ORIGINAL RESEARCH

Assessment of the Effectiveness of Different Fluoride-releasing Bonding Agents on Prevention of Enamel Demineralization around Orthodontic Bracket: An *In Vitro* Study

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Abstract

Aim: The aim of the current research was to evaluate the efficacy of different fluoride-releasing bonding products in preventing enamel demineralization around orthodontic brackets by using a scanning electron microscope (SEM).

Materials and methods: This research was performed using 80 healthy human premolar teeth that were extracted in course of orthodontic therapy. Until use, the sample premolars were subjected to storage in 0.1% thymol. Each premolar was thereafter cleansed with pumice for 10 seconds. Stainless steel brackets for premolars were employed. The 80 samples were allocated at random to one of the four groups (20 in each) as follows: Group I, control; group II, Transbond Plus color change adhesive; group III, GC Fuji Ortho LC; and group IV, Vitremer. An hour following bonding, all samples were subjected to pH cycling at a temperature of 37°C for a 14-day period. The premolar teeth were assessed below SEM. Analysis was performed with the one-way analysis of variance. Statistical significance was set at a *p*-value less than 0.05.

Results: The extreme area of demineralization was abridged by the use of Transbond™ Plus color change adhesive (108.19 ± 0.68), trailed by GC Fuji Ortho LC (119.24 ± 0.37) use, Vitremer (121.56 ± 0.92) as well as the control group (141.88 ± 1.09) in that order. And there was a statistically significant difference found between the groups ($p < 0.001$). Tukey's honestly significant difference (HSD) was employed in an overall comparison of mean areas of enamel demineralization, which depicted that differences were significant statistically with the exception of group III and group IV.

Conclusion: The current research came to a conclusion that the Transbond Plus color change adhesive group was more potent in significant inhibition of demineralization areas in comparison to GC Fuji Ortho LC group and Vitremer group.

Clinical significance: In course of fixed orthodontic therapy, demineralization of enamel is an inherent occurrence. Multiple approaches are being continually developed to avoid the formation of white spot lesions (WSLs) that compromise esthetics and cause deprived remineralization that enhances the menace of dental caries. Bonding agents that can release fluorides are thus considered highly efficacious.

Keywords: Bonding agents, Enamel demineralization, Fluoride release, Premolar brackets.

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Introduction

Formation of areas of demineralization surrounding brackets is among the chief glitches pertaining to fixed orthodontic treatment that is documented to transpire in a short span and with much higher frequency amid patients seeking orthodontic therapy. Archwires, O-ring, brackets as well as metal ligature ties are essential constituents of fixed orthodontic employment. Nevertheless, they produce extra spots of retention, leading to enhanced plaque accretion.[1]

Following the use of brackets, alterations in the microbial flora such as the higher quantity of caries causing species like *Streptococcus mutans* attribute to a greater demineralization of enamel, as noted clinically adjoining the orthodontic appliances. As time progresses, this in addition to any state of the patient that enhances risk parameters to develop caries leads to active white spot lesions (WSLs) which if not treated in time can cause carious areas with frank cavitation.[2]

Fluoride (F) use is indeed unparalleled although a varied number of efficacious treatment and preventive products exist that have indeed positively impacted the patients' well-being besides the enhanced quality of life. Research involving products that release fluoride has shown promising results, reflecting their capability to control caries development either through inhibition of demineralization or by activating the remineralization of enamel/dentin.[3]

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Numerous approaches to administer fluoride throughout orthodontic therapy have been employed. These include oral rinses, varnishes, gels as well as kinds of toothpaste. Additionally, products that deliver fluoride during therapy have been launched like resin-altered, composite-bonding agents that release fluoride, glass Ionomer cements, compomers, fluoride devices that slowly release fluoride as well as elastomeric ligatures capable of disseminating fluoride.[4]

Transbond™ Plus color change adhesive contains a fluoroaluminosilicate glass as the fluoride source. The hydrophilic nature of the adhesive allows fluoride diffusion through the cured cross-linked matrix in an aqueous medium. Vitremer is a resin-modified glass ionomer cement; this penetrates through the smear layer into the dentinal tubules, providing micromechanical interlocking.[3] GC Fuji Ortho LC is an enamel protector by the sustained and rechargeable fluoride release, significantly reducing the risk of white spots developing during orthodontic treatment. Fluoride-carrying agents have been added to these orthodontic adhesive agents with the goal of releasing fluoride in the area surrounding the bracket to avoid demineralization. For a fluoride-liberating orthodontic bonding product to offer a protective effect against enamel decalcification adjoining the orthodontic bracket, the fluoride release should employ localized microbial outcomes or get unified with the surrounding enamel, rendering it impervious to acidic insults. The fluoride quantity of the bonding product does not determine its caries-arresting capacity, but rather it has been noted that the same is associated with the extent of release of fluoride. A number of orthodontic-bonding substances that contain fluoride have been launched to avoid enamel surrounding the brackets to decalcify.[5] Thus, the current research was performed to evaluate the efficacy of different fluoride-releasing bonding agents on deterrence of enamel demineralization around orthodontic brackets by using a scanning electron microscope (SEM).

Materials and Methods

Preparation of Samples

The current *in vitro* research was performed in the Department of Orthodontics and Dentofacial Orthopedics. The sample size was calculated by using $n = \frac{z_1^2 - \alpha/2}{d^2}$ Formula, where n is the required sample size, $z_1 \_ \alpha/2$ is a constant, its value for a two-sided test is 1.96 for 95%, and d is absolute precision of 20% = 0.2. After sample size calculation, this research included 80 healthy human premolar teeth that were extracted in course of orthodontic therapy. Healthy and sound tooth structure, absence of caries/discoloration/fracture, or hypoplastic changes constituted the inclusion criteria. The exclusion criteria were teeth with dental caries, developmental disorders, evident cracks/fractures, as well as restored buccal surfaces. Until use, the sample premolars were subjected to storage in 0.1% thymol. Each premolar was thereafter cleansed with pumice (S.S. White) for 10 seconds. Two patterns of adhesive tape measuring 3 × 4 mm and 1 × 4 mm were glued to the vestibular areas of the premolars to demarcate the bonding region. The samples were subjected to painting with nail varnish. Following the drying of the varnish over the next 2 hours, the 3 × 4 mm adhesive tape was removed. Stainless steel premolar brackets (American Orthodontics, United States) were utilized. Brackets with a mean base area of approximately 8.686 mm^2 were subjected to bonding in harmony with the recommendations of the manufacturer.

Bonding Procedure

The 80 sample premolars were randomly allocated (20 in each group) to one of the below groups using random allocation table:

Group I: Control (nonfluoride releasing): Subsequent to preparing the tooth surface with 37% phosphoric acid gel (3M Dental Products; St Paul, Minnesota, United States), the brackets were positioned in place. Following this, application of the fluid primer—Transbond XT (3M Unitek; Monrovia, California, United States) —to the etched surface was performed. Premolar brackets used in orthodontics that were made up of stainless steel were subjected to bonding to the premolars using the traditional nonfluoride-liberating Transbond™ XT. Prior to resin polymerization, the surplus resin was eliminated with the aid of an explorer.

Group II: Transbond™ Plus color change adhesive: Thirty-seven percent phosphoric acid gel for thirty seconds was employed for etching the teeth after which the premolars were subjected to rinsing and drying with an air spray devoid of oil for 20 seconds. Following this, the Transbond Plus Primer (3M Unitek) was coated on teeth for about 3 to 5 seconds. An air spray devoid of oil as well as moisture was employed for 1 to 2 seconds to parch the primer to a slim film. After this, bonding applied [Transbond™ Plus color change adhesive (3M Unitek, Monrovia, California, United States)] on stainless steel orthodontic premolar brackets to the central part of the coronal buccal surface was done and subjected to light-curing for 20 seconds by means of the light-curing element.

Group III: GC Fuji Ortho LC: The entire base of the bracket was enclosed with Fuji Ortho LC (GC International Corp., Tokyo, Japan), ensuring that there are not any spaces or bubbles, on the bracket mesh. Subsequently, the bracket was placed on the premolar by employing sufficient forces to eliminate surplus adhesive around the bracket leaving a steady width of the adhesive. The brackets were then settled to their ultimate location and pushed resolutely. The additional adhesive existing about the premolars surface was detached. Each portion of the premolar surface was subjected to light curing for 20 seconds.

Group IV: Vitremer™: Upon a slab, a cement spatula was used to mix an entire spoon of powder with one complete drop of the conforming liquid for about 45 seconds. The Vitremer (3M ESPE, Seefeld, Germany) mix thus prepared was applied on the complete base of the bracket prior to its placement on the premolar tooth with tweezers under gentle pressure. Additional cement was eliminated using an explorer followed by 20 seconds of light curing.

Demineralization and pH Cycling Procedure

Sixty minutes following bonding, the specimens were subjected to pH cycling for 14 days at a temperature of 37°C to replicate the environment within the oral cavity. The premolars were subjected to immersion in a demineralizing solution composed of 2.2 mM $CaCl_2$, 2.2 mM NaH_2PO_4, 1 ppm NaF, 100 mM NaCl, 50 mM acetic acid, and 0.02% NaN_3 for a period of 6 hours as well as for a period of 18 hours in artificial saliva composed of KH_2PO_4 0.9 mmol/L, potassium chloride (KCl) 50 mmol/L, calcium chloride ($CaCl_2$) 1.5 mmol/L, and Tris buffer 20 mmol/L, which were subjected to change on a day-to-day basis.

Evaluation of Samples under SEM

To evaluate the samples under the SEM analysis, they were coated with 40–60 nm of gold by means of a sputter coater followed by

observation under the microscope (Carl Zeiss EVO 40) at 2000× magnifying power (Fig. 1). The buccal surface of very premolar was assessed meticulously along the occlusal, proximal as well as the gingival surface up to the area of the orthodontic bracket bonding. SEM observation was done by experienced, standardized, and trained two observers who were blinded with regard to the experimental groups. The scores were then documented by employing the criteria below[6]:

Score 0—Enamel surface persisted flawlessly integral without grooves, pits, or porosity
Score 1—Existence of surface indiscretions upon the surface of enamel, devoid of demineralization of prismatic and/or interprismatic enamel
Score 2—Existence of wrinkles along with demineralized areas of prismatic/interprismatic enamel
Score 3—Diffuse demineralization involving the rod core, with breakdown of prism morphology

Statistical Analysis

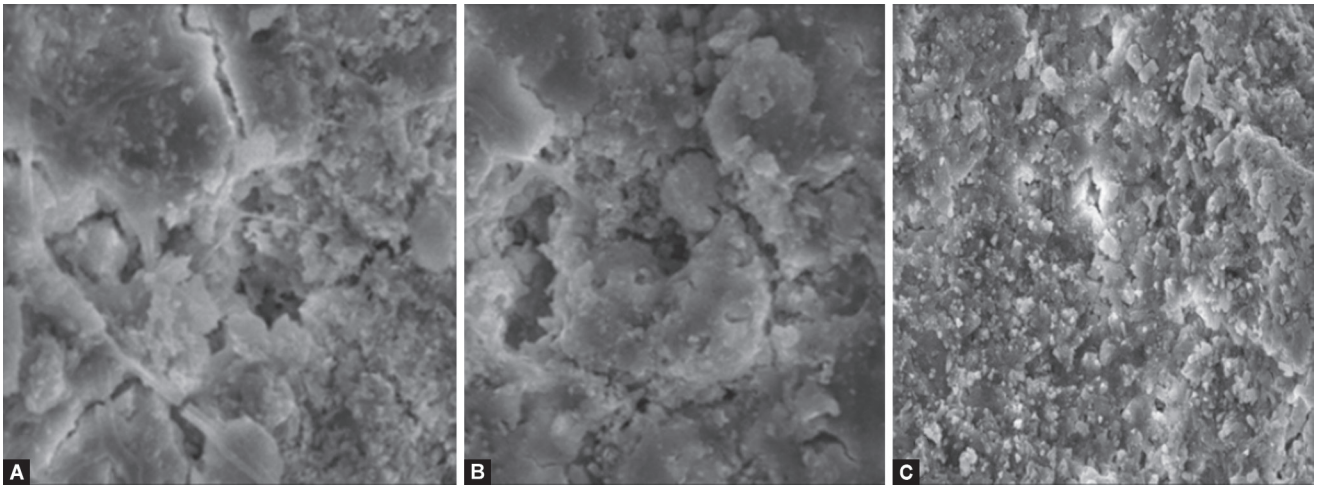
Data were analyzed using the SPSS version 20.0. Calculation of the mean as well as standard deviation was performed. The assessments amid different bonding agent groups in the deterrence of enamel demineralization around orthodontic brackets were calculated employing the one-way analysis of variance. Statistical significance was set at a *p*-value less than 0.05.

Results

Table 1 depicts inter- as well as intra-examiner Kappa scores employed in the assessment of mean enamel demineralization regions. These scores are high with most beyond 0.80, 0.84, and 0.80, which suggest concordance between the observers. The scores delineate a kappa value with near-complete harmony amid observer 1 and observer 2 without significant difference.

Table 2 delineates the mean area of demineralization among the control as well as investigational groups. Higher areas of demineralization were noted in the control group (128.10 ± 0.32), in pursuit by GC Fuji Ortho LC (127.49 ± 0.11) group, Transbond™ Plus color change adhesive (125.62 ± 0.18) group as well as Vitremer (125.22 ± 0.84) group. The differences amid the groups were not statistically significant.

The mean demineralization area amid the control and investigational groups subsequent to 14 days is depicted in Table 3. The extreme area of demineralization was abridged by the use of Transbond™ Plus color change adhesive (108.19 ± 0.68), trailed by GC Fuji Ortho LC (119.24 ± 0.37) use, Vitremer (121.56 ± 0.92) as well as the control group (141.88 ± 1.09) in that order. The difference among the groups was found to be statistically significant (p <0.001).



Figs 1A to C: SEM images of (A) Transbond Plus; (B) GC Fuji Ortho LC; (C) Vitremer

Table 1: Evaluation of intra- and interobserver variability using kappa test

Type of observation	*Symmetric measures kappa value*	*Significance*
Intra-examiner agreement (observer 1)	0.80	0.522
Intra-examiner agreement (observer 2)	0.84	0.194
Inter-examiner agreement	0.80	0.510

Table 2: Comparison of mean enamel demineralization between control group and experimental groups

Groups	*N*	*Mean ± std. deviation (µm)*	*F*	*p value*
Group I: Control	20	128.10 ± 0.32	24.378	0.694
Group II: Transbond™ Plus color change adhesive	20	125.62 ± 0.18		
Group III: GC Fuji Ortho LC	20	127.49 ± 0.11		
Group IV: Vitremer	20	125.22 ± 0.84		

Table 3: Comparison of mean enamel demineralization between control group and experimental groups after 14 days

Groups	*N*	*Mean ± std. deviation (μm)*	*F*	*p value*
Group I: Control	20	141.88 ± 1.09	23.114	0.001
Group II: Transbond™ Plus color change adhesive	20	108.19 ± 0.68		
Group III: GC Fuji Ortho LC	20	119.24 ± 0.37		
Group IV: Vitremer	20	121.56 ± 0.92		

Table 4: Overall comparison of mean enamel demineralization using Tukey's HSD

Group	*Compared with*	*Mean difference (I–J)*	*Sig.*
Group I	Group II	33.69	0.001
	Group III	22.64	0.001
	Group IV	20.32	0.001
Group II	Group I	−33.69	0.001
	Group III	−11.05	0.001
	Group IV	−13.37	0.001
Group III	Group I	−22.64	0.001
	Group II	11.05	0.001
	Group IV	−2.32	0.07
Group IV	Group I	−20.32	0.001
	Group II	13.37	0.001
	Group III	2.32	0.07

Tukey's honestly significant difference (HSD) was employed in an overall comparison of mean areas of enamel demineralization as noted in Table 4. The differences were significant statistically with the exception of group III and group IV.

Discussion

The average duration of orthodontic therapy is about 2 years during which approximately one-half of the patients show clinically evident white spot areas. It has been noted that in patients on orthodontic therapy, particularly teenagers, the decalcification rate is greater than those not seeking orthodontic treatment. Following debonding, these areas of white spots decline over the first 2 years after orthodontic therapy. Nevertheless, parts of demineralization may still persist on teeth for as long as 5 years subsequent to orthodontic therapy being cosmetically problematic.[7]

Numerous reasons are credited to the formation of demineralizing areas surrounding the brackets. These include the following: Higher plaque build-up on the surface of teeth surrounding the orthodontic attachment as their presence makes effective cleansing of teeth tough; the quantity and regularity of consuming carbohydrates in the diet in course of orthodontic therapy enhanced plaque microflora like *S. mutans* as well as lactobacillus due to carbohydrates in diet that reduce the pH and are chief contributory factor; and areas on the teeth with reduced action of saliva show a predominance of demineralized zones.[8]

Widely used techniques in preventing areas of enamel demineralization include appropriate advices on maintenance of oral hygiene, use of resin sealants to shield the surface of teeth, decreasing the solubility of enamel, as well as the inhibiting effect of bacteria by topically applying fluoride agents among others. Unfortunately, the majority of these techniques depend on patient cooperation and are of inadequate importance clinically. Hence, a prevention technique that is not patient dependent and releases low amounts of fluoride topically where there is an utmost requirement such as the area of plaque accumulation surrounding the brackets would be ideal. Bonding agents that release fluoride to encounter such decalcified zones of enamel around the orthodontic attachments have been established.[9]

In the present study, pH cycling was implemented for a period of 14 days to provide sufficient time to generate modifications in the demineralized enamel specimens. This research model permitted the enamel surfaces to be exposed to demineralization by means of acetic acid buffer at a pH of 4.5. This is in harmony with the technique adapted by Ten Cate et al.[10] to perform pH cycling in order to evaluate the anti-carious activity of materials on the phases of enamel demineralization and remineralization.

Multiple methods have been tested by numerous investigators to regulate the process of demineralization like fluoride-releasing products, lasers, and other fluoride treatment.[11] The cycles of demineralization and remineralization (decrease or uptake in mineral) on the surface of the enamel have been concluded after a research by Cury and Tenuta[12] and Arnold et al.[13] to be an active physiochemical course as oral microflora result in the formation of a biofilm on the tooth surface. When carbohydrates in the diet that can be fermented come in contact with this biofilm, a crucially small pH remains for some duration that can cause tooth disintegration as a consequence.

Transbond™ Plus color change adhesive was most efficacious in decreasing the demineralized areas in the current research, next by GC Fuji Ortho LC group, Vitremer group as well as the control group in that order. These findings are in harmony to the investigations of Wilson et al.[14] and Passalini et al.[15] These researches depicted that the fluoride component of Transbond Plus color change adhesive was capable of deterring the formation of WSLs adjoining the orthodontic brackets, substantiating the facts in the literature pertaining to the situation of large cariogenic test in acidic saliva at a pH of 4.5 throughout the 14-day tenure of the pH cycling. When the samples were challenged with a high cariogenic environment at a pH leading to hydroxyapatite disintegration, no WSLs were seen in any of the tried resins. It is likely that fluoride played the role of a demineralization inhibitor in such situations. It appears that all through the demineralization process, the resin releases fluoride that combines with the free calcium of artificial saliva and again incorporates itself within the dental substrate, leading to fluoridated hydroxyapatite as well as calcium fluoride formation on the enamel surface.

Nevertheless, this is not in accordance with the research of da Silva et al.[3] The fluoride-releasing products were assessed as employed for bracket bonding in an *in vitro* environment. The resin-modified glass ionomer cement (Vitremer) depicted the maximum anticariogenic capacity. Transbond™ Plus color change adhesive group displayed abridged areas of demineralization as compared to GC Fuji Ortho LC group, in this research. These results are in harmony with the conclusion of Wandera et al.[16] Fuji

Ortho LC released fluoride at a level 27% lesser in artificial saliva than in distilled water on the first day. Chemical constituents of artificial saliva, like calcium, carbonate, hydrogen, phosphate, besides sodium, may interact with fluoride or get adsorbed by the cement, thus possibly acting as a blockade to decrease preliminary solubility.

Claydon[17] and Derks et al.[18] reported that the premolars are the teeth most affected by demineralization during orthodontic treatment. This is because during orthodontic treatment, the buccal surface of the teeth is difficult to clean and is less protected by saliva. Hence only premolars were used in the present study.

The inability to simulate completely the real intraoral environment is a limitation of the current research as this is an *in vitro* study. Research with longer durations must be performed to judge the durability of the procured results in effectively avoiding demineralization during the entire time of orthodontic therapy.

Conclusion

The current research came to a conclusion that Transbond Plus color change adhesive group was more potent in significant inhibition of demineralization areas in comparison to GC Fuji Ortho LC group and Vitremer group. This superiority makes either bonding agent a possible improvement for usage as an adjunct in preventive dentistry in clinically tolerable limits.

References

1. Demling A, Elter C, Heidenblut T, et al. Reduction of biofilm on orthodontic brackets with the use of a polytetrafluoroethylene coating. Eur J Orthod 2010;32(4):414–418. DOI: 10.1093/ejo/cjp142.
2. Al Mulla AH, Al Kharsa S, Kjellberg H, et al. Caries risk profiles in orthodontic patients at follow-up using cariogram. Angle Orthod 2009;79(2):323–330. DOI: 10.2319/012708-47.1.
3. da Silva SR, da Silva LAH, Basting RT, et al. Evaluation of the anticariogenic potential and bond strength to enamel of different fluoridated materials used for bracket bonding. Rev Odontol UNESP 2017;46(3):138–146. DOI: 10.1590/1807-2577.06716.
4. Benson PE, Parkin N, Millett DT, et al. Fluorides for the prevention of white spots on teeth during fixed brace treatment. Cochrane Database Syst Rev 2004;3:CD003809. DOI: 10.1002/14651858.CD003809.pub2.
5. Rix D, Foley T, Banting D, et al. A comparison of fluoride release by resin-modified GIC and polyacid modified composite resin. Am J Orthod Dentofacial Orthop 2001;120(4):398–405. DOI: 10.1067/mod.2001.116083.
6. Chandru TP, Yahiya MB, Peedikayil FC, et al. Comparative evaluation of three different toothpastes on remineralization potential of initial enamel lesions: a scanning electron microscopic study. Indian J Dent Res 2020;31(2):217–223. DOI: 10.4103/ijdr.IJDR_745_18.
7. White SN, Paine ML, Ngan AY, et al. Ectopic expression of dentin sialoprotein during amelogenesis hardens bulk enamel. J Biol Chem 2007;282(8):5340–5345. DOI: 10.1074/jbc.M604814200.
8. Swapna G, Sharma S, Soni VP, et al. Demineralization adjacent to orthodontic brackets with fluoride releasing and conventional bonding agents. Indian J Dent Res 2016;27(4):426–432. DOI: 10.4103/0970-9290.191894.
9. Barik AK, Duggal R. Comparative evaluation of fluoride release from chemically cured and light-cured orthodontic bonding agents and surface alteration of enamel: an in vitro study. J Indian Orthodont Soc 2020;54(3):233–239. DOI: 10.1177/0301574220937525.
10. Ten Cate JM, Buijs MJ, Miller CC, et al. Elevated fluoride products enhance remineralization of advanced enamel lesions. J Dent Res 2008;87(10):943–947. DOI: 10.1177/154405910808701019.
11. Hu W, Featherstone JD. Prevention of enamel demineralization: an *in-vitro* study using light-cured filled sealant. Am J Orthod Dentofacial Orthop 2005;128(5):592–600. DOI: 10.1016/j.ajodo.2004.07.046.
12. Cury JA, Tenuta LM. Enamel remineralization: controlling the caries disease or treating early caries lesions? Braz Oral Res 2009;23(Suppl. 1):23–30. DOI: 10.1590/s1806-83242009000500005.
13. Arnold WH, Haase A, Hacklaender J, et al. Effect of pH of amine fluoride containing toothpastes on enamel remineralization *in vitro*. BMC Oral Health 2007;7:14. DOI: 10.1186/1472-6831-7-14.
14. Wilson RM, Donly KJ. Demineralization around orthodontic brackets bonded with resin-modified glass ionomer cement and fluoride-releasing resin composite. Pediatr Dent 2001;23(3):255–259. Available from: https://pubmed.ncbi.nlm.nih.gov/11447960/
15. Passalini P, Fidalgo TK, Caldeira EM, et al. Preventive effect of fluoridated orthodontic resins subjected to high cariogenic challenges. Braz Dent J 2010;21(3):211–215. DOI: 10.1590/s0103-64402010000300006.
16. Wandera A, Spencer P, Bohaty B. In vitro comparative fluoride release, and weight and volume change in light-curing and self-curing glass ionomer materials. Pediatr Dent 1996;18(3):210–214. Available from: https://pubmed.ncbi.nlm.nih.gov/8784911/
17. Claydon NC. Current concepts in toothbrushing and interdental cleaning. Periodontol 2000 2008;48:10–22. DOI: 10.1111/j.1600-0757.2008.00273.x.
18. Derks A, Kujipens-Jagtman AM, Frencken JE. Caries preventive measures used in orthodontic practices: an evidence-based decision? Am J Orthod Dentofacial Orthop 2007;132(2):165–170. DOI: 10.1016/j.ajodo.2005.10.028.