



RESEARCH ARTICLE

COMPARATIVE EVALUATION OF THE EFFECT OF THREE REMINERALIZING AGENTS AND RESIN INFILTRATION ON THE SURFACE MICROHARDNESS OF ARTIFICIALLY DEMINERALIZED ENAMEL- AN IN VITRO STUDY

¹Dr. Tamanna, ²Dr. Jagvinder Singh Mann, ³Dr. Navjot Singh Khurana, ^{4,*}Dr. Mansi Aggarwal, ⁵Dr. Naveen Prasath and ⁶Dr. Jaspreet Kaur

^{1,4,5,6}PG (Conservative Dentistry & Endodontics), Department of Conservative Dentistry & Endodontics, Government Dental College & Hospital, Patiala; ²Professor & Head of Department, Department of Conservative Dentistry & Endodontics, Government Dental College & Hospital, Patiala; ³Assistant Professor, Department of Conservative Dentistry & Endodontics, Government Dental College & Hospital, Patiala

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*Corresponding author:

Dr. Mansi Aggarwal

ABSTRACT

Aim: The aim of this in-vitro study was to compare the efficacy of three different remineralizing agents, namely: - casein phosphopeptide -amorphous calcium phosphate (CPP-ACP), calcium sodium phosphosilicate (CaSP), calcium silicate sodium phosphate salts (CSSP), and resin infiltrate (RI) in restoring the hardness of demineralized enamel using Vickers microhardness testing machine. **Method:** Ninety non-carious central incisors were decoronated, embedded in acrylic, and an enamel window (4mm × 4mm) was exposed. Specimens were divided into three groups: Group 1 (intact enamel), Group 2 (demineralized, no treatment), and Group 3 (demineralized with treatment), further split into four sub-groups treated with CPP-ACP, Novamin, Calcium Silicate + Sodium Phosphate, and resin infiltration. Treatments except resin were applied twice daily for 28 days. All samples were stored in artificial saliva and tested for surface microhardness using a Vickers machine. Data was statistically analyzed to evaluate enamel hardness changes. **Results:** The highest surface hardness was seen in Group 1 (intact enamel), while Group 2 (demineralized) showed the lowest. Among treated samples, Group 3D (resin infiltration) had the greatest hardness, followed by 3C (CSSP), 3B (CaSP), and 3A (CPP-ACP), showing that resin infiltration was most effective. **Conclusion:** The study found that resin infiltration is the most effective in restoring enamel hardness, closely matching that of healthy enamel. CPP-ACP, CaSP, and CSSP also improved hardness and helped prevent decay. Early use of these treatments can strengthen teeth and stop cavities from progressing.

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INTRODUCTION

Dental caries is defined as an irreversible microbial disease of the calcified tissues of the teeth, characterized by demineralization of the inorganic portion and destruction of the organic portion of the tooth, which often leads to cavitation.^[1] Dental caries is a dynamic, multifactorial microbial disease, driven by biofilm and frequent sugar intake, leading to alternating cycles of demineralization and remineralization in dental hard tissues.^[2] Oral bacteria ferment carbohydrates to produce organic acids which lower the pH and cause the sub-surface dissolution of the hydroxyapatite crystals. Under normal physiological conditions (pH 7), saliva is supersaturated with calcium and phosphate ions which diffuse into the porosities created during acid mediated demineralisation episodes^[3,4].

The imbalance between demineralization and remineralization leads to early enamel caries. White spot lesions represent the first clinical stage of dental caries.^[5] These early-stage lesions can often be stopped from progressing. A range of minimally invasive treatment options exist, with remineralization of non-cavitated lesions being one of the most widely adopted approaches. The aim of remineralization is to supply ions like calcium, phosphate, and fluoride directly onto the demineralized tooth structure. Various Remineralizing agents with casein phosphopeptide -amorphous calcium phosphate (CPP-ACP), calcium sodium phosphosilicate (CaSP), calcium silicate sodium phosphate salts (CSSP), hydroxyapatite, self-assembling peptide, and resin infiltrates (RI) have been used to remineralize the enamel further, aiding in increasing the microhardness of enamel. CPP-ACP, a milk-derivative, comprises peptide fragments that are rich in phosphorylated seryl and glutamic acid residues that bind to amorphous calcium

phosphate nanoparticles. [6] CPP binds with ACP to form a stable complex that retains the bioavailability of calcium and phosphate ions in the oral environment. It maintains a supersaturated state with minerals to promote remineralization, and also hinders cariogenic bacterial colonization. The NovaMin™ technology is based on calcium sodium phosphosilicate bioactive glass which is claimed to release calcium and phosphate ions intra-orally to help the self-repair process of teeth. [7] It mainly consists of calcium, sodium, phosphorus, and silica which becomes highly reactive when it comes in contact with saliva, maintaining the super-saturated environment around the tooth. Additionally, it forms a protective layer of hydroxycarbonate apatite (HCA) on enamel which makes it more resistant to future demineralization. A promising approach for restoring enamel is the use of calcium silicate combined with sodium phosphate salts, using NR-5 technology. Calcium silicate, recognized for its bioactive capabilities, interacts with saliva to release calcium ions and Sodium phosphate salts complement this by acting as a plentiful source of phosphate ions, which are essential for forming hydroxyapatite and restoring enamel's hardness.

Another non-invasive alternative treatment based on the experiments conducted by Robinson et al. 1976 on caries infiltration with resorcinol-formaldehyde resin has also been advocated. Caries infiltration product ICON-DMG™ was introduced in the year 2009 in Germany; it is a low-viscosity resin that fills, strengthens, and stabilizes demineralized enamel, without causing any damage to healthy tooth structure. It also inhibits caries progression in initial carious lesions. [8] Both, remineralizing agents and resin infiltrates, have demonstrated significant efficacy in restoring enamel hardness. Remineralizing agents like fluoride, CPP-ACP, bioactive glass, and calcium silicate-sodium phosphate formulations replenish essential minerals, filling crystal voids and increasing enamel hardness. Resin infiltrates, on the other hand, provide a robust barrier that protects against future damage. Hence, the purpose of this in vitro study was to evaluate and compare the effect of CPP-ACP (GC Tooth Mousse), CaSP (Sensodyne repair and protect with Novamin), CSSP (Regenerate advance tooth paste, Enamel Science, France) and Resin Infiltration (ICON smooth surface kit DMG, Hamburg, Germany) on the surface microhardness of demineralized enamel.

SUBJECTS AND METHODS

This in-vitro study was conducted in the Department of Conservative Dentistry and Endodontics of Government Dental College and Hospital, Patiala, after obtaining the requisite clearance from the Institutional Ethics Committee. This study evaluated the efficacy of three different remineralizing agents, namely: -casein phosphopeptide -amorphous calcium phosphate (CPP-ACP), calcium sodium phosphosilicate (CaSP), calcium silicate sodium phosphate salts (CSSP), and resin infiltrate (RI) in restoring the hardness of demineralized enamel using Vickers microhardness testing machine.

Preparation of Specimens: A total of 90 non-carious human maxillary central incisors extracted for therapeutic purpose were selected for the study. All the specimens were decoronated & the crowns were sectioned and mounted in acrylic resin. An



Figure 1.

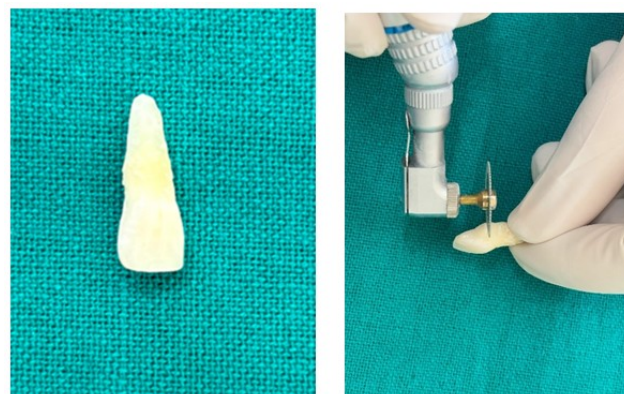


Figure 2

acid-resistant nail varnish was applied on exposed enamel leaving a window of enamel measuring 4mm x 4mm. All the specimens were stored in distilled water.

Inclusion criteria:

- Non-carious permanent maxillary central incisors with intact enamel.

Exclusion criteria

- Teeth with any carious lesion or prior restoration.
- Teeth with any crack or fracture.
- Teeth with any hypoplastic defect.
- Teeth with any crown discoloration.

Preparation of Demineralizing Solution: The demineralizing solution will be prepared with 2.2 mM calcium, 2.2 mM phosphate and 50 mM buffer (acetic acid/ K acetate) at pH of 4.7.

Preparation of Artificial saliva: The artificial saliva was prepared with Na₃PO₄- 3.90 mmol, NaCl - 4.29 mmol, KCl - 17.98 mmol, CaCl₂- 1.10 mmol, MgCl₂ - 0.08 mmol, H₂SO₄ - 0.50 mmol, NaHCO₃ - 3.27 mmol, and distilled water, and the pH was set at a level of 7.2.

Grouping Of Specimens: All the 90 specimens were randomly divided into 3 groups out of which the 3rd group was further divided into 4 sub-groups, each consisting 15 specimens.

Group 1- (Control group) Intact enamel with no surface treatment (n=15)

Group 2- Specimens with artificially demineralized enamel with no surface treatment. Samples were kept for 96 hours in the demineralizing solution and were washed with distilled water and dried. (n=15)

Group 3- Specimens with artificially demineralized enamel with surface treatment. (n=60)

Group 3 was further divided into 4 sub-groups with 15 specimens each as per the surface treatment to be carried out.

Sub-group 3(A) – The specimens were immersed in demineralizing solution for 96 hours and were then washed with distilled water. A thin layer of GC tooth mousse crème (CPP-ACP) was applied with an applicator tip and was left for 3 minutes. Thereafter, specimens were washed with distilled water and kept in artificial saliva. The treatment was carried out twice daily for 28 days.



Figure 3

Sub-group 3(B) – The specimens were immersed in demineralizing solution for 96 hours and then were washed with distilled water. A thin layer of Calcium Sodium Phosphosilicate (CaSP) (Sensodyne repair and protect with Novamin) was applied with an applicator tip and was left for 3 minutes. Each specimen was then washed with distilled water and was kept in artificial saliva. The treatment was carried out twice daily for 28 days.

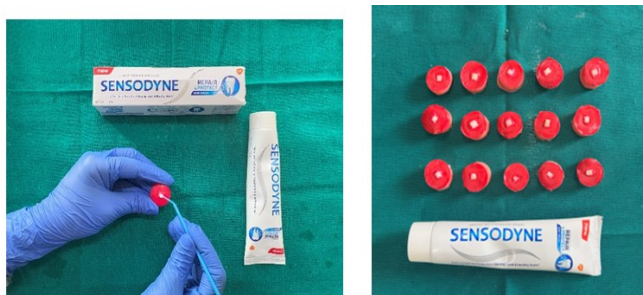


Figure 4

Sub-group 3(C): The specimens were immersed in demineralizing solution for 96 hours and then were washed with distilled water. A thin layer of Calcium silicate and Sodium phosphate (CSSP) salt (Regenerate advance science tooth paste) was applied with an applicator tip and was left for 3 minutes. Each specimen was then washed with distilled water and was kept in artificial saliva. The treatment was carried out twice daily for 28 days.

Sub-group 3(D): The specimens were immersed in demineralizing solution for 96 hours and then were washed with distilled water. Specimens were subjected to Resin infiltration treatment by etching the samples with 15% HCl for 2 minutes, followed by drying with ethanol solution for 30 seconds, and finally resin infiltrant was applied and light-cured for 40 seconds. Each specimen was then stored in artificial saliva.

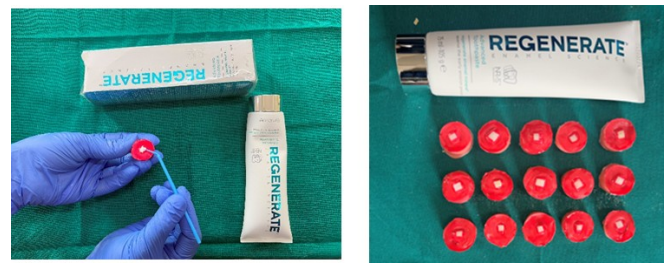


Figure 5

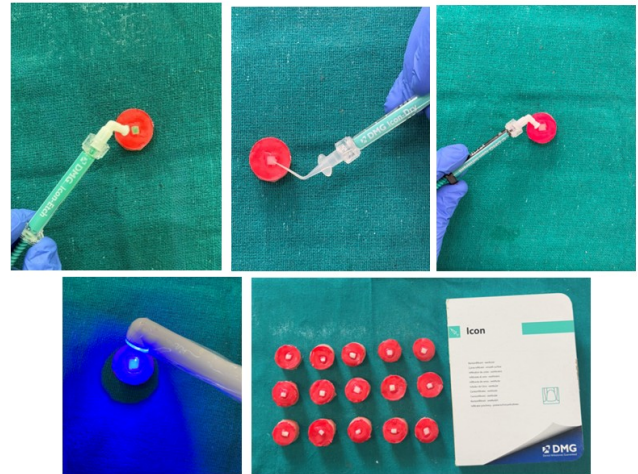


Figure 6. etch, dry, infiltrate and cure.

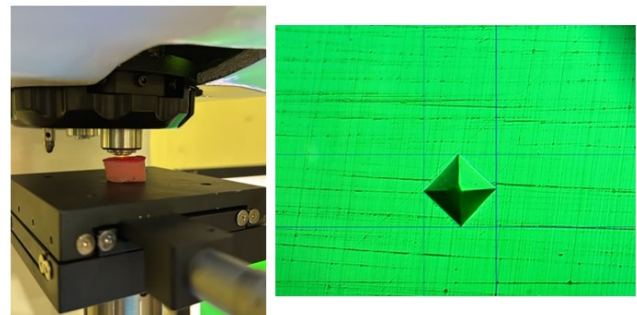


Figure 7

Microhardness Testing: All specimens from each group underwent surface microhardness testing using a Vickers microhardness testing machine. The micro hardness values of the all the specimens were obtained, compiled and were put to statistical analysis.

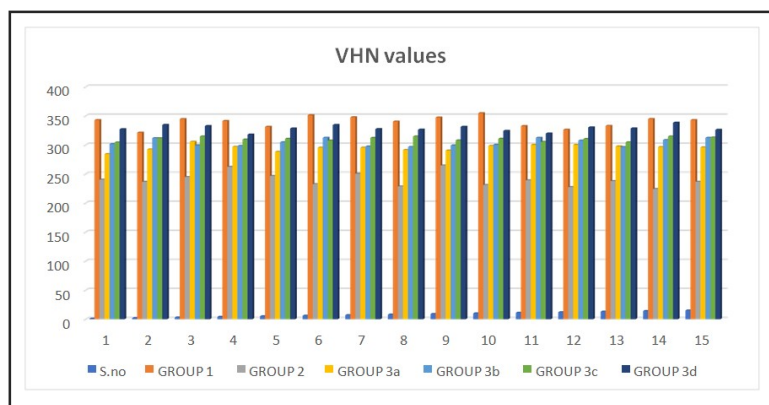
RESULTS

Statistical analysis of the study revealed that:

- There is a notable increase in the mean surface microhardness of artificially demineralized enamel after different surface treatments in groups 3(a), 3(b), 3(c), and 3(d). This indicates that all three remineralizing agents (CPP-ACP, CaSP namely Novamin, Calcium silicate and sodium phosphate salts) and resin infiltration respectively, have the potential to enhance the surface microhardness of demineralized enamel. (Table 1 & Graph 1)
- The mean surface microhardness following the application of different surface treatments in Group 3 is in the order of

Table 1. Shows VHN values of all the samples of each group

S.no	GROUP 1	GROUP 2	GROUP 3a	GROUP 3b	GROUP 3c	GROUP 3d
1	342.7	240.2	284.2	301.7	304.2	327.1
2	321.1	236.5	292.3	311.6	311.4	334.5
3	344.5	244.7	305.5	299.4	314.5	332.5
4	341.2	262.3	296.7	298.3	309.2	317.8
5	331.2	246.6	288.2	304.6	310.6	328.3
6	351.2	232.5	295.2	312.3	307.5	334.4
7	347.6	250.5	295.3	297.3	312.1	327.3
8	340.2	228.6	291.3	296.5	314.5	326.3
9	347.2	264.6	290.4	299.2	307.8	331.2
10	354.6	231.1	298.3	300.5	310.7	324.2
11	332.4	239.1	300.2	312.2	305.2	319.7
12	326.2	227.4	300.2	307.4	310.2	330.2
13	332.8	237.8	297.5	296.2	304.5	328.4
14	344.6	224.2	296.3	308.5	314.6	338.3
15	342.7	236.6	295.7	312.2	312.8	326.1



Graph 1. Shows VHN values of all the samples of each group

Table 2 & Graph 2. show the mean VHN of Group 1, Group 2, Group 3a, Group 3b, Group 3c and Group 3d

	Groups	N	Mean	Std. Deviation
VHN	Group 1	15	340.01	9.41
	Group 2	15	240.18	11.89
	Group 3a	15	295.15	5.28
	Group 3b	15	303.86	6.26
	Group 3c	15	309.98	3.53
	Group 3d	15	328.42	5.46

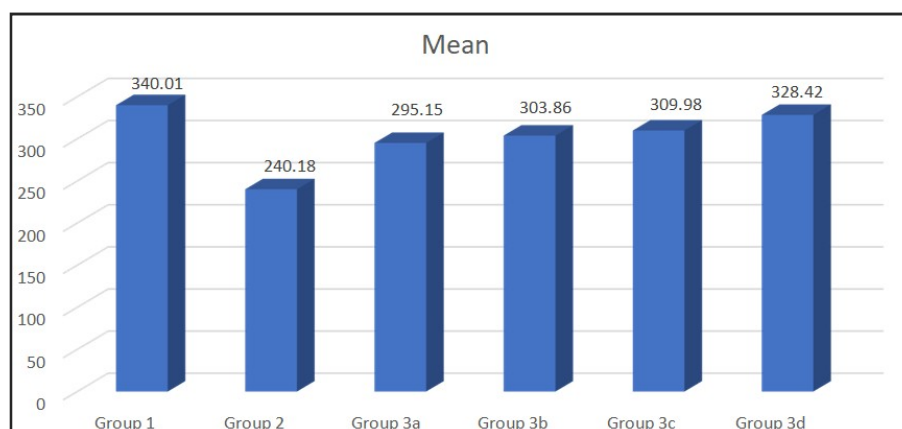


Table 3. Shows Analysis of variance (ANOVA) for mean VHN among different groups

VHN	Sum of Squares	df	Mean Square	F	P
Between Groups	91104.21	5	18220.84	321.88	<.0001
Within Groups	4754.91	84	56.60		
Total	95859.12	89			

(Table 2 & Graph 2): Group 3d (Resin infiltration) > Group 3c (CSSP) > Group 3b (CaSP) > Group 3a (CPP-ACP).

- The microhardness of the Resin infiltration group (Group 3d) was nearly identical to that of the Control group (Group 1), which represents intact, healthy enamel. This indicates that resin infiltration successfully restored the microhardness of artificially demineralized enamel to a level comparable to healthy enamel.

The other three remineralizing agents also positively contribute in restoring the microhardness of demineralized enamel, enhancing enamel's resistance to acid attacks and halting further demineralization, thus effectively preventing cavity formation.

GROUP 1 (Intact enamel with no surface treatment)

GROUP 2 (Artificially demineralized enamel with no surface treatment)

GROUP 3a (Artificially demineralized enamel + surface treatment with CPPACP)

GROUP 3b (Artificially demineralized enamel + surface treatment with Calcium Sodium Phosphosilicate)

GROUP 3c (Artificially demineralized enamel + surface treatment with Calcium silicate and Sodium phosphate salt)

GROUP 3d (Artificially demineralized enamel + surface treatment with resin infiltration)

Graph 1: shows VHN values of all the samples of each group: The mean VHN of Group 1, Group 2, Group 3a, Group 3b, Group 3c and Group 3d are 340.01 ± 9.41 , 240.18 ± 11.89 , 295.15 ± 5.28 , 303.86 ± 6.26 , 309.98 ± 3.53 , 328.42 ± 5.46 respectively.

Graph 2: ANOVA was used to compare mean VHN between the 6 groups. The p value was less than 0.05 i.e., there was a significant difference between the 6 groups. To further understand the significantly different groups Tukey's post hoc test was performed.

DISCUSSION

Dental caries is an infectious microbiological disease of teeth that results in localized dissolution and destruction of calcified tissue (Sturdevant). Dental caries proceeds when salivary pH drops below 5.5, initiating the demineralization of enamel. This early stage of demineralization clinically appears as White spot lesions, in which there is sub-surface loss of minerals while the outer enamels remain intact. However, if left untreated, this layer can eventually break down, leading to the formation of a cavity (Mann and Dickinson, 2006) [9]. At this pre-cavitation stage, the caries process can be halted or reversed through therapeutic intervention, primarily via remineralization (Featherstone, 2008) [10]. Remineralization serves as a non-invasive approach to manage early carious lesions. Various agents, both fluoridated and non-fluoridated are available to promote the remineralization of these early enamel lesions which further helps in restoring the enamel micro-hardness. Another novel treatment concept known as caries infiltration has been developed to address white spot lesions. It works by

blocking the pathways through which cariogenic acids diffuse, effectively sealing the lesion. Unlike traditional caries-sealing methods (Griffin et al., 2008) [11], this technique creates a diffusion barrier within the lesion itself rather than on its surface. Resin infiltration increases the microhardness of enamel in white spot lesions by penetrating the porous, demineralized enamel with a low-viscosity resin. The resin penetrates the tiny gaps in the enamel, by infiltrating deeply, the resin replaces the lost mineral matrix and prevents further progression of demineralization. Additionally, it forms a smooth, hardened surface that enhances the enamel's resistance to acid attacks and mechanical wear.

Hence, the purpose of this in vitro study was to evaluate and compare the effect of CPP-ACP (GC Tooth Mousse), CaSP (Sensodyne repair and protect with Novamin), CSSP (Regenerate advance tooth paste, Enamel Science, France) and Resin Infiltration (ICON smooth surface kit DMG, Hamburg, Germany) on the surface microhardness of demineralized enamel. The statistical analysis of the present in-vitro study reveals that all three remineralizing agents—CPP-ACP, Calcium Sodium Phosphosilicate (CaSP), and Calcium Silicate & Sodium Phosphate (CSSP) salt, along with Resin infiltration, effectively enhance the surface microhardness of demineralized enamel. In the present study, on comparing mean surface microhardness of Group 1 (intact enamel) with Group 2 (artificially demineralized enamel) there was reduction in the mean surface microhardness of Group 2, which attribute the loss of surface microhardness of artificially demineralized lesions to the depletion of calcium and phosphate from enamel's hydroxyapatite structure.

When comparing Group 2 (artificially demineralized enamel) with Group 3 (artificially demineralized enamel treated with surface treatments), Group 3 exhibited a significantly higher mean surface microhardness.

Group 3, which was subdivided into four sub-groups consisting of three remineralizing agents and resin infiltration, exhibited the mean surface microhardness in the following descending order:

Group 3d (Resin infiltration group) > Group 3c (CSSP group) > Group 3b (CaSP group) > Group 3a (CPP-ACP group).

In the present study, the Resin Infiltration group (3D) showed the highest surface microhardness after enamel demineralization. The microhardness of the Resin infiltration group (Group 3d) was nearly identical to that of the Control group (Group 1), which represents intact, healthy enamel. This indicates that resin infiltration successfully restored the microhardness of artificially demineralized enamel to a level comparable to healthy enamel.

These findings align with Kingston Chellapandian et al. (2020) [12]. Their research demonstrated that resin infiltrant outperformed CPP-ACP, nanohydroxyapatite, and the control group in terms of acid resistance and preventing caries progression, because resin infiltrant works by deeply penetrating the enamel and bonding strongly, filling the porous lesions and creating a protective barrier against acid attacks.

The results also align with the studies of Jyothi Mandava et al. (2017) [13], Torres CRG et al. (2012) [14] and Paris S et al. (2007) [15], Rana N et al (2021) [16], who found that resin infiltration

significantly improved the microhardness of carious lesions following demineralization.

The study also found that the remineralizing agents in Group 3(c) (CSSP group), Group 3(b) (CaSP; Novamin) and Group 3(a) (CPP-ACP) help in enhancing the enamel's resistance to acid attacks and halting further demineralization, thus effectively preventing cavity formation. However, their effectiveness was lower than that of the Resin infiltration group.

CONCLUSION

Remineralizing agents and resin infiltration possess the unique ability to restore the microhardness of demineralized enamel effectively. Resin infiltration has the ability to restore enamel hardness to levels similar to healthy enamel, making it an ideal treatment option for early caries and white spot lesions. Its resistance to acid and durability contribute to long-term effectiveness, reducing the need for more invasive procedures. CSSP and CaSP are highly effective in restoring enamel microhardness and preventing cavity formation, with their bioactive properties making them suitable for patients at high risk or those with compromised oral environments. Despite its lower performance compared to other agents, CPP-ACP remains a valuable preventive measure, and its compatibility with fluoride further enhances its utility in maintaining enamel health.

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