

Effect of Fluoride-based Varnishes with Added Calcium and Phosphate on Microhardness of Esthetic Restorative Materials: An *In Vitro* Study

Raksha S Shetty¹ , Sham S Bhat² , Sundeep Hegde K³ , Vidya S Bhat⁴ 

ABSTRACT

Background and objectives: Fluoride varnishes are being used to prevent caries in children. The high concentration of fluoride in varnishes apart from caries prevention may cause changes in surface properties of esthetic restorations. The study aims to evaluate and compare the effect of four commercially available fluoride varnishes with added calcium and phosphate on microhardness of three esthetic materials namely conventional GIC (Fuji II), high viscosity GIC (Fuji IX), and nanocomposite (Filtek Z350).

Materials and methods: A total of 28 pellets were made of each material and stored in distilled water at 37 °C for 48 hours. The microhardness of the pellets was tested which served as a baseline. These were then randomly divided into four subgroups. In one subgroup Profluorid varnish was applied, second subgroup MI varnish was applied, third subgroup Embrace varnish was applied, and in the fourth subgroup Enamel Pro varnish was applied as per protocol. Thereafter, all the pellets were subjected to microhardness testing (load = 100 g for 15 seconds).

Results: The fluoride varnishes increased the microhardness of conventional GIC (Fuji II) whereas in case of high viscosity GIC (Fuji IX) the application of varnishes reduced the microhardness. In case of nanocomposite restorative material (Filtek Z350) only Profluorid varnish increased its microhardness.

Conclusion: Fluoride varnish and calcium-phosphate containing fluoride varnish effect on the microhardness of restorative material is material dependent. So, the choice of fluoride varnish with or without proprietary additives depends on the nature and composition of the restorative material.

Keywords: CPP-ACP, Glass ionomer cements, Microhardness, Nanocomposites, Varnishes.

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INTRODUCTION

Dental caries occurs when acids from bacterial metabolism seep into enamel and dentin.¹ It is a chronic, multifactorial, transmissible infectious dental disease that has an adverse effect on a great deal of the world's population, causing both pain and discomfort.² In the primary dentition, glass ionomer cement and composites are the most commonly used tooth-colored restorative materials.³

Glass ionomer cement is a popular restorative material in primary dentition due to its properties of esthetic value, biocompatibility, sustained release of fluoride, rechargeability, and chemical adhesion to enamel and dentin.⁴

Resin-based composites are also becoming more and more popular as restorative materials due to the increase in demands for esthetics and also outstanding development in the field of adhesive dentistry. Although there has been significant development in restorative materials, restorations in the oral cavity are affected by numerous conditions that may affect the physical and mechanical properties such as color and microhardness per se.⁵

In dental practice, restorative filling materials need to be long-lasting in the oral cavity. Surface hardness correlates well with compressive strength and abrasion resistance and is one of the most significant physical properties of restorative filling materials.⁶

Typically, fluoride varnish is applied as an adherent material that consists of a high fluoride content as a salt or silane preparation in a fast-drying alcohol and resin base.⁷ Varnishes were developed to extend the contact time between fluoride and enamel while adhering to the surface of the tooth for a longer time in a thin

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layer and to avoid the loss of fluoride after the application. Upon application, fluoride is slowly released from varnish.⁸

Calcium and phosphate ions are naturally present in saliva, but they are present in low concentrations. Mineral deposition only occurs at the surface of enamel as a result of a low ion concentration gradient in saliva. Surface mineralization on the enamel alone may not have the desired effect of improving the structural properties of incipient carious lesions. As a result, calcium phosphate-based delivery systems containing a high concentration of calcium phosphate was introduced.⁹ Amorphous calcium phosphate (ACP) is a precursor of hydroxyapatite and casein phosphopeptide (CPP) which contains a peptide sequence. The incorporation of CPP-ACP in varnish acts as a reservoir for calcium and phosphate ions.¹⁰

Incorporation of CPP-ACP component in the leachable restorative material like glass ionomer (GI) system increases the concentration of reservoir and increases the efficiency of the transport of calcium and phosphate ions into the enamel subsurface lesions.^{11,12}

Following exposure to topical fluorides, glass ionomer cements are able to acquire even more fluoride ions, making them a rechargeable fluoride release system. Nevertheless, topically applied fluoride agents can cause the surface properties of esthetic restorative materials to deteriorate. This may negatively affect the clinical durability of dental restorations.⁴

Literature search showed few studies have shown the result of fluoride varnish on surface hardness of restorative materials. Therefore, the present study evaluated and also compared the effect of four commercial fluoride-based varnishes with added calcium and phosphate on microhardness of three types of esthetic restorative materials.

MATERIALS AND METHODS

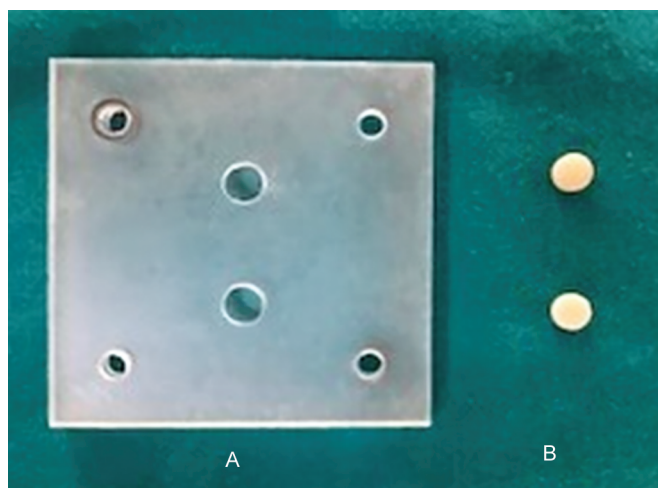
The study proposal was reviewed and cleared by the Yenepoya ethics committee and the study was carried out in the Department of Pedodontics and Preventive Dentistry and Department of BioMaterials, Yenepoya Dental College, Mangaluru.

Materials used in the study are:

- Fluoride varnishes:
 - Profluorid varnish (5% sodium fluoride)
 - MI varnish (5% sodium fluoride, CPP-ACP)
 - Embrace varnish (5% sodium fluoride, Xylitol coated calcium and phosphate (CXP))
 - Enamel Pro varnish (5% sodium fluoride, ACP)
- Restorative materials:
 - Conventional GIC: Fuji II
 - High viscosity GIC: Fuji IX
 - Nanocomposite: Filtek Z350
- Vickers microhardness tester

Sample Preparation

Test pellets were prepared from a custom-made acrylic plate mold of diameter 6.5 mm and 2 mm thickness (Fig. 1). A total of 28 pellets



Figs 1A and B: (A) Acrylic plate mold (B) Test pellet

each were prepared of the 3 restorative materials: conventional GIC (Fuji II), high viscosity GIC (Fuji IX), and nanocomposite (Filtek Z350). Petroleum jelly was coated on the lateral walls of the mold to prevent material adhesion. Fuji II and Fuji IX are available in powder liquid form and as per the manufacturer's instructions, it was mixed in a ratio of 3:1 on a paper pad. In a custom-made mold, the mixed cement was then placed by slightly overfilling them and by using an acrylic plate on both the ends the material was compressed between mylar strips followed by which the pellets were kept at room temperature for 15 minutes. The same procedure was carried out with Filtek Z350 which is available as a single-component paste and cured for 20 seconds.

A total of 84 pellets of each restorative material was prepared and grouped as:

- Group I: 28 pellets of Fuji II
- Group II: 28 pellets of Fuji IX
- Group III: 28 pellets of Filtek Z350

All the pellet was immersed in a plastic vial with 2 mL of distilled water and incubated at 37 °C for 48 hours.

After 48 hours, pellets were dried and the microhardness was tested using Vickers microhardness tester. A force of 100 g for 15 seconds was applied with an indenter diameter of 45 mm onto the surface of restorative material at three points and the average of readings was obtained as Vickers microhardness number which served as a baseline.

Each group was further divided into four subgroups of seven pellets each. All four subgroups were treated with different fluoride varnishes using microbrushes.

- Subgroup A: Profluorid varnish applied on each pellet
- Subgroup B: MI varnish applied on each pellet
- Subgroup C: Embrace varnish applied on each pellet
- Subgroup D: Enamel Pro varnish applied on each pellet

Rinsing and drying of pellets were done after the application of varnish. The microhardness was assessed using Vickers hardness testing machine and values were obtained. The collected data was expressed in terms of Vickers microhardness number (VHN) and was subjected to statistical analysis.

RESULTS

One-way ANOVA followed by Tukey Post hoc test was applied to assess and compare the microhardness of esthetic restorative materials namely, conventional glass ionomer cement (Fuji II), high viscosity glass ionomer cement (Fuji IX), and Nanocomposite (Filtek Z350).

Microhardness of Fuji II increased after all fluoride varnish and more significantly evident after MI varnish (82.057 ± 10.070) application as shown by higher mean VHN in comparison to Baseline (59.700 ± 4.902) (Table 1).

In the case of Fuji IX, the mean microhardness value reduced after each of the fluoride varnish treatment as compared to their baseline (Table 1).

In the case of Filtek Z350, profluorid varnish treatment increased the microhardness (96.100 ± 10.948) as compared to the baseline (84.186 ± 5.044), whereas, the microhardness of the restorative material was reduced in the order of MI varnish (83.100 ± 11.959), Enamel Pro varnish (71.257 ± 10.488), and Embrace varnish (68.771 ± 10.814) (Table 1).

Overall a statistically significant change in the microhardness ($p < 0.05$) was observed after different fluoride varnish treatment in all the esthetic restorative materials as compared to their respective baseline (Fig. 2).

Among the tested fluoride varnishes, MI varnish significantly increased the microhardness of Fuji II in comparison to profluorid varnish with 5% sodium fluoride, and calcium and phosphorus containing Embrace varnish and Enamel Pro varnish. Among Embrace varnish (5% sodium fluoride, CXP) and Enamel Pro varnish (5% sodium fluoride, ACP) Enamel Pro varnish significantly increased the microhardness of Fuji II (Table 2).

Profluorid varnish reduced microhardness (60.386 ± 8.752) as compared with baseline (78.300 ± 10.913), whereas, Enamel Pro varnish retained the microhardness (75.457 ± 12.141) of Fuji IX.

In contrast, MI varnish and Embrace varnish did not result in any significant change in the microhardness of Fuji IX (Table 3).

Among the varnishes tested on Filtek Z350, the fluoride varnish containing calcium and phosphate, that is, Embrace and Enamel Pro varnish significantly decreased the microhardness as compared to the profluorid treatment (Table 4). MI varnish application showed no significant difference suggesting that profluorid and MI varnish were comparable and safer to use.

DISCUSSION

We compared the effect of application of fluoride varnish alone or fluoride varnish with added calcium and phosphate on the microhardness of three restorative materials, namely, conventional GIC

Table 1: Microhardness of esthetic restorative materials Fuji I, Fuji IX, and Filtek Z350 following fluoride varnish application

Restorative materials/Fluoride varnish	Mean	Std. Deviation	F	P value
<i>Conventional GIC: Fuji II</i>				
Baseline	59.700	4.902	14.244	0.000
Profluorid varnish	63.000	8.768		
MI varnish	82.057	10.070		
Embrace varnish	53.029	4.980		
Enamel Pro varnish	66.000	7.733		
<i>High viscosity GIC: Fuji IX</i>				
Baseline	78.300	10.913	4.232	0.008
Profluorid varnish	60.386	8.752		
MI varnish	68.186	3.882		
Embrace varnish	68.557	6.909		
Enamel Pro varnish	75.457	12.141		
<i>Nanocomposite: Filtek Z350</i>				
Baseline	84.186	5.044	8.262	0.000
Profluorid varnish	96.100	10.948		
MI varnish	83.100	11.959		
Embrace varnish	68.771	10.814		
Enamel Pro varnish	71.257	10.488		

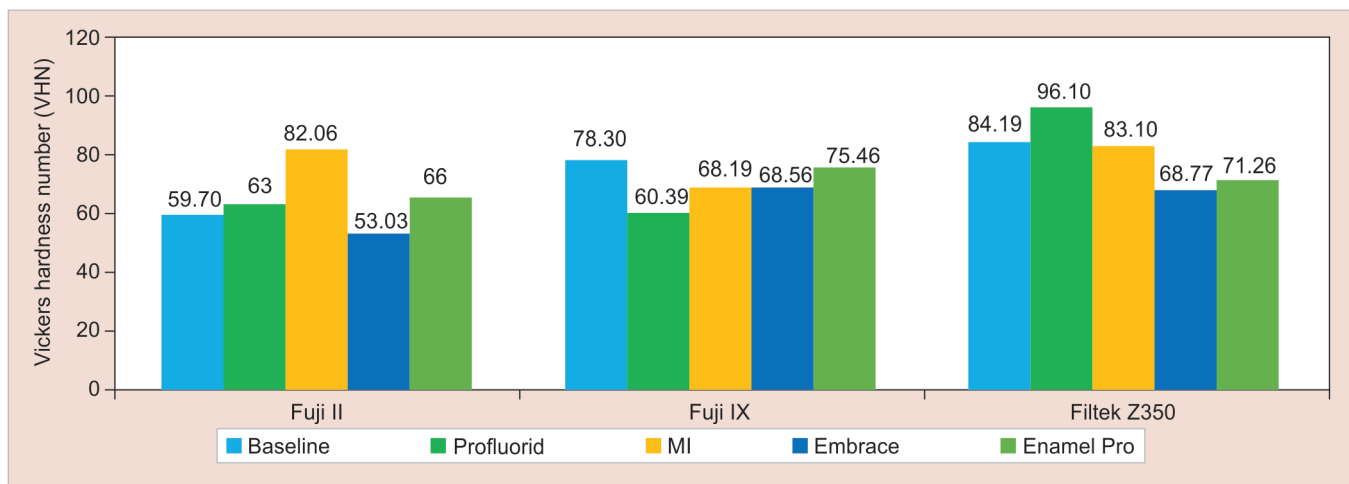


Fig. 2: Microhardness of esthetic restorative materials following fluoride varnish application

Table 2: Multiple comparison of microhardness of conventional GIC (Fuji II) following fluoride varnish application

Group		Conventional GIC: Fuji II			
		Mean difference	SEM	P value	Sig.
Baseline	Profluorid varnish	-3.300	4.049	>0.05	NS
	MI varnish	-22.357	4.049	<0.05	S
	Embrace varnish	6.671	4.049	>0.05	NS
	Enamel Pro varnish	-6.300	4.049	>0.05	NS
Profluorid varnish	Baseline	3.300	4.049	>0.05	NS
	MI varnish	-19.057	4.049	<0.05	S
	Embrace varnish	9.971	4.049	>0.05	NS
	Enamel Pro varnish	-3.000	4.049	>0.05	NS
MI varnish	Baseline	22.357	4.049	<0.05	S
	Profluorid varnish	19.057	4.049	<0.05	S
	Embrace varnish	29.029	4.049	<0.05	S
	Enamel Pro varnish	16.057	4.049	<0.05	S
Embrace varnish	Baseline	-6.671	4.049	>0.05	NS
	Profluorid varnish	-9.971	4.049	>0.05	NS
	MI varnish	-29.029	4.049	<0.05	S
	Enamel Pro varnish	-12.971	4.049	<0.05	S
Enamel Pro varnish	Baseline	6.300	4.049	>0.05	NS
	Profluorid varnish	3.000	4.049	>0.05	NS
	MI varnish	-16.057	4.049	<0.05	S
	Embrace varnish	12.971	4.049	<0.05	S

(Fuji II), high viscosity GIC (Fuji IX), and nanocomposite (Filtek Z350). Both conventional GIC (Fuji II) and high viscosity GIC (Fuji IX) sets by acid–base reaction. High viscosity GIC has a longer survival rate when compared to the low viscosity restorative materials thus conferring it highly suitable for the restorative material^{13,14} and the viscosity is high due to the incorporation of polyacrylic acid to the powder.⁴

Technological progress has encouraged topical fluoride varnish manufacturers to add different proprietary additives like amorphous calcium phosphate (ACP), xylitol-coated calcium phosphate (CXP), and casein phosphopeptide–amorphous calcium phosphate (CPP–ACP) in the fluoride varnish to increase the availability of calcium and phosphate along with fluoride on the surface.¹⁵

Profluorid varnish (pH 6.4) used in the present study is a resin-based varnish containing 5% sodium fluoride (22600 ppm fluoride). It effectively seals the dentinal tubules by the accumulation of both fluoride ions and calcium ions, which precipitate into calcium fluoride.¹⁶ In the present study, the three tested restorative materials showed some significant difference after the application of profluorid varnish. The high viscosity GIC (Fuji IX) showed reduced microhardness after profluorid varnish application.

In our study, the application of MI varnish increased the microhardness of conventional GIC (Fuji II) whereas in case of high viscosity GIC (Fuji IX) the application of Enamel Pro had the ability to maintain the microhardness of restorative material.

MI varnish contains 5% sodium fluoride, ACP as a precursor of hydroxyapatite, and CPP represents a peptide sequence. The incorporation of CPP–ACP in varnish is widely due to the fact that they act as a resource of calcium and phosphate ions. During surface demineralization, CPP stabilizes the level of ACP and increases the chances of calcium ion uptake into the demineralized area.¹⁰ The preventive effect of fluoride varnish containing 1–5% CPP–ACP in enamel erosion and demineralization because of higher

release of calcium, phosphate, and fluoride ions in the surface is reported.^{17,18} The values obtained in the present study conclude that the microhardness of conventional GIC (Fuji II) was statistically different after the application of MI varnish containing 5% sodium fluoride and CPP–ACP. Our study corroborates with Bayrak et al.¹⁸ and Shen et al.¹⁷ suggesting that increased calcium, phosphate, and fluoride ion release will have increased microhardness. Babu et al.¹⁹ showed that agents such as CPP–ACP can help to compensate the adverse effect of high fluorine from only fluoride varnish thus serving as a better alternative. MI varnish has higher effect on microhardness in comparison to Enamel Pro varnish containing ACP due to higher release of calcium and fluoride ions.²⁰ The feasibility to bind directly with dentin and enamel, biocompatibility, and ability to act as a fluoride reservoir had made GIC an ideal candidate for the restorative material. However, the highly viscous nature of GIC as in the case of Fuji IX makes it susceptible to gap formation and increases the microleakage.

Enamel Pro varnish contains 5% sodium fluoride and ACP. We found that Enamel Pro varnish increased the microhardness of conventional GIC (Fuji II) which could be possibly due to the presence of ACP crystals and increased formation of hydroxyapatite on the surface of GIC thereby resulting in increased phosphate, calcium, and fluoride ion release.²¹ Moshaverinia et al.²² showed that the incorporation of nanoparticles fluoroapatite in high viscosity GIC (Fuji IX) can also increase the microhardness of the material.

Similar to our result Gill and Pathak⁴ did not find any significant difference in the microhardness of conventional GIC (Fuji II) after sodium fluoride (NaF) gel treatment; however, their result on Fuji IX contradicted with our result, which could be due to the different efficacy and reactivity of fluoride components of sodium fluoride gel and varnish with the matrix of restorative material.²³

Embrace varnish contains xylitol-coated calcium and phosphate (CXP) which prevents the reaction of these ions with fluoride in the

Table 3: Multiple comparison of microhardness of high viscosity GIC (Fuji IX) following fluoride varnish application

<i>Group</i>		<i>High viscosity GIC:Fuji IX</i>			
		<i>Mean difference</i>	<i>SEM</i>	<i>P value</i>	<i>Sig.</i>
Baseline	Profluorid varnish	17.914	4.816	>0.05	S
	MI varnish	10.114	4.816	<0.05	NS
	Embrace varnish	9.743	4.816	>0.05	NS
	Enamel Pro varnish	2.843	4.816	>0.05	NS
Profluorid varnish	Baseline	-17.914	4.816	>0.05	S
	MI varnish	-7.800	4.816	<0.05	NS
	Embrace varnish	-8.171	4.816	>0.05	NS
	Enamel Pro varnish	-15.071	4.816	>0.05	S
MI varnish	Baseline	-10.114	4.816	<0.05	NS
	Profluorid varnish	7.800	4.816	<0.05	NS
	Embrace varnish	-0.371	4.816	<0.05	NS
	Enamel Pro varnish	-7.271	4.816	<0.05	NS
Embrace varnish	Baseline	-9.743	4.816	>0.05	NS
	Profluorid varnish	8.171	4.816	>0.05	NS
	MI varnish	0.371	4.816	<0.05	NS
	Enamel Pro varnish	-6.900	4.816	<0.05	NS
Enamel Pro varnish	Baseline	-2.843	4.816	>0.05	NS
	Profluorid varnish	15.071	4.816	>0.05	S
	MI varnish	7.271	4.816	<0.05	NS
	Embrace varnish	6.900	4.816	<0.05	NS

Table 4: Multiple comparison of microhardness of nanocomposite (Filtek Z350) following fluoride varnish application

<i>Group</i>		<i>Nanocomposite:Filtek Z350</i>			
		<i>Mean difference</i>	<i>SEM</i>	<i>P value</i>	<i>Sig.</i>
Baseline	Profluorid varnish	-11.914	5.426	>0.05	NS
	MI varnish	1.086	5.426	<0.05	NS
	Embrace varnish	15.414	5.426	>0.05	NS
	Enamel Pro varnish	12.929	5.426	>0.05	NS
Profluorid varnish	Baseline	11.914	5.426	>0.05	NS
	MI varnish	13.000	5.426	<0.05	NS
	Embrace varnish	27.329	5.426	>0.05	HS
	Enamel Pro varnish	24.843	5.426	>0.05	HS
MI varnish	Baseline	-1.086	5.426	<0.05	NS
	Profluorid varnish	-13.000	5.426	<0.05	NS
	Embrace varnish	14.329	5.426	<0.05	NS
	Enamel Pro varnish	11.843	5.426	<0.05	NS
Embrace varnish	Baseline	-15.414	5.426	>0.05	NS
	Profluorid varnish	-27.329	5.426	>0.05	HS
	MI varnish	-14.329	5.426	<0.05	NS
	Enamel Pro varnish	-2.486	5.426	<0.05	NS
Enamel Pro varnish	Baseline	-12.929	5.426	>0.05	NS
	Profluorid varnish	-24.843	5.426	>0.05	HS
	MI varnish	-11.843	5.426	<0.05	NS
	Embrace varnish	2.486	5.426	<0.05	NS

absence of saliva. On account of sustained-time release property for fluoride ions and upon dissolution of xylitol in saliva, free calcium and phosphorus ions react with fluorine to continuously form fluorapatite on the teeth. In our study, Embrace varnish reduced the microhardness of conventional GIC (Fuji II), which could likely be due to the increased

fluoride release, significant rates of fluoride depletion, and low substantivity of Embrace varnish as reported by Milburn et al.¹⁵

The nanocomposite restorative systems with the newer generations of bonding agents and resin composite formulations are widely used in restorative dentistry. Nanocomposites are filled

with nanoparticles and their small size, high surface area, optical and mechanical properties make them suitable for restorative material compared to the glass ionomer.²⁴ In our study, Profluorid increased the microhardness of nanocomposite Filtek Z350, but the Embrace and Enamel Pro varnish reduced the microhardness. Yeh et al.²⁵ found that 60 Second Taste Gel, an APF gel reduced the microhardness of nanocomposite whereas other gels like Topex and Zap did not affect the microhardness.²⁶ However, nanocomposite Filtek Z350 has increased mechanical properties compared to conventional GIC. The difference in the pH and fluoride ion concentration may cause decline in the hardness of the material.²⁶

Between composite and fluorides, three major interactions can be noticed, that is, with organic matrix (BisGMA), filler matrix coupling agent and with reinforcing fillers.²

It can be inferred that though nanocomposites are better restorative materials, careful selection of fluoride varnish is necessary to maintain the microhardness of composite resins.

We found a statistically significant change in the microhardness ($p < 0.05$) after different fluoride varnish treatment with or without calcium phosphate in all the esthetic restorative materials as compared to their respective baseline. The loss of hardness in restorative material will result in the failure of the material causing raise in surface roughness and retention of plaque, deterioration of anatomical form, and discoloration which will remarkably lessen the duration of restoration.

CONCLUSION

This study suggests that the effectiveness of fluoride varnish and calcium-phosphate containing fluoride varnish on the microhardness of restorative material is material-dependent. The MI varnish was suitable for the conventional GIC, whereas Enamel Pro was found to be suitable for high viscosity GIC. In the case of nanocomposite restorative material, only fluoride varnish had resulted in increased microhardness. The choice of fluoride varnish with or without proprietary additives depends on the nature and composition of restorative material.

CLINICAL SIGNIFICANCE

We infer that careful selection of fluoride varnish is essential to maintain the microhardness of various esthetic restorative materials.

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