h remineralization, 6-h demineralization in a day). After pH cycling, microhardness and laser fluorescence measurements were repeated. Following that; a post-treatment cycle was performed 7 days to assess the long-term effects of remineralization treatments. According to the obtained data, the initial microhardness values showed no significant differences between groups ($p > 0.05$). Statistical analysis revealing a significant difference between demineralization and remineralization processes ($p < 0.05$) and the most significant microhardness increase was seen in the nano-HAP gel + Ozone group ($p > 0.05$). Laser fluorescence measurements showed that lesion size decreased in all groups. However, after the post-treatment cycle, the nano-HAP gel + Ozone group was found to have the lowest microhardness ($p > 0.05$), indicating that although this combination provided the best remineralization, its effect was not permanent over time.

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Nanohydroxyapatite Gel; Ozone; PH Cycle; Remineralization

Introduction

Dental caries is a chronic condition that remains prevalent in society despite significant advancements in dental science (Sicca et al. 2016). The equilibrium between demineralization and remineralization of hard tissues of the teeth is crucial; any disruption of this balance favoring demineralization triggers caries formation. The cycle of these demineralization and remineralization processes determines the prognosis of carious lesions (Sezer and Kargül 2020). During demineralization, minerals such as calcium and phosphate are dissolved from the tooth structure. Conversely, remineralization refers to the process through which these lost minerals are reincorporated into the tooth tissue (Fontana et al. 2010). This remineralization process is initiated by the precipitation of calcium and phosphate ions from saliva onto the tooth surface, allowing the enamel tissue to recover its lost minerals (Taştan, Güler and Aytaç Bal 2021). Over the past 30 years, there has been a significant increase in understanding tooth decay and its progression. Throughout this period, numerous studies (Fontana et al. 2010; Moi, Tenuta and Cury 2008; Sezer and Kargül 2020; Taştan, Güler and Aytaç Bal 2021) have been conducted by dental professionals to maintain the demineralization-remineralization balance within the tooth. The primary objective of these studies was to halt the demineralization process through minimally invasive treatment procedures and subsequently initiate remineralization. This approach enhances the durability of the tooth's hard tissues and preserves both aesthetics and functionality.

Fluoride is the most widely used and proven effective remineralization agent for the prevention of dental caries formation and progression (Moi, Tenuta and Cury 2008). The fluoride ion in the oral environment exhibits caries-preventive and remineralizing effects due to its

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various properties. These include inhibiting plaque formation, preventing acid production by microorganisms within the plaque, reacting with hydroxyapatite in enamel to form a more caries-resistant fluorapatite compound, and facilitating the precipitation of calcium (Ca) and phosphate (PO_4) ions onto the tooth surface. While fluoride can exert its effects both systemically and topically, it is well-established that topical fluoride applications are more effective (Moi, Tenuta and Cury 2008). Among the most commonly used topical fluoride applications are fluoride toothpastes, dental flosses, and mouthwashes, which are suitable for individual use. In contrast, fluoride solutions, gels, and varnishes are designed for professional use. Numerous studies in the literature have demonstrated that various materials, either used alone or in combination with fluoride, are highly effective in reducing initial carious lesions. Fluoride varnishes are particularly recommended as the most suitable topical fluoride agents for professional application, especially for individuals at high risk for caries. This is because fluoride varnishes adhere to the tooth surface for a longer duration time compared to other agents and serve as a slow-release fluoride reservoir (Mishra et al. 2017).

There are some factors that limit the use of fluoride and fluoride compounds, which are highly effective in caries prevention and are considered the gold standard. The enamel protection potential of fluoride is approximately limited to the 30-micron outer surface of the tooth, and it has been reported that mineralization or regeneration in the subsurface layer of the lesion may not be fully achieved with such treatments (Alkilzy et al. 2018). Therefore, in recent years, researchers have focused on the development of more effective and safe agents with remineralization properties.

Ozone, a triatomic molecule composed of three oxygen atoms, exists in the environment as a gas. Due to its instability, ozone gas cannot be stored, and its half-life varies with changes in temperature and pressure depending on ambient conditions. In nature, ozone is formed when activated oxygen atoms, generated from the photolysis of diatomic oxygen molecules, react with additional oxygen molecules (Taştan, Güler and Aytaç Bal 2021). In dentistry, ozone is utilized as an alternative treatment option for the remineralization of initial carious lesions and is available in both gaseous and liquid forms. Its applications include preventing caries on root and occlusal surfaces, providing bactericidal effects, and mitigating dentin sensitivity (Baysan and Lynch 2004; Dähnhardt et al. 2008). Ozone's strong oxidizing properties enable it to open dentinal tubules affected by carious lesions, thereby facilitating the penetration of remineralization agents into the dentin (Azarpazhooh et al. 2009).

Hydroxyapatite is a calcium phosphate compound and is one of the various forms of calcium phosphate found in nature. It is, however, the most stable and least soluble form. Hydroxyapatite nanoparticles typically range from 1 to 100 nm in size, with particles derived from these dimensions exhibiting varying activities. The increased surface area and reduced size enhance the material's hydration, thereby improving its physical and chemical properties. The synthesis of nano-hydroxyapatite (nano-HAP) is technically demanding, involving methods such as sol-gel synthesis, solid-state reactions, co-precipitation, wet chemical precipitation, and hydrothermal reactions. Due to its structural similarity to the inorganic components of bone, the use of hydroxyapatite in dentistry is steadily increasing (Bordea et al. 2020). Nano-hydroxyapatite (nano-HAP) is considered one of the most widely used biocompatible and bioactive materials. Nanoparticles of hydroxyapatite show morphological and crystallographic similarities to HAP crystals found in tooth enamel and thus penetrate into the enamel microstructure and restore tooth structure and surface composition (Huang, Gao and Yu 2009).

Nano-HAP is utilized for the remineralization of dentin and enamel affected by caries. In the early stages of caries, hard tissue loses mineral ions due to acid attacks from bacterial metabolism, while the collagen network remains largely intact. Remineralization efforts aim to restore the organic structure by directly depositing minerals or using nanoparticles as carriers to facilitate the recovery of ions lost during carious processes (Bordea et al. 2020). For caries prevention, nano-HAP is incorporated into toothpastes to supply ions that mitigate demineralization and promote remineralization. These nanoparticles can infiltrate onto pores and form a protective layer on the tooth surface (Souza et al. 2015).

The aim of this study was to evaluate the remineralizing effect of various remineralizing agents on artificial caries lesions by laser fluorescence and Vickers microhardness measurement methods. It was aimed to evaluate whether the application of ozone gas before nanohydroxyapatite gel would increase the penetration of nanohydroxyapatite gel by evaluating the individual and combined effects of nano-HAP gel and ozone, which have become popular recently. In this direction, the null hypothesis of the study is: *"The application of ozone gas*

Table 1. Groups.

Groups	Group 1: Ozone (HealOzone, Curozone, KaVo, Biberach, Germany) Group 2: Nano-HAP gel (Biodent Medikal, Istanbul, Turkey) Group 3: Ozone (HealOzone, Curozone, KaVo, Biberach, Germany) + Nano-HAP gel(Biodent Medikal, Istanbul, Turkey) Group 4: Nano-HAP gel(Biodent Medikal, Istanbul, Turkey) + Ozone(HealOzone, Curozone, KaVo, Biberach, Germany) Group 5: Fluoride varnish (Proshield, President Dental Germany) Group 6: Control group (Non-treated)

before nanohydroxyapatite gel increases the remineralization in artificial caries."

Materials and methods

Ethical approval was received for the study from Bezmialem Vakıf University Dentistry Ethics Committee (Grant Number: 2024–270).

For this study, 60 orthodontically extracted teeth without caries, fracture or restoration were embedded into acrylic blocks with the buccal surfaces on top and exposed. The samples were standardized by smoothing the surfaces with 800, 1000 and 1200 grit silicon carbide sandpaper. Initial values of laser fluorescence and microhardness of samples were recorded with FluoreCam (Therametric; Indianapolis, IN, USA) and Vickers microhardness device (HMV-2; Shimadzu, Japan).

A demineralization solution developed by Amaechi (Amaechi 2019) consisting of 314.2 mg/L Ca $(NO_3)_2 \cdot 4\,H_2O$, 174.18 mg/L KH_2PO_4, 1225 mg/L CH_3COOH with the pH adjusted to 4.5 using 1 M KOH was used. The remineralization solution (artificial saliva) was composed of 3.8 mg/L Mg^{2+} ($MgCl_2 \cdot 6\,H_2O$), 84.36 mg/L $(PO_4)_3$ – (K_2HPO_4/KH_2PO_4) 50 mg/L Ca^{2+} (calcium lactate), 0.05 mg/L fluoride, 625 mg/L KCl, 400 mg/L carboxymethylcellulose, and 2000 mg/L methyl4-hydroxybenzoate, with the pH adjusted to 7.2 using 1 M KOH. Then samples were divided into 6 groups, tested materials are shown in Table 1.

Group 1: ozone

2.100 ppm ±10% concentration of ozone gas, ozone generation system was used with a silicone applicator cups to prevent any air contact of the teeth during 40 seconds of application ($n = 10$).

Table 2. The pH cycle (Chen and Huang 2009).

08:00-10:00	Demineralization Solution
10:00-10:10	Application of Remineralization Agent
10:10-12:00	Remineralization Solution
12:00-14:00	Demineralization Solution
14:00-14:10	Application of Remineralization Agent
14:10-16:00	Remineralization Solution
16:00-18:00	Demineralization Solution
18:00-18:10	Application of Remineralization Agent
18:10-08:00	Remineralization Solution

Group 2: nano-HAP gel

Nano-HAP gel was applied on the specimen for 1 min at 500 rpm with a slow speed handpiece using a rubber cap under dry conditions, according to the manufacturers' instructions (after hydrochloric acid application, nano-HAP gel was applied) ($n = 10$).

Group 3: ozone + nano-HAP gel

2.100 ppm ±10% concentration of ozone gas, ozone generation system was used with a silicone applicator cups to prevent any air contact of the teeth during 40 seconds of application. Then, Nano-HAP gel was applied to the specimen for 1 min at 500 rpm with a slow speed handpiece using a rubber cap under dry conditions, according to the manufacturers' instructions ($n = 10$).

Group 4: nano-HAP gel + ozone

Nano-HAP gel was applied to the specimen for 1 min at 500 rpm with a lowspeed handpiece using a rubber cap under dry conditions according to the manufacturers' instructions. Then 2.100 ppm ± 10% concentration of ozone gas ozone generation system was used, and silicone application cups were used to prevent air contact with the teeth during ozone application and ozone application was performed for 40 seconds ($n = 10$).

Group 5: fluoride varnish

Fluoride varnish was applied onto the specimen with a micro-brush under dry conditions. It was allowed to be absorbed for 4 min and was then air-dried according to the manufacturers' instructions ($n = 10$).

Group 6: control group (non-treated)

The samples were placed in a shaking incubator with the buccal surfaces facing upwards and kept in the demineralization solution for 72 h to create an artificial caries lesion.

pH cycle was performed for 7 days with 18 h of remineralization and 6 h of demineralization as shown in Table 2 (McKnight-Hanes and Whitford 1992).

After the pH cycling, FluoreCam and microhardness measurements of the samples were repeated. Following the remineralization measurements, for 7 days (post cycle) without any treatment stages to evaluate the permanence of the treatments, and then microhardness and FluoreCam measurements were performed.

The data obtained were analyzed using SPSS Statistics Version 25.0 (IBM SPSS Statistics 25.0, IBM Corporation, Armonk, NY, USA) software using repeated measures analysis of variance and Bonferroni test for pairwise comparisons. In addition, the delta difference between demineralization and remineralization was calculated using one-way analysis of variance and Tukey test for pairwise comparisons. $p < 0.05$ was accepted for significance level.

Results

According to the obtained results (Table 3), there were no significant differences between initial (T_0) microhardness values of the samples ($p > 0.05$). After the demineralization process (T_1), microhardness values of all groups were decreased ($p < 0.05$). The laser fluorescence results (Table 4) between T_0 - T_1 showed that artificial enamel lesions were increased in all tested material groups. A statistically significant increase was observed in the microhardness values of all tested material groups after the remineralization process (T_2) ($p < 0.05$), and supporting these results, a decrease in lesion sizes was observed in the laser fluorescence measurements performed after remineralization. After remineralization processes, microhardness values of all groups except the negative control group returned to their initial microhardness values. The most notable increase in microhardness values with the remineralization treatment was observed in the group receiving nano-HAP gel + Ozone treatment, but this increase was not statistically significant. When the durability of remineralization treatments

Table 3. Hardness values of initial, demineralization, remineralization and post-cycle treatments.

	Initial (T_0)	After demineralization (T_1)	After remineralization (T_2)	Post-cycle (T_3)	p
Ozone	259.13 ± 21.70^{B}	198.46 ± 17.96^{bA}	251.02 ± 24.71^{bB}	248.24 ± 18.83^{B}	<0.001
Nano-HAP Gel	271.16 ± 22.52^{B}	193.28 ± 18.60^{abA}	253.54 ± 52.03^{bB}	265.33 ± 23.93^{B}	<0.001
Ozone + Nano-HAP Gel	267.70 ± 35.09^{C}	187.56 ± 20.96^{abA}	266.71 ± 32.48^{bBC}	234.68 ± 30.77^{B}	<0.001
Nano-HAP Gel + Ozone	256.75 ± 26.14^{B}	168.93 ± 19.61^{aA}	269.32 ± 57.19^{bB}	233.96 ± 29.04^{B}	<0.001
Fluoride varnish	249.06 ± 19.72^{B}	191.16 ± 12.78^{abA}	257.93 ± 45.93^{bB}	240.82 ± 27.20^{B}	<0.001
Control	275.47 ± 21.51^{C}	194.76 ± 18.35^{bA}	193.13 ± 27.57^{aA}	239.21 ± 17.09^{B}	<0.001
p	0.182	0.011	0.002	0.066	

[a]Different lowercase letters indicate statistical difference between columns.
[b]Different uppercase letters indicate statistical difference between rows.

Table 4. Percent changes of lesion size with FluoreCam values of initial, demineralization, remineralization and post-cycle treatments (%).

	Initial(T_0)/Demineralization(T_1) (Δ)	Demineralization(T_1)/Remineralization(T_2) (Δ)	Remineralization (T_2)/Post Cycle(T_3) (Δ)
Ozone	426%	– 64%	49%
Nano-HAP Gel	377%	– 33%	1%
Ozone + Nano-HAP Gel	234%	– 72%	28%
Nano-HAP Gel + Ozone	405%	– 79%	104%
Fluoride	364%	– 13%	– 42%
Control	251%	– 16%	– 19%

was evaluated following the post cycle, the nano-HAP gel + Ozone group showed the lowest hardness results.

According to the statistical result of the delta difference between demineralization and remineralization, a non-significant difference was found in the microhardness measurements in the nano-HAP gel + ozone treatment. ($p = 0.589 \pm 0.282$) (Table 5).

According to the data obtained from the FluoreCam measurements, there was a decrease in the lesion size in all remineralization treatment groups. The highest decrease in the lesion size (– 79%) was obtained in the Nano-HAP Gel + Ozone group (Table 4).

Discussion

Numerous studies have assessed the remineralization efficacy of various materials on extracted teeth (Burwell, Litkowski and Greenspan 2009; Chen and Huang 2009; Faller, Eversole and Yan 2010; Huang et al. 2013; Karlinsey et al. 2011; Reynolds 1997). This study aimed to determine the remineralization effects of different remineralization agents. According to the results, no significant difference was found between the effectiveness of the tested materials. Since no difference was found between the microhardness values of the samples treated with ozone and nano-HAP gel after the remineralization process, the null hypothesis of the study, "*The application of ozone gas before nanohydroxyapatite gel increases the remineralization in artificial caries*", was rejected.

This study aimed to determine how the single and combined use of materials available in the market for remineralization therapy affects the success of remineralization, utilizing modern diagnostic methods. The null hypothesis established at the beginning of the study was as follows: The sequence of applying nano-hydroxyapatite gel and ozone in combination to the tooth surface alters the remineralizing effect of ozone. According to the results obtained from this study, all remineralization groups increased hardness and were statistically significant, however combined application of ozone and nano-HAP gel results were similar on remineralization in the order of application; therefore, the null hypothesis was rejected.

Table 5. Percent changes in microhardness values between demineralization and remineralization treatments (%).

Ozone	0.276 ± 0.197^{ab}
Nano-HAP Gel	0.323 ± 0.294^{b}
Ozone + Nano-HAP Gel	0.436 ± 0.294^{b}
Nano-HAP Gel + Ozone	0.589 ± 0.282^{b}
Fluoride varnish	0.357 ± 0.265^{b}
Control	-0.007 ± 0.119^{a}
p	<0.001

Based on the microhardness results of the study, the lowest remineralization was observed with the single application of ozone gas (0.276 ± 0.197), while the highest remineralization amount was seen with nano-HAP gel + ozone (0.589 ± 0.282), followed by ozone + nano-HAP gel (0.436 ± 0.294). Although the differences between the obtained microhardness values were not statistically significant, the combination of ozone and nano-hydroxyapatite gel appears to be effective in remineralizing on demineralized tooth surfaces. Grocholewich et al. (Grocholewicz et al. 2020) conducted an *in vivo* study assessing the effects of nano-HAP, ozone, and their combined application on initial enamel caries on the approximal surfaces of premolars and molars. Their findings support the results of the present study indicating that ozone and nano-HAP gel, the combined application, promote more remineralization on initial caries

The least value obtained by the control group highlights the importance of remineralization agents on initial enamel lesions. These results could provide a robust foundation for the use of agents such as ozone and nano-HAP gel as chemical dental treatment. Additionally, the effectiveness of fluoride varnish applications in remineralization processes is demonstrated (He et al. 2016; Mohd Said, Ekambaram and Yiu 2017). These findings offer valuable insights for future research.

Ozone exerts its effects by attacking glycoproteins, glycolipids, and other amino acids disrupting enzymatic control systems in cells. Consequently, cell membrane permeability increases, leading to membrane expansion and ultimately cell death. It is hypothesized that the oxidative effect of ozone removes glycoproteins from demineralized areas, facilitates increased diffusion of calcium and phosphate ions, and thereby supports the remineralization of lesions (Baysan and Lynch 2007). Although the current study data show that ozone alone has the lowest remineralization efficacy, its significant oxidative effect, when combined with another remineralizing agent, substantially supports remineralization. Erçin et al. (Erçin et al. 2024), in their *in vitro* study, found no statistical difference between the surface hardness values of the group treated with ozone alone and the control group, whereas groups with combined applications of ozone and remineralizing agents exhibited significantly higher surface hardness values, supporting the findings of the present study. The reason for the lowest hardness value obtained with ozone alone might be low amount of minerals in the pH cycling model since porotic areas left need to be filled with for the adequate remineralization value. Additionally, if the study is done as in vivo, minerals from saliva could provide minerals for the remineralization process.

Nie et al. (Nie, Li and Hu 2007) applied distilled water, fluoride, and ozone treatments to teeth with initial carious lesions. Evaluation of the fluorescence images of the lesions showed no significant difference between the deionized water and ozone groups, with fluoride being the most effective treatment. According to the present study, ozone and fluoride treatments showed similar remineralization efficacy with pH cycling. On the other side, a significant drop on remineralization values was observed in the fluoride group after a 7-day post-acid challenge, whereas the ozone group did not show such decline. The surface enamel layer contains a higher amount of fluorapatite because fluorapatite becomes highly saturated at pH levels of 4.5–5 and contributes to remineralization. Different fluoride compounds result with increased fluoride uptake on the surface enamel layer but may be less effective in the body of the lesion. According to the FluoreCam values in the present study, it can be thought that the lesion values in the fluoride-applied group decreased more than the control group, which may be due to the higher fluoride concentration in the superficial layer and the fact that FluoreCam measurements are based on light scattering from the surface layer of the lesion.

The current study is an in vitro study and cannot fully mimic oral conditions due to the absence of many external factors such as saliva and bacteria that are found in the human mouth and have great effects on remineralization and demineralization. At the same time, the remineralization activity in our study was evaluated for only 7 days, and therefore only the short-term activities of the tested materials could be evaluated. Further studies are needed to evaluate the long-term effects of the materials and the permanence of this activity.

Conclusion

According to the results of this study, all tested remineralization agents showed efficacy. In this in vitro study, where 7-day remineralization conditions were evaluated, it was concluded that ozone application before nano-HAP gel did not show any positive effect. To eliminate possible bias due to sample size, future studies with larger sample sizes and longer effects are needed.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The data that support the findings of this study are available from the corresponding author, MK, upon request.

Ethical approval

Ethical approval was received for the study from Bezmialem Vakıf University Dentistry Ethics Committee (Grant Number: 2024–270).

References

Alkilzy, M., R. M. Santamaria, J. Schmoeckel, and C. H. Splieth. 2018. “Treatment of Carious Lesions Using Self-Assembling Peptides.” *Advances in Dental Research* 29 (1): 42–47. Feb. https://doi.org/10.1177/0022034517737025.

Amaechi, B. T. 2019. “Protocols to Study Dental Caries in Vitro: pH Cycling Models.” In *Methods in Molecular Biology*, 379–92. Vol. 1922. Humana Press Inc. https://doi.org/10.1007/978-1-4939-9012-2_34.

Azarpazhooh, A., H. Limeback, H. P. Lawrence, and E. D. Fillery. 2009. “Evaluating the Effect of an Ozone Delivery System on the Reversal of Dentin Hypersensitivity: A Randomized, Double-Blinded Clinical Trial.” *Journal of Endodontics* 35 (1): 1–9. Jan. https://doi.org/10.1016/j.joen.2008.10.001.

Baysan, A., and E. Lynch. 2004. “‘Management of Root Caries Using Ozone’.” Accessed December 4, 2024. [Online]. Available: https://anamoreira.km35633-02.keymachine.de/cmi/wp-content/uploads/2019/12/Gest%C3%A3o-de-C%C3%A1rie-Radicular-Usando-Ozono.pdf.

Baysan, A., and E. Lynch. 2007. "Clinical Reversal of Root Caries Using Ozone: 6-Month Results." *American Journal of Dentistry* 20 (4): 203–08. Aug.

Bordea, I. R., S. Candrea, G. T. Alexescu, S. Bran, M. Băciuţ, G. Băciuţ, O. Lucaciu, C. M. Dinu, and D. A. Todea. 2020. "Nano-Hydroxyapatite Use in Dentistry: A Systematic Review." *Drug Metabolism Reviews* 52 (2): 319–32. Apr. https://doi.org/10.1080/03602532.2020.1758713.

Burwell, A. K., L. J. Litkowski, and D. C. Greenspan. 2009. "Calcium Sodium Phosphosilicate (NovaMin ®): Remineralization Potential." *Advances in Dental Research* 21 (1): 35–39. Aug. https://doi.org/10.1177/0895937409335621.

Chen, C.-C., and S.-T. Huang. 2009. "The Effects of Lasers and Fluoride on the Acid Resistance of Decalcified Human Enamel." *Photomedicine and Laser Surgery* 27 (3): 447–52. Jun. https://doi.org/10.1089/pho.2008.2312.

Dähnhardt, J. E., M. Gygax, B. Martignoni, P. Suter, and A. Lussi. 2008. "Treating Sensitive Cervical Areas with Ozone. A Prospective Controlled Clinical Trial." *American Journal of Dentistry* 21 (2): 74–76. Apr.

Erçin, Ö., D. Kopuz, E. Alkan, D. Tağtekin, and F. Yanikoğlu. 2024. "Evaluation of the Effect of Different Remineralizing Agents and Ozone Application on Artificial Carises." *Ozone: Science & Engineering* 46 (6): 613–21. May. https://doi.org/10.1080/01919512.2024.2355879.

Faller, R. V., S. L. Eversole, and J. Yan. 2010. "Anticaries potential of a stabilized stannous-containing sodium fluoride dentifrice." *American Journal of Dentistry* 23 (B): 32B–8B. Sep.

Fontana, M., D. A. Young, M. S. Wolff, N. B. Pitts, and C. Longbottom. 2010. "Defining Dental Caries for 2010 and Beyond." *Dental Clinics of North America* 54 (3): 423–40. Jul. https://doi.org/10.1016/j.cden.2010.03.007.

Grocholewicz, K., G. Matkowska-Cichocka, P. Makowiecki, A. Droździk, H. Ey-Chmielewska, A. Dziewulska, M. Tomasik, G. Trybek, and J. Janiszewska-Olszowska. 2020. "Effect of Nano-Hydroxyapatite and Ozone on Approximal Initial Caries: A Randomized Clinical Trial." *Scientific Reports* 10 (1): 11192. Jul. https://doi.org/10.1038/s41598-020-67885-8.

He, T., X. Li, Y. Dong, Na Zhang, Y. Zhong, W. Yin, and D. Hu. 2016. "Comparative Assessment of Fluoride Varnish and Fluoride Film for Remineralization of Postorthodontic White Spot Lesions in Adolescents and Adults Over a 6-Month Period: A Single-Center, Randomized Controlled Clinical Trial." *American Journal of Orthodontics and Dentofacial Orthopedics* 149 (6): 810–19. Jun. https://doi.org/10.1016/j.ajodo.2015.12.010.

Huang, S. B., S. S. Gao, and H. Y. Yu. 2009. "Effect of Nano-Hydroxyapatite Concentration on Remineralization of Initial Enamel Lesion *In Vitro*." *Biomedical materials* 4 (3): 034104. Jun. https://doi.org/10.1088/1748-6041/4/3/034104.

Huang, Y., Y. Duan, Y. Qian, R. Huang, Z. Yang, Y. Li, and Z. Zhou. 2013. "Remineralization Efficacy of a Toothpaste Containing 8% Arginine and Calcium Carbonate on Enamel Surface." *American Journal of Dentistry* 26 (5): 291–97. Oct.

Karlinsey, R. L., A. C. Mackey, T. J. Walker, K. E. Frederick, D. D. Blanken, S. M. Flaig, and E. R. Walker. 2011. "In vitro Remineralization of Human and Bovine White-Spot Enamel Lesions by NaF Dentifrices: A Pilot Study." *Journal of Dentistry and Oral Hygiene* 3 (2): 22–29. Feb.

McKnight-Hanes, C., and G. M. Whitford. 1992. "Fluoride Release from Three Glass Ionomer Materials and the Effects of Varnishing with or without Finishing." *Caries Research* 26 (5): 345–50. https://doi.org/10.1159/000261466.

Mishra, P., N. Fareed, H. Battur, S. Khanagar, M. Bhat, and J. Palaniswamy. 2017. "Role of Fluoride Varnish in Preventing Early Childhood Caries: A Systematic Review." *Dental Research Journal* 14 (3): 169. https://doi.org/10.4103/1735-3327.208766.

Mohd Said, S. N. B., M. Ekambaram, and C. K. Y. Yiu. 2017. "Effect of Different Fluoride Varnishes on Remineralization of Artificial Enamel Carious Lesions." *International Journal of Paediatric Dentistry* 27 (3): 163–73. May. https://doi.org/10.1111/ipd.12243.

Moi, G. P., L. M. A. Tenuta, and J. A. Cury. 2008. "Anticaries Potential of a Fluoride Mouthrinse Evaluated in vitro by Validated Protocols." *Brazilian Dental Journal* 19 (2): 91–96. https://doi.org/10.1590/S0103-64402008000200001.

Nie, L., X. Li, and D. Hu. 2007. "Effect of Ozone on the Remineralization of Enamel in Vitro]." *Zhonghua Kou Qiang Yi Xue Za Zhi* 42 (2): 102–05. Feb.

Reynolds, E. C. 1997. "Remineralization of Enamel Subsurface Lesions by Casein Phosphopeptide-Stabilized Calcium Phosphate Solutions." *Journal of Dental Research* 76 (9): 1587–95. Sep. https://doi.org/10.1177/00220345970760091101.

Sezer, B., and B. Kargül. 2020. "Current Remineralizing Agents in Caries Management." *Turkiye Klinikleri Journal of Dental Sciences* 26 (3): 472–86. https://doi.org/10.5336/dentalsci.2019-71685.

Sicca, C., E. Bobbio, N. Quartuccio, G. Nicolò, and A. Cistaro. 2016. "Prevention of Dental Caries: A Review of Effective Treatments." *Journal of Clinical and Experimental Dentistry*. https://doi.org/10.4317/jced.52890.

Souza, B. M., L. P. Comar, M. Vertuan, C. Fernandes Neto, M. A. R. Buzalaf, and A. C. Magalhães. 2015. "Effect of an Experimental Paste with Hydroxyapatite Nanoparticles and Fluoride on Dental Demineralisation and Remineralisation in situ." *Caries Research* 49 (5): 499–507. https://doi.org/10.1159/000438466.

Taştan, E., E. Güler, and F. Aytaç Bal. 2021. "An in-Vitro Investigation of the Effects of Different Remineralization Agents Combined with Laser and Ozone Therapy on the Initial Caries Lesions." *Turkiye Klinikleri Journal of Dental Sciences* 27 (2): 269–79. https://doi.org/10.5336/dentalsci.2020-74741.