

The Addition of CPP-ACP Paste after Dental Polishing in Glass Ionomer Adhesion as a Fissure Sealant Material

Paramita, Annisa Listya¹, Rahmitasari, Fitria², Akwan, Yulie Emilda³

^{1,3}Department of Pediatric Dentistry, Faculty of Dentistry, Hang Tuah University, Indonesia

²Department of Dental Material, Faculty of Dentistry, Hang Tuah University, Indonesia

ABSTRACT

Background: Fissure sealant prevented dental caries by applying material over pit and fissure in posterior teeth, so plaque and debris wouldn't be retain over it. Nowadays, resin based and glass ionomer based used widely as a fissure sealant materials. Glass ionomer based has an advantages in anti-caries effect by fluoride releasing. The adhesion of dental material to the tooth was consider as a factor of material choice. Shear bond strength was a material test to show the attachment of dental material into tooth. **Objective:** This study was aimed to determine the effect of CPP-ACP paste addition after dental polishing in glass ionomers Fuji VII shear bond strength as a material for fissure sealants.

Method: This study was a laboratory experimental research with post-test only group design. Twenty one GIC Fuji VII applied in putty box consist of dental enamel divided into 3 group, each groups consist of 7 samples. The control group was GIC Fuji VII applied without CPP-ACP paste addition after dental polishing. Group 1 was GIC Fuji VII applied with CPP-ACP paste addition after dental polishing and group 2 was GIC Fuji VII applied with CPP-ACFP paste addition after dental polishing. Shear bond strength calculated with UTM machine. Data analysis using Kruskal-wallis followed by Mann-Whitney test.

Result: There were significant differences ($p < 0.05$) seen from the GIC Fuji VII shear bond strength on the groups 1 compared with control group. But, there were no significant different seen in control group compare to group 2 and in group 1 compare to group 2. **Conclusion:** The addition of CPP-ACP paste after dental polishing increased Fuji VII shear bond strength as fissure sealant material.

KEYWORDS: Fissure sealant, Glass Ionomer, CPP-ACP, Shear bond strength

ARTICLE DETAILS

Published On:
07 December 2023

Available on:
<https://ijmscr.org/>

INTRODUCTION

Oral common chronic infectious disease, namely dental caries starting with primarily Streptococcus Mutans, the cariogenic bacteria adherent in the tooth surface, then metabolize sugars to produce acid. The acidogenic environment then demineralizing the tooth structure, started with enamel over time¹ which leads to the formation of dental caries lesion. In permanent dentition, the development of new caries lesion associated with the depth of first permanent molars's fissure. The preventive method for patients should be chosen by assessing the importance of the fissure anatomy² in developing dental caries.

Dental fissure sealants or dental sealant and fluoride varnishes are used widely to prevent caries³ Sealants act to prevent caries by sealing the depth fissure with flowing material. When fissure are sealed, they will no bacterial

growth then continued to become dental decay. Evidence shows that fissure sealants are effective in preventing caries in many teeth of children and adolescents compared to teeth with no sealants. Nowadays, the choice of material for fissure sealant is resin-based or glass ionomer-based. Glass ionomer materials is known to have advantages over resin materials, including: could be applied in pre-erupted teeth, could be used in younger children and another advantage is the release of fluoride (an anti-caries agent). In addition, adequate isolation of the GIC material is not required. Moreover, fissure sealants based on glass ionomer are more effective than composite resins in preventing the attachment of *S. mutans* bacteria.⁵ Research on the attachment of Fuji VII glass ionomer cement, which is sometimes used as a glass ionomer-based fissure sealant material towards tooth structure, has not been widely found. However, in a previous study by

The Addition of CPP-ACP Paste after Dental Polishing in Glass Ionomer Adhesion as a Fissure Sealant Material

Kucukyilmaz and Savas, it was found that the highest shear bond strength was found in Fuji VII EP (glass ionomer material with CPP-ACP content) compared to other types of glass ionomer materials.⁶ Problems arise when this material is not available in the market. In the Indonesian market, this problem encourage the authors to conduct research about the effect of adding CPP-ACP paste on the adhesion of fissure sealants based on glass ionomer.

METHODS

This study was an experimental laboratory research with post test only control group design. Twenty-one samples were divided into 3 groups (control, treatment 1, treatment 2). In the control group we only stained and applied GIC Fuji VII material on tooth samples. Samples in the treatment group 1 were added CPP-ACP after polishing then applied GIC Fuji VII material, while in treatment group 2 we added CPP-ACFP (CPP-ACP with fluoride) after polishing, then applied GIC Fuji VII material.

Samples were made by selecting premolars according to the following criteria: no caries, no decalcification, never installed orthodontic brackets, no enamel defects, and no fillings—Samples then prepared the buccal portion of the premolars to the extent of enamel using a wheel diamond bur until smooth and even. The root of tooth was cut and then implanted in putty impression material measuring length x width x height of 10 x 10 x 25 mm. The teeth that had been embedded in the putty beam were then stained with pumice and treated with the addition of CPP-ACP paste and CPP-ACFP paste after polishing and filled with GIC Fuji VII material with 4 mm diameter and 4 mm height of object. Shear bond strength was measured in megapascals (MPa) using a universal testing machine (UTM) of the Shimadzu brand at a speed of 1 mm/minute until the sample and specimen were separated into 2 parts between the fissure sealant material and the teeth. After hitting the specimen, the number on the load will increase until the sample is split into 2 parts and the crosshead will automatically stop. The number on the load will indicate the maximum force which is then used to calculate the shear bond strength using the formula below, (Anusavice et al., 2013):

$$\sigma = \frac{F}{A} \times 0,09807 \text{ MPa}$$

Description:

- σ = Shear bond strength (MPa)
- F = Force (Kgf)
- A = Surface area (cm²)

RESULT

The result of shear bond strength measurement in 21 sample was divided into 3 groups in the following table:

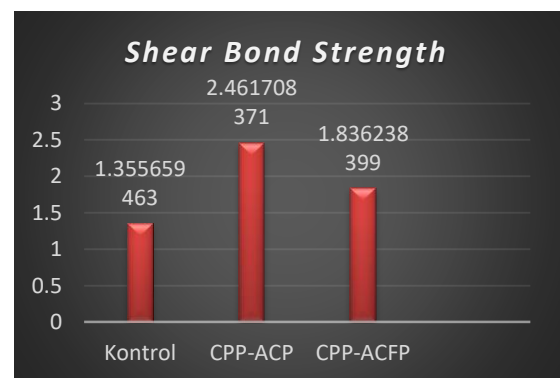
Table 1. Shear bond strength measurement in 3 groups

Groups	Sample	Maximum force (N)	Surface area (mm ²)	Shear strength (MPa)
			3.14 x 2 mm x 2 mm	Maximum force / surface area
Control	1	5.22868	12.56	0.416
	2	12.4946	12.56	0.995
	3	24.3276	12.56	1.937
	4	29.311	12.56	2.334
	5	16.8354	12.56	1.340
	6	17.2044	12.56	1.370
	7	13.7879	12.56	1.098
Treatment-1 (added with CPP-ACP after polishing)	1	20.0739	12.56	1.598
	2	16.9921	12.56	1.353
	3	38.7143	12.56	3.082
	4	39.4963	12.56	3.145
	5	41.7701	12.56	3.326
	6	39.3661	12.56	3.134
	7	20.0206	12.56	1.594
Treatment-2 (added with CPP-ACFP after polishing)	1	0.60288	12.56	0.048
	2	36.9302	12.56	2.940
	3	6.639	12.56	0.529
	4	43.4113	12.56	3.456
	5	27.058	12.56	2.154
	6	25.5593	12.56	2.035
	7	21.2414	12.56	1.691

Meanwhile, the average of shear bond strength in 3 groups can be seen in table 2 and graph 1.

Table 2. The average of shear bond strength in 3 groups

Variable : Shear Bond Strength	Groups	Results
	Control	1,3557 ± 0,629
	Treatment-1	2,4617 ± 0,892
	Treatment-2	1,8362 ± 1,219



Picture 1. Comparison of the average shear bond strength in the control group, group 1 (CPP-ACP) and group 2 (CPP-ACFP)

Table 2 and Graph 1, demonstrated that the average of shear bond strength in the treatment group with the addition of CPP-ACP paste after polishing (P1), and with the addition of CPP-ACFP paste after polishing (P2) were higher than in the control group. The highest result of shear bond strength was obtained in treatment group 1, namely the group with the addition of CPP-ACP paste after polishing.

The next stage is Kruskal Wallis and Mann Whitney test to find out the difference between the 3 groups. From the test above, the significance value of $p = 0.117$ ($p > 0.05$). We can conclude that there is no significant difference in the three groups.

The Addition of CPP-ACP Paste after Dental Polishing in Glass Ionomer Adhesion as a Fissure Sealant Material

Table 3. Kruskal Wallis test result in 3 different groups

	Groups	Sig.
Shear Bond Strength different test	Control - Treatment 1 – Treatment 2 (3 Groups)	0.117

The last statistical test used was the Mann Whitney test to compare one group to the other. We used the test to establish the comparison between:

- Control group vs treatment group 1 (CPP-ACP paste addition after polishing)
- Control group vs treatment group 2 (CPP-ACFP paste addition after polishing)
- Treatment group 1 vs treatment group 2 (CPP-ACP paste addition after polishing vs CPP-ACFP paste addition after polishing)

The comparison of shear bond strength in control group and treatment group 1 (CPP-ACP) were significantly different ($p = 0.025 < 0.05$), though the comparison between control group and treatment group 2 (CPP-ACFP) showed no significant difference ($p = 0.338 > 0.05$). The similar significantly indifference were found in the comparison between treatment group 1 and treatment group 2 ($p = 0.482 > 0.05$)

Table 4. Mann Whitney test result among groups

	Groups	Sig.
Shear Bond Strength	Control and treatment 1	0.025
	Control and treatment 2	0.338
	Treatment 1 and treatment 2	0.482

Based on the results of the different tests carried out between each group, it was found that only the control group and group 1 (treatment with the addition of CPP-ACP after smearing) had significant differences, while between other groups, the differences were not significant. The absence of a significant difference does not mean that there is no difference at all because when viewed from the average there is a difference in numbers. However, statistical calculations based on the results of differences in result was not significant ($p > 0.005$)

DISCUSSION

Fissure sealant is a preventive method in dental caries which is carried out by applying a layer of cover which will protect pits and fissures from soft food components.⁷ Fissure sealants have been used in the last 5 decades as a preventive and control method of dental caries both in primary and permanent teeth.⁸ The incidence of caries in the occlusal surfaces of permanent molars in children and adolescents reduces for almost 80% in treatment group which applied the occlusal surface with sealant compared with control groups that did not have sealants in the fissures. When comparing to

fluoride varnishes, carious lesions in the occlusal surfaces reduced approximately 70% in permanent molars that applied with sealant, but it was supported only by low-quality evidence.⁹

Use of glass ionomer as a sealant agent gives advantage as this material continuously releases fluoride and gives continuous protection from dental caries while still attached. Fluoride release is a result of acid-base reaction between the fluoro-aluminosilicate glass powder and the water-based polyacrylic liquid.¹⁰ Glass ionomer can be applied as a sealant agent on the deep fissures of primary molars which are difficult to isolate in less cooperative pediatric patients, or in partially erupted first permanent molars that are prone to dental caries. In some cases glass ionomer material needs to be evaluated and possibly replaced with resin-based material if isolation is adequate.¹¹

Fluoride release from the GIC material influenced on shear bond strength between GIC material and tooth. extrinsic and intrinsic factors mixed in a complex process happened when fluoride release from GIC. Intrinsic factors such as the formulation of glass ionomers, p/w ratio or powder and liquid composition in the GIC material, the geometry of the sample, duration for blending powder and liquid (mixing time), material solubility or porosity, temperature, surface finishing, and treatment. Extrinsic factors playing part in fluoride release include type and acidity (pH) of storage media, the room or environmental temperature, study design, analysis method of the study. Surface loss, fluoride diffusion through cracks and material pores, and bulk diffusion are three mechanism that responsible in fluoride releasing in glass ionomers.¹² The lowest shear bond strength was observed in research by Somani et al in 2016 was Fuji II. It could be because during the initial setting period, they are susceptible to attack by moisture. They also have short working time, long setting time and maturation time.¹³ However, this study did not involve GIC Fuji VII as a comparison used in this study. More research is needed involving the Fuji VII GIC and other types in the future.

Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) was a new type of bioactive material which derived from casein (milk protein). It formulated from two parts: casein phosphopeptides (CPP) and amorphous calcium phosphate (ACP) that blended and formed CPP-ACP. It acts as a reservoir of bio-available calcium and phosphate, promoting their precipitation on the enamel surface and effectively enhancing remineralization.^{14,15} Based on study in clinical research and in vitro, CPP-ACP showed excellent remineralization effects, demonstrated improvements in morphology, aesthetics, and function in treating white spot lesions case.¹⁶

CPP-ACP causes decrease in demineralization and increase of remineralization in the surface of an early dental caries.¹⁷ An example of naturally-sourced agent that affects

The Addition of CPP-ACP Paste after Dental Polishing in Glass Ionomer Adhesion as a Fissure Sealant Material

enamel remineralization is the free-range chicken eggshells, usually applied as a paste.¹⁸

In other countries the mix of GIC and CPP-ACP based fissure sealant agents have been marketed and are ready to use. Laboratory studies show that the addition of CPP-ACP on a GIC-based agent can increase compressive strength and micro-tensile bond strength on dentin, and increase release of calcium and phosphate in acidic and neutral environment.¹⁹ This correlates with the higher average shear bond strength in treatment group 1 in our study (addition of CPP-ACP paste after polishing), compared to control group (without addition). The result was significant, which indicates the addition of CPP-ACP paste after polishing will increase shear bond strength on GIC material.

Glass Ionomer combined with 3% CPP-ACP significantly reduced development of *S. mutans* biofilm, thus indicating another potential anticariogenic mechanism of CPP-ACP. The formation of *S. mutans* biofilms disrupted by aqueous CPP-ACP addition. The use of CPP-ACP containing GIC together with regular CPP-ACP treatment can reduce the number of *S. mutans*.²⁰ Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) merges into conventional GIC as a bioactive additional agent.²¹ GIC with a CPP-ACP modification shows increase in enamel resistance against acid demineralization, without any mechanical effect that weakens GIC. Moreover, GIC with a CPP-ACP modification can also prevent the advancement of secondary root caries as the biofilm undergoes a cariogenic multi-species change in a laboratory experiment. Other study also showed the addition of CPP-ACP on GIC material can increase shear bond strength (SBS) on enamel compared to conventional GIC. Adhesion of CPP-ACP modified GIC on a caries lesion or a demineralized dentin is still yet to be described. However, studies showed the agent is still more superior to conventional GIC in initiating remineralization of dentin.²²

In our study, in our treatment group 2 we added CPP-ACFP, or CPP-ACP with fluoride after polishing. Statistic study showed no significant difference in shear bond strength between control and treatment 2 groups. Theoretically, CPP-ACFP combines 2 remineralization agent, CPP-ACP and fluoride.²³ CPP-ACP can localize ACP in a dental structure, and increase calcium phosphate in dental plaque, thus maintaining the activity of calcium and free phosphate ion. This will help maintain dental enamel supersaturation resulting in decreased dental demineralization and increased remineralization.¹⁴ The presence of fluoride ion causes the unstable ACP to produce fluoroapatite. The formation of intraoral fluoroapatite will eventually take up existing fluoride ions and reduce dental enamel demineralization ability in an acidic environment.²⁴ Attigupe et al in 2019 found that the addition of CPP-ACP increased the fluoride release from the fluoride varnish which might have further enhanced its remineralization property.²⁵ The enhanced remineralization will impacted the shear bond strength. Shear bond strength values reduces after pre-treatment of enamel

with 1.23% APF and 5% SFV prior to fixing orthodontic brackets²⁶

After application of CPP-ACP agent, we used a 10% polyacrylic acid as a conditioner for 20 seconds. Conditioners are described as an agent (usually acidic) used to condition enamel/dentin surface by removing smear layer which on a certain level can stimulate demineralization of enamel or dentin surface. Smear layer itself is described as an amorphous and relatively smooth layer of microcrystalline debris, accumulated in the surface that cannot be seen with the naked eye. The American Association of Endodontics describe smear layer as a surface film or debris retained on enamel, dentin, and cementum after instrumentation with either rotary instruments or endodontic files.²⁷ Use of conditioning agent is usually expected to increase the interaction between tooth and restoration agents.

Based on both theories above, the addition of fluoride ion in CPP-ACFP form will prevent demineralization process on enamel caused by conditioning agents. It is still possible that this may prevent the adhesion of glass ionomer cement agent used in this study as fissure sealant. However, further study on the effect of fluoride application on the efficiency of conditioner agents is still needed.

In this study we found a widely fluctuative shear bond strength among treatment groups. This may be caused by several uncontrollable factors during the making of study samples, such as the condensation force of GIC agent application or the varied timing of GIC application. However, this does not indicate lack of control during the process. We controlled w:p ratio as suggested by manufacturing factory in each sample. Factors that may affect mechanical characteristics including shear bond strength of GIC include mixing technique which may minimize porosity, material manipulation of powder and liquid until setting phase, powder-liquid ratio, viscosity, and material homogeneity. Porosity is one factor that may affect GIC material strength. Porosity results from an imperfect mixing technique, that causes trapped air inside the mix, which will eventually affect adhesion of GIC material to dental surface.²⁸

CONCLUSION

In this research we found that the addition of CPP-ACP paste after dental polishing increased Fuji VII shear bond strength as fissure sealant material.

REFERENCES

- I. Rathee M, Sapra A. Dental Caries. [Updated 2022 Jun 12]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK551699/>
- II. Sánchez-Pérez L, Irigoyen-Camacho ME, Molina-Frechero N, Zepeda-Zepeda M. Fissure Depth and Caries Incidence in First Permanent Molars: A Five-Year Follow-Up Study in Schoolchildren. *Int J*

- Environ Res Public Health. 2019 Sep 23;16(19):3550
- III. (Kashbour W, Gupta P, Worthington HV, Boyers D. Pit and fissure sealants versus fluoride varnishes for preventing dental decay in the permanent teeth of children and adolescents. *Cochrane Database Syst Rev.* 2020 Nov 4;11(11):CD003067.
 - IV. Ahovuo-Saloranta A, Forss H, Walsh T, Nordblad A, Mäkelä M, Worthington HV. Pit and fissure sealants for preventing dental decay in permanent teeth. *Cochrane Database Syst Rev.* 2017 Jul 31;7(7):CD001830.
 - V. Zettira, Nazala Zetta; Probsari, Niken; Lestari, Pujiana Endah. (2017). Perlekatan Streptococcus mutans pada Aplikasi Fissure Sealant Berbahan Resin Dibandingkan dengan Ionomer Kaca Fuji VII (The Attachment of Streptococcus mutans for Fissure Sealant application Made of Resin Compare With Glass Ionomer Fuji VII). *Pustaka Kesehatan*, [S.l.], v.5, n. 3, p. 441-448, nov. ISSN 2355-178X. Available at: <<https://jurnal.unej.ac.id/index.php/JPK/article/view/6012>>.
 - VI. Kucukyilmaz, E dan Savas, S. (2016). Evaluation of shear bond strength, penetration ability, microleakage and remineralisation capacity of glass ionomer-based fissure sealants. *European Journal of Paediatric Dentistry*. vol. 17/1. Hal: 17-23
 - VII. Herawati. (2018). *Senyum Menawan dengan Gigi Sehat dan Rapi Mungkinkah?*. Surabaya: Revka Prima Medika.
 - VIII. AAPD. Evidence-based Clinical Practice Guideline for the Use of Pit-and-Fissure Sealants. *PEDIATRIC DENTISTRY V 38 / NO 5 SEP / OCT 16* . 263-279
 - IX. Wright JT, et all. Sealants for Preventing and Arresting Pit-and-fissure Occlusal Caries in Primary and Permanent Molars A systematic review of randomized controlled trials—a report of the American Dental Association and the American Academy of Pediatric Dentistry. *PEDIATRIC DENTISTRY V 38 / NO 4 JUL / AUG 16*
 - X. Anusavice, K. J., Shen, C., & Rawls, R. H. (2013). *Phillips' Science of Dental Materials* (12th ed.). St. Louis, Missouri: Mosby Elsevier.
 - XI. Dean, Jeffrey A, Avery, David R and Ralph E. McDonald. (2011). *McDonald and Avery. Dentistry for the Child and Adolescent*. 9th edition. Missouri: Mosby Inc.
 - XII. Ajob JN, Aref NS, Al-Wakeel EE. Effect of casein phosphopeptide-amorphous calcium phosphate on fluoride release and micro-shear bond strength of resin-modified glass ionomer cement in caries-affected dentin. 2018. *Restorative Dentistry & Endodontics* 2018; 43(4): e45. Published online: 30 October 2018
 - XIII. Somani R, Jaidka S, Singh DJ, Sibal GK. Comparative Evaluation of Shear Bond Strength of Various Glass Ionomer Cements to Dentin of Primary Teeth: An in vitro Study. *Int J Clin Pediatr Dent.* 2016 Jul-Sep;9(3):192-196. doi: 10.5005/jp-journals-10005-1362. Epub 2016 Sep 27. PMID: 27843248; PMCID: PMC5086004.
 - XIV. Reema SD, Lahiri PK, Roy SS. Review of casein phosphopeptides-amorphous calcium phosphate. *Chin J Dent Res.* 2014;17(1):7-14. PMID: 25028684.
 - XV. Zhou C, Zhang D, Bai Y, Li S. Casein phosphopeptide-amorphous calcium phosphate remineralization of primary teeth early enamel lesions. *J Dent.* 2014;42(1):21–9
 - XVI. Ma, X., Lin, X., Zhong, T. et al. Evaluation of the efficacy of casein phosphopeptide-amorphous calcium phosphate on remineralization of white spot lesions in vitro and clinical research: a systematic review and meta-analysis. *BMC Oral Health* 19, 295 (2019). <https://doi.org/10.1186/s12903-019-0977-0>
 - XVII. Rahayu, Yani Corvianindya. PERAN AGEN REMINERALISASI PADA LESI KARIES DINI. *STOMATOGNATIC - Jurnal Kedokteran Gigi*, [S.l.], v. 10, n. 1, p. 25-30, dec. 2015. ISSN 2442-4935. Available at: <<https://jurnal.unej.ac.id/index.php/STOMA/article/view/2019>>. Date accessed: 26 July 2021
 - XVIII. Setyawati, Ani. Pengaruh Pasta Cangkag Telur Ayam Negeri Terhadap Remineralisasi Email. *Denta Jurnal*. Vol 13, No. 2 (2019)
 - XIX. Zalizniak, I.; Palamara, J.E.; Wong, R.H.; Cochrane, N.J.; Burrow, M.F.; Reynolds, E.C. Ion release and physical properties of CPP-ACP-modified GIC in acid solutions. *J. Dent.* 2013, 41, 449–454.
 - XX. Dashper SG, Catmull DV, Liu SW, Myroforidis H, Zalizniak I, Palamara JE, Huq NL, Reynolds EC. Casein Phosphopeptide-Amorphous Calcium Phosphate Reduces Streptococcus mutans Biofilm Development on Glass Ionomer Cement and Disrupts Established Biofilms. *PLoS One.* 2016 Sep 2;11(9):e0162322. doi: 10.1371/journal.pone.0162322. PMID: 27589264; PMCID: PMC5010225.
 - XXI. Li, J.; Xie, X.; Wang, Y.; Yin, W.; Antoun, J.S.; Farella, M.; Mei, L. Long-term remineralizing effect of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) on early caries lesions in vivo: A systematic review. *J. Dent.* 2014, 42, 769–777.
 - XXII. Zhao IS, Mei ML, Zhou ZL, Burrow MF, Lo EC, Chu CH. Shear Bond Strength and Remineralisation Effect of a Casein Phosphopeptide-Amorphous

The Addition of CPP-ACP Paste after Dental Polishing in Glass Ionomer Adhesion as a Fissure Sealant Material

Calcium Phosphate-Modified Glass Ionomer Cement on Artificial "Caries-Affected" Dentine. *Int J Mol Sci.* 2017 Aug 7;18(8):1723. doi: 10.3390/ijms18081723. PMID: 28783130; PMCID: PMC5578113

- XXIII. Arifa MK, Ephraim R, Rajamani T. Recent Advances in Dental Hard Tissue Remineralization: A Review of Literature. *Int J Clin Pediatr Dent.* 2019;12(2):139-144. doi:10.5005/jp-journals-10005-1603
- XXIV. Walsh LJ. Contemporary technologies for remineralization therapies: A review. *Int Dent SA.* 2009;11:6-16
- XXV. Attiguppe P, Malik N, Ballal S, Naik SV. CPP-ACP and Fluoride: A Synergism to Combat Caries. *Int J Clin Pediatr Dent.* 2019 Mar-Apr;12(2):120-125. doi: 10.5005/jp-journals-10005-1608. PMID: 31571784; PMCID: PMC6749876.
- XXVI. Leódidio Gda R, Fernandes HO, Tonetto MR, Presoto CD, Bandéca MC, Firoozmand LM. Effect of fluoride solutions on the shear bond strength of orthodontic brackets. *Braz Dent J.* 2012;23(6):698-702.
- XXVII. Berman RH, Hargreaves K. 2015. *Cohen's Pathways of the Pulp.* 11th edition. Elsevier.
- XXVIII. McCabe JF, Walls AWG. 2008. *Applied Dental Material.* 9th ed. Oxford: Blackwell Publishing Ltd, h. 6, 200, 246, 354, 358.