ORIGINAL RESEARCH

Comparative Evaluation of Effect of Different Antioxidants on Shear Bond Strength of Composites on Bleached Enamel: An *In Vitro* Study

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Abstract

Aim: To evaluate the effect of antioxidants on the shear bond strength of composite resin to bleached enamel.

Materials and methods: A total of 120 extracted permanent anterior teeth were split into four major groups, one among them being a control group. Group I (bleaching + without antioxidant), group II (bleaching + 10% sodium ascorbate), group III (bleaching + 5% grape seed extract), and group IV (bleaching + 5% pine bark extract). All of the groups were bleached using a gel containing 35% carbamide peroxide, and then they were divided into two subgroups depending on when the bonding operation was to be completed—subgroup A was done in 24 hours, while subgroup B took 3 weeks. The universal testing machine was used to measure the shear bond strength.

Results: Subgroup IA showed poor shear bond strength than other groups, there was no statistically significant difference between subgroup IB, IIA, and IVA. Subgroup IIIA showed the highest shear bond strength than the remaining groups. Between subgroups IIA and IIB, IIIA and IIIB, and IVA and IVB there was no statistically significant difference. This shows that delaying the bonding procedure for 3 weeks shows similar shear bond strength when compared to groups that were immediately bonded after antioxidant applying groups.

Conclusion: All the antioxidants' immediate application could reverse compromised bond strength. Among them, 5% grape seed extract shows the highest increase in shear bond strength.

Keywords: Antioxidants, Bleaching, Composites, Grape seed extract, Pine bark extract, Sodium ascorbate, Universal testing machine.

International Journal of Clinical Pediatric Dentistry (2023): 10.5005/jp-journals-10005-2480

Introduction

Esthetic dentistry is one of the most advanced and challenging branches of the dental field. The need for esthetic dentistry has grown significantly.[1] Patients pay more concern to smile esthetics and their color appearance than before.[2] Tooth color is a significant issue for dental practitioners and for patients who wish to enhance the appearance of their smiles. There are many reasons for the discoloration of the teeth that might be due to extrinsic or intrinsic stains.[3] There are different treatment modalities to remove discoloration, which include removal of surface stains, micro and macro abrasion, veneering, and placement of porcelain crowns.[4] From the number of services listed to enhance the appearance of the discolored teeth, bleaching is an option which preserves as much of the natural tooth as possible.[5] But one of the prime complications for performing the bleaching procedure is compromised composite resin bond strength to enamel immediately after the bleaching procedure.[3] Due to the presence of free radicals of oxygen, which interfere with resin tag formation, inhibit resin polymerization, and compromise the function of the adhesive system.[6] The most commonly known approach to overcome this compromised bond strength was to delay the bonding procedure for 24 hours to 3 weeks.[7] Numerous methods are there to achieve the compromised bond strength immediately after performing the bleaching technique, such as applying alcohol to bleached enamel before the restoration procedure, use of organic solvent-containing adhesives, and removing the outermost layer of enamel. Other methods are exposing the bleached enamel surface to water, saline solution, or artificial saliva and the use of antioxidants like ascorbic acid, sodium ascorbate, bicarbonate, or α-tocopherol. In recent years, interest in plant-based natural antioxidants has increased[7]; antioxidants include aloe vera extract, pedicularis extract, mangosteen extract, grape seed extract, pine bark extract, lycopene, and green tea extract.[8] Even though studies have shown improved bond strength by using sodium ascorbate to bleached enamel, natural antioxidants have not yet been thoroughly studied as a potential replacement for sodium ascorbate. In order

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How to cite this article: Maddula DC, Vasepalli M, Martha S, *et al.* Comparative Evaluation of Effect of Different Antioxidants on Shear Bond Strength of Composites on Bleached Enamel: An *In Vitro* Study. Int J Clin Pediatr Dent 2023;16(1):87–92.

Source of support: Nil

Conflict of interest: None

to improve the shear bond strength of composite resin repair to bleached enamel. Therefore, the intent of this study is to determine how sodium ascorbate, pine bark extract, and grape seed extract affect the shear bond strength of composite resin restorations to bleached enamel.

Materials and Methods

Preparation of the Antioxidant Solution

To make 10% sodium ascorbate, 100 mL of distilled water was used to dissolve 10 gm of sodium ascorbate (SD Fine Chem Limited, Mumbai, Maharashtra, India) in powder form. To make 5% grape seed extract, 100 mL of distilled water was used to dissolve 5 gm of powdered grape seed extract (iAYUR, Swmabhan Commerce Pvt Ltd). To make 5% pine bark extract, 100 mL of distilled water was used to dissolve 5 gm of powdered pine bark extract (Biotrex Nutraceuticals).

Specimen Preparation

A total of 120 extracted permanent anterior teeth were included in the study. Teeth without caries and cracks were taken in the study. Teeth were brushed, and a 0.5% sodium hypochlorite solution was used to disinfect them. Following disinfection, the teeth were stored in a saline solution at room temperature. Roots were embedded in self-cure acrylic resin blocks of 2 × 2 cm in size to cementoenamel junction, keeping just the coronal section visible. Before beginning the technique, the teeth were polished with pumice. Samples were split into four groups, one control group and three study groups. All the groups were bleached with 35% carbamide peroxide gel (24 carat, tooth-whitening system) for 30 minutes according to the manufacturer's instructions and rinsed with water. Then specimens were randomly divided into four groups of 30 teeth each group:

- Group I ($n = 15$): No antioxidants, only bleaching (control group).
- Groups II, III, and IV ($n = 30$): These three groups' teeth were bleached first and then 10% sodium ascorbate, 5% grape seed extract, and pine bark extract were applied for 10 minutes for groups II, III, and IV, respectively.

Prior to executing the bonding technique, all the groups were separated into two subgroups based on the application period:

- Subgroup A: Within 24 hours ($n = 15$).
- Subgroup B: After 3 weeks ($n = 15$).

Application of Antioxidant Solution

Immediately after the bleaching procedure to experimental subgroup IIA, 10% sodium ascorbate was applied; to experimental subgroup IIIA, 5% grape seed extract was applied; and to experimental subgroup IVA, 5% pine bark extract was applied using a brush.

After storing in artificial saliva of 300 mL for 3 weeks to experimental subgroup IIB, 10% sodium ascorbate was applied; to experimental subgroup IIIB, 5% grape seed extract was applied; to experimental subgroup IVB, 5% pine bark extract was applied with a brush.

Bonding Procedure

After applying antioxidant solutions, all of the specimens were etched with 37% phosphoric acid gel for 15 seconds before being cleaned and allowed to air dry. All specimens were coated with Single Bond Universal Adhesive (3M Espe Dental Products, Saint Paul, Minnesota, United States) in accordance with the manufacturer's instructions. A plastic mold of 2 mm diameter and 2 mm height was placed on bonded specimen before the adhesive was light-cured for 30 seconds. Composite (Fltek Z 250 XT USA) was filled into the plastic mold and light-cured for 40 seconds. Next, the plastic tube was taken out. Prior to shear bond strength testing, all specimens were kept in distilled water for 24 hours on the universal testing machine (Dak Series 7200 system Inc). The chiseled model of the machine would lie perpendicular to composite cylinders. With a force of 1 mm/minute, composite restorations were pulled away from the enamel surface. Shear bond strength measurements were then tabulated and statistically analyzed using the student's unpaired *t*-test, one-way analysis of variance, *post hoc* with IBM Statistical Package for the Social Sciences 23.0 software, and comparative analysis test. It is significant when the *p*-value is 0.000.

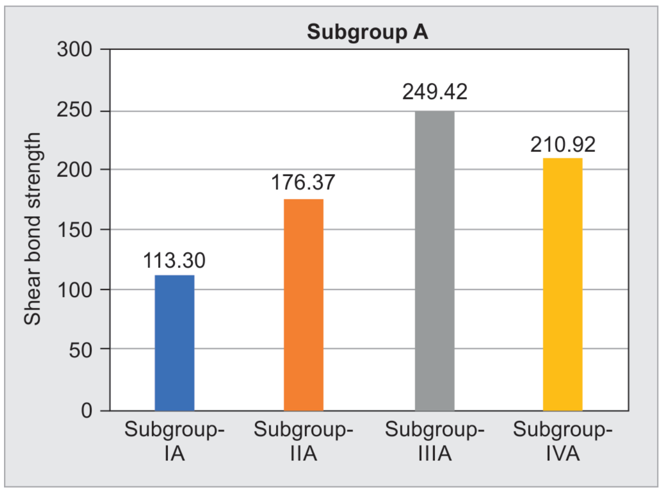

Fig. 1: Comparison of mean values of subgroup A within 24 hours

Table 1: Comparison of mean shear bond strength of the control group with experimental groups

		Mean	*N*	*Standard deviation*	*Standard error mean*	*t-value*	*p-value*
Group I	Group IA	113.3200	15	9.73339	2.51315	–7.504	0.000[b]
	Group IB	204.4733	15	44.76037	11.55708		
Group II	Group IIA	176.3733	15	42.38525	10.94382	1.342	0.201
	Group IIB	161.2000	15	8.22114	2.12269		
Group III	Group IIIA	249.4267	15	26.12693	6.74594	1.718	0.108
	Group IIIB	232.3667	15	22.95327	5.92651		
Group IV	Group IVA	210.9267	15	52.05428	13.44036	1.843	0.087
	Group IVB	182.0867	15	23.49617	6.06668		

$P = 0.000$ is statistically significant difference

Results

As shown in Table 1, there was a statistically significant difference between subgroup IA and IB ($p = 0.000$), and there was no statistically significant difference between subgroup IIA and IIB ($t = 1.342$, $p = 0.201$), subgroup IIIA and IIIB ($t = 1.718$, $p = 0.108$), and subgroup IVA and IVB ($t = 1.843$, $p = 0.087$). As shown in Table 2, the mean shear bond strength of subgroup IIIa was seen to be statistically higher (249.42 ± 26.12) as compared with other experimental groups. As shown in (Table 2 and Fig. 1), *post hoc* comparison revealed the significance between subgroups IA–IIA, IA–IIIA, IA–IVA, and subgroups IIA–IIIA being at 0.000 subgroups IIIA–IVA to be at 0.026. As shown in (Table 3 and Fig. 2) subgroup IIB, where restoration was delayed for 3 weeks after bleaching, showed higher bond strength (204.47 ± 44.76). However, among the experimental groups, subgroup IIIB (232.3667 ± 22.95) showed the highest mean shear bond strength.

Discussion

In this esthetic dentistry era, tooth bleaching is the most commonly used procedure for discolored teeth. Carbamide peroxide is the most widely used bleaching agent, which, when applied on dental structures, causes the release of free radicals in the form of

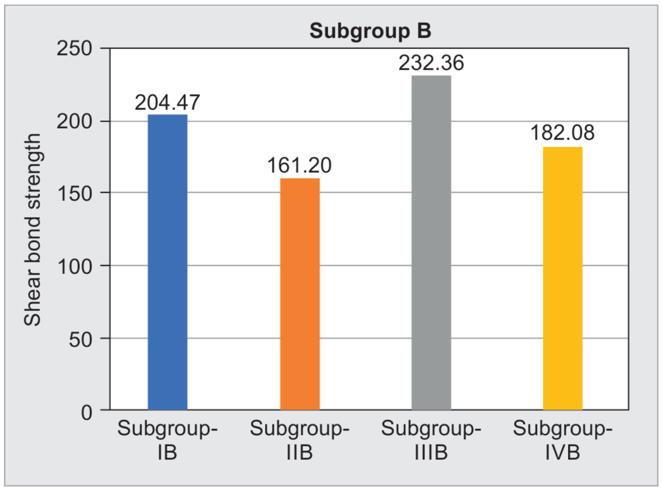

Fig. 2: Comparison of mean values of subgroup B after 3 weeks

Table 2: *Post hoc* comparison of subgroup A

					95% confidence interval	
Group	*Subgroup A*	*Mean difference (I–J)*	*Standard error*	*Significance*	*Lower bound*	*Upper bound*
Group IA	Group IIA	−63.05333[a]	13.27091	0.000	−98.1932	−27.9135
	Group IIIA	−136.10667[a]	13.27091	0.000	−171.2465	−100.9668
	Group IVA	−97.60667[a]	13.27091	0.000	−132.7465	−62.4668
Group IIA	Group IA	63.05333[a]	13.27091	0.000	27.9135	98.1932
	Group IIIA	−73.05333[a]	13.27091	0.000	−108.1932	−37.9135
	Group IVA	−34.55333	13.27091	0.056	−69.6932	0.5865
Group IIIA	Group IA	136.10667[a]	13.27091	0.000	100.9668	171.2465
	Group IIA	73.05333[a]	13.27091	0.000	37.9135	108.1932
	Group IVA	38.50000[a]	13.27091	0.026	3.3601	73.6399
Group IVA	Group IA	97.60667[a]	13.27091	0.000	62.4668	132.7465
	Group IIA	34.55333	13.27091	0.056	−0.5865	69.6932
	Group IIIA	−38.50000[a]	13.27091	0.026	−73.6399	−3.3601

[a]The mean difference is significant at the 0.05 level

Table 3: *Post hoc* comparison of subgroup B

					95% confidence interval	
Group	*Subgroup B*	*Mean difference (I–J)*	*Standard error*	*Significance*	*Lower bound*	*Upper bound*
Group IB	Group IIB	43.27333[a]	10.24695	0.001	16.1406	70.4061
	Group IIIB	−27.89333[a]	10.24695	0.042	−55.0261	−0.7606
	Group IVB	22.38667	10.24695	0.140	−4.7461	49.5194
Group IIB	Group IB	−43.27333[a]	10.24695	0.001	−70.4061	−16.1406
	Group IIIB	−71.16667[a]	10.24695	0.000	−98.2994	−44.0339
	Group IVB	−20.88667	10.24695	0.186	−48.0194	6.2461
Group IIIB	Group IB	27.89333[a]	10.24695	0.042	0.7606	55.0261
	Group IIB	71.16667[a]	10.24695	0.000	44.0339	98.2994
	Group IVB	50.28000[a]	10.24695	0.000	23.1472	77.4128
Group IVB	Group IB	−22.38667	10.24695	0.140	−49.5194	4.7461
	Group IIB	20.88667	10.24695	0.186	−6.2461	48.0194
	Group IIIB	−50.28000[a]	10.24695	0.000	−77.4128	−23.1472

[a]The mean difference is significant at the 0.05 level

hydroxy, per-hydroxy, or nascent oxygen ions. Any molecule that has one unpaired electron on its outermost shell is a free radical, which accounts for its extreme reactivity.[6] These molecules can interact with the electron-rich areas of the pigment structure, dissolving giant pigment molecules into smaller, less pigmented ones.[9] Pigmented molecules' double bonds are broken, causing size or structural changes. These alterations change the tooth color's optical qualities, giving teeth a whiter appearance.[10] After the application of carbamide peroxide gel, dissociation of the bleaching agent takes place, that releases a high amount of oxygen that remains on enamel prisms and dentin surface. Peroxide and oxygen free radicals present on the surface of dentine and dentinal fluid serves as a reservoir, holding them until they get eliminated by pulpal microcirculation or released later through surface diffusion. Poorly refined resin tags were formed on the bleached enamel.[11] Numerous investigations have demonstrated resin tags in bleached enamel were short, imperfectly formed, structurally defective, or altogether missing. Chng[12] Several methods have been proposed to diminish these problems, such as pretreatment of bleached enamel with alcohol, removal of the superficial enamel layer, use of adhesives containing organic solvents, cavity cleansing with catalase, or sodium ascorbate antioxidant are used to improve bond strength of composite to bleached enamel.

Since the reduction in bond strength after bleaching is reversible, the better method is to delay the bonding procedure after the bleaching process, which varies from 24 hours to 1, 2, or even up to 4 weeks.[13] In all cases, bleaching alone may not attain esthetics, some procedures like diastema and veneers composite restorations are needed. The longevity of restoration depends on appropriate adhesion to the teeth structure.[14] Several authors suggested delaying the procedure for 1 day to 3 weeks.[12] But in all cases, a patient can't wait for a long time due to practical reasons. Therefore, the use of antioxidants like ascorbic acid or natural antioxidants like grape seed extract, etc., immediately increases the bond strength of the composite to bleached enamel.[15] In this current study, we have taken both the time intervals of 1 day and 3 weeks to compare the effect of the time interval between these materials.

Antioxidants like ascorbic acid and its salts are excellent at reducing different oxides. In order for the bonding agents to maintain free radical polymerization and eventually regain the reduced bond strength to bleached dentin, Lai et al.[16] assumed that sodium ascorbate could recover the change in the oxidation–reduction potential of the oxidized tooth substance.[17] By using a process known as passive detoxification, sodium ascorbate can donate two highly energetic electrons to scavenge the free radicals. According to Kimyai and Valizadeh, the application of sodium ascorbate antioxidant solution for 10 minutes effectively restores the reduced bond strength of the composite.[18]

Although oligomeric proanthocyanidins (OPC) have been proposed as a viable sodium ascorbate substitute, there is not currently enough evidence to support their usage. Because of this, this study compared the usage of OPC as an antioxidant before bonding on bleached enamel.

In the present study, the bleaching procedure was carried out for 30 minutes using 35% carbamide peroxide, followed by the application of antioxidant solution for 10 minutes, and composite cylinders were built up on the middle third of the tooth surface using filtek Z250 XT. Shear bond strength was performed and Megapascal (MPa) values were recorded. Results revealed that subgroup IA (113.32 ± 9.73) showed the least shear bond strength when compared with subgroup IB (204.47 ± 44.76). This could be attributed to the formation of peroxide apatite after the bleaching process, which interferes with resin polymerization, and delaying the bonding process after bleaching might restore the bond strength that has been compromised by the removal of residual oxygen.[7] This result was in concurrence with the study conducted by Bulut et al.,[19] where they found a reduction in the shear bond strength of brackets immediately after bleaching. Another reason might be due to the fact that after 3 weeks of storage, peroxide ions replace the hydroxyl radicals in the apatite lattice, eliminating structural alterations. Consequently, similar studies show that delaying the bonding process might restore the bond strength that has been compromised.

The use of antioxidants may reverse the inclusion of peroxide ions. This antioxidants usage has been supported by the study conducted by Dabas et al.[20] About 10% sodium ascorbate subgroup IIA (176.37 ± 42.38) showed significantly highest bond strength in comparison to subgroup IA (113.32 ± 9.73), this result was similar to that of Murali Mohan et al.[21] and Manoharan et al.[4] No significant statistical difference between subgroup IIA (176.37 ± 42.38) and subgroup IB (204.47 ± 44.76) has been observed, this means the application of sodium ascorbate antioxidant within 24 hours effectively reverses the compromised bond strength as similar to that of delay in bonding procedure for 3 weeks; this result was identical to that of Subramonian et al.[7] There was no significant statistical difference between subgroup IIA (176.37 ± 42.38) and IIB (161.20 ± 8.22), this implies that delaying restoration for 3 weeks after the antioxidant application was of no clinical significance, this result was in concurrence with Nair et al., where they achieved similar bond strength between immediate antioxidant application group and stored for 2 weeks group.[22]

Subgroup IIA (176.37–42.38) samples were unable to restore the compromised bond strength to the same extent as subgroup IIIA (249.42–26.12) and subgroup IVA (210.92–52.05), which could be explained by the fact that the OPC present in grape seed and pine bark extract are much more potent than the common antioxidants like vitamin C, E, and β-carotene.[23] This finding is in line with a study by Subramonian et al.[7] They found that sodium ascorbate was less efficient in restoring the shear bond strength.

The present study result was conflicting with the study conducted by Arumugam et al.,[6] who stated that bleached teeth treated with 10% sodium ascorbate show the highest shear bond strength when compared with natural antioxidants like 6.5% proanthocyanidin and 5% lycopene extract. The reason behind this may be the low molecular weight of sodium ascorbate, which has a better penetration ability than proanthocyanidin.

In this study, treatment with 5% grape seed extract subgroup IIIA (249.42 ± 26.12) has shown a significant increase in bond strength compared to subgroup IA (113.32 ± 9.73), which suggests that using an antioxidant soon after bleaching might counteract the damaging effects of residual oxygen on bleached enamel. This study's findings were consistent with those of Vidhya et al.,[24] where they showed increased shear bond strength compared with bonding done immediately without applying antioxidants. A statistically significant increase in bond strength has been observed between subgroup IB (204.47 ± 44.76) and IIIA (249.42 ± 26.12), this study result was comparable to a study conducted by Subramonian et al.,[7] but no statistically significant difference was observed. There was a statistically significant difference between subgroup IIA (176.37 ± 42.38) and IIIA (249.42 ± 26.12). This result was similar to the study conducted by Shashibhushan et al.[25] and

Lokhande et al.,[26] where they showed better shear bond strength using proanthocyanidin containing antioxidants. The possible reason could be due to the OPC groups having multiple donor sites on their outermost shell that trap hydroxyl free radicals, and those are removed by esterification of epicatechin by gallic acid, which enhances the free radicals' scavenging ability.[27] In the current study, obtained superior bond strength compared to sodium ascorbate by treatment with OPCs. This result was similar to Abraham et al. study.[28] There was statistically no significant difference between subgroup IIIA (249.42 ± 26.12) and IIIB (232.36 ± 22.95), this result was similar to the study conducted by Nair et al.,[22] where they showed similar results between the immediate antioxidant application group and store for 2 weeks group.

There was statistical significance between subgroup IIIA (249.42 ± 26.12) and IVA (210.92 ± 52.05), where subgroup IIIA (249.42 ± 26.12) showed higher shear bond strength compared to that of subgroup IVA, this could be due to the presence of trace amounts of epicatechin and taxifolin, a flavan-3-ol, which are responsible for free radicals scavenging ability.[29] The present study result was contradictory to the study conducted by Subramonian et al.,[7] where they stated that pine bark extract showed high shear bond strength when compared to grape seed extract.

A study conducted by Vidhya et al.[24] showed that 5% grape seed extract showed high shear bond strength when compared to 10% sodium ascorbate, this result is similar in both the cases where bonding was performed immediately and bonding was performed after stored in distilled water for 2 weeks. This study result was in accordance with the present study, where group III (5% grape seed extract) showed an increase in shear bond strength when compared to group II (10% sodium ascorbate).

In this study, treatment with 5% pine bark extract subgroup IVA (210.92 ± 52.05) has shown a substantial increase in bond strength when compared to subgroup IA (113.32 ± 9.73), this indicates that the application of antioxidants immediately on bleached enamel surface could alter the compromised bond strength of the composite resin. This experimental result was in line with the study conducted by Mukka et al.,[30] where they showed an increase in shear bond strength after the immediate application of antioxidants formed using 5% pine bark extract. Subgroup IVA (210.92 ± 52.05) displayed the highest mean shear bond strength value compared to that of subgroup IB (204.47 ± 44.76) and was not statistically significant. There was no significance in the bond strength between subgroup IIA (176.37 ± 42.38) and subgroup IVA (210.92 ± 52.05), this result was identical to the study conducted by Al-Hassani and Al-Shamma,[31] who stated that there were no statistically significant differences between sodium ascorbate and pine bark extract antioxidant treated groups when compared to each other. This shows that the harmful effects of bleaching may be neutralized by all of these antioxidants and are equally effective.

In the present study, subgroup IVA (210.92 ± 52.05) showed the least shear bond strength compared with subgroup IIIA (249.42 ± 26.12), this result was inconsistent with the study conducted by Mukka et al.,[30] and Subramonian et al.,[7] where they showed the highest shear bond strength value with 10% pine bark extract compared to 10% grape seed extract. There was no statistically significant difference observed when subgroup IVA (210.92 ± 52.05) compared with subgroup IVB (182.08 ± 23.49). This conclusion was consistent with a Nair et al. study, demonstrating that postponing restoration for 3 weeks after antioxidant treatment had no significant difference.[22]

In a related investigation, Aksakalli et al.[32] found that using pine bark extract as an antioxidant produced nearly identical bond strengths to 10% sodium ascorbate, this finding is consistent with the findings of the current study.

Material safety analyses state that the health hazard level of sodium ascorbate is greater than OPC. Furthermore, there was no carcinogenic effect of OPC when their material safety data were examined.[7] While sodium ascorbate exhibits a mutagenic effect on mammalian somatic cells. However, the use of natural antioxidants as an effective substitute for chemical and synthetic antioxidants has been encouraging. In this study, an attempt was made to find the natural ingredient that could restore the compromised bond strength after bleaching. Hence, choosing pine bark extract and grape seed extract.

The loading procedure used for the bond strength test might be the reason for the variation in results. The loading method can influence relative strength measurements. However, the study was conducted *in vitro* and the *in vivo* could not be replicated. The restoration-to-tooth interface is subjected to numerous forces acting at once in the oral cavity. A restoration is loaded cyclically over the course of its lifetime. Even though a single load won't cause a failure, it could eventually cause slight deterioration and the loss of restoration over time. Nevertheless, to confirm the laboratory findings, extensive clinical trials are still required. Additional clinical trials are required to validate these results.

Limitations

- More cumbersome and time-consuming to prepare antioxidant solutions.
- Being an *in vitro* study does not replicate the oral cavity environment.
- The effect of temperature changes were not studied.
- Natural antioxidants used, such as grape seed and pine bark extract, have a tendency to stain composites.

Conclusion

All the antioxidants' immediate application could reverse compromised bond strength. So this can help avoid a waiting period before bonding to bleached enamel, which makes it clinically significant. Delaying the bonding process for 3 weeks after bleaching resulted in almost the same shear bond strength as that achieved after antioxidant treatment.

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