

Volume 46, Number 1, March 2013

ISSN 1978-3728

Dental Journal

Published quarterly per year

Majalah Kedokteran Gigi



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Accredited No. 56/DIKTI/Kep./2012

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Research Report

Shear strength of orthodontic bracket bonding with GIC bonding agent after the application of CPP-ACPF paste

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ABSTRACT

Background: White spot lesion is a major problem during fixed orthodontic treatment. This problem can be solved by minimizing white spot lesion before the treatment and using a fluoride-releasing bonding agent. The application of casein phosphopeptides-amorphous calcium phosphate fluoride (CPP-ACPF) paste as remineralization agent before treatment and GIC as orthodontic bonding agent is expected to overcome this problem as well as to strengthen GIC bonding. **Purpose:** To measure the shear strength of fix orthodontic appliance using GIC bonding with CPP-ACPF application prior treatment. **Methods:** In this study, 50 extracted premolars were randomly divided into 2 groups: group I as treatment group and group II as control group that was not given CPP-ACPF pretreatment. After having been cut and put into acrylic device, the samples in group I were given pretreatment with CPP-ACPF paste on enamel surface for 2 minutes twice a day as instructed in product label for 14 days. Orthodontic brackets were bonded with GIC bonding agent on all samples in both groups as instructed in product label. Then, the shear strength was measured by Autograph Shimatzu with crosshead speed 0.5 mm/minute. The data was analyzed with Independent t-test. **Results:** The mean shear bond strength in treatment group was 19.22 ± 4.04 MPa and in control group was 12.97 ± 3.97 MPa. Independent t-test analysis showed that there was a significant difference between treatment and control group ($p < 0.05$). **Conclusion:** CPP-ACPF pretreatment could increase GIC orthodontic bonding shear strength.

Key words: Shear strength, GIC bonding, CPP-ACPF

ABSTRAK

Latar belakang: Lesi putih karies merupakan masalah utama selama perawatan dengan peranti cekat ortodonti. Hal ini dapat diatasi dengan cara mengurangi lesi putih sebelum perawatan dengan menggunakan bahan bonding yang mengandung fluorida. Aplikasi pasta casein phosphopeptides-amorphous calcium phosphate fluoride (CPP-ACPF) sebagai bahan remineralisasi sebelum perawatan dan bahan bonding GIC diharapkan dapat mengatasi masalah ini sekaligus menambah kekuatan cekat bahan bonding GIC. **Tujuan:** Mengukur kekuatan geser piranti cekat ortodonti menggunakan bonding GIC dengan aplikasi pasta CPP-ACPF. **Metode:** 50 gigi premolar dibagi menjadi 2 kelompok, yaitu kelompok I sebagai kelompok perlakuan dan kelompok II sebagai kelompok kontrol. Setelah gigi dipotong dan ditanam dalam tabung akrilik, diaplikasikan pasta CPP-ACPF pada permukaan enamel sampel pada kelompok I selama 2 menit 2 kali sehari selama 14 hari. Aplikasi pasta CPP-ACPF tidak dilakukan pada kelompok kontrol. Kemudian breket ortodonti direkatkan dengan bahan bonding GIC pada semua sampel di kelompok I dan kelompok II. Kekuatan geser diukur dengan menggunakan alat Autograph Shimatzu dengan kecepatan cross head 0.5 mm/menit. Data dianalisis dengan statistik independent t-test. **Hasil:** Rerata kekuatan geser pada kelompok perlakuan $19,22 \pm 4,04$ MPa dan pada kelompok kontrol $12,97 \pm 3,97$ MPa. Analisis Independent t-test menunjukkan bahwa terdapat perbedaan signifikan antara kelompok perlakuan dan kontrol ($p < 0.05$). **Kesimpulan:** Kekuatan geser antara piranti cekat ortodonti dengan bahan bonding GIC meningkat setelah aplikasi pasta CPP-ACPF.

Kata kunci: Kekuatan geser, bonding GIC, CPP-ACPF

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INTRODUCTION

Recently, orthodontic treatment is a viable option to repair teeth arrangement and aesthetics, however, risks of caries will increase on patients using fixed orthodontic appliances. White spot lesions, which are an indication of early caries activity, usually occur after orthodontic treatment in varying degrees. In the first few weeks after the extrication of brackets, an exponential decrease of the white spot lesions was found remineralized, albeit only around half the initial lesions were remineralized after 6 months without any specific therapy. Topical fluoride is often used by dentists to help remineralization.¹ To prevent the worsening of occurring caries lesions, an oral hygiene improvement and a minimization of early caries lesions are needed before performing any orthodontic treatment. Besides, bonding agents with cariostatic properties or those containing fluoride can also be used to deter this problem.

Fluoride may influence the strength of glass ionomer cement (GIC) based orthodontic bracket adhesive, whereas the higher the fluoride content within the GIC, the lower its shear strength.^{2,3} GIC bonding agent has the lowest bonding strength and is also prone to debonding.⁴ However, it is also able to prevent caries better as compared with other bonding agents due to its fluoride-releasing property.⁵ Moreover, having low bonding failure is a priority in fixed orthodontic treatments due to the frequent debonded bracket replacement which is inefficient, time-consuming and costly.⁶

Because of this problem, an alternative remineralization agent is needed, one that does not lower the bonding strength of the orthodontic brackets. It is stated that casein phosphopeptides – amorphous calcium phosphate (CPP-ACP) paste can help remineralization.⁷ CPP-ACP acts as a remineralization and anti-cariotic agent due to its plaque-preventing property and also plays the role of calcium and phosphate reservoir on dental surface.⁸ Casein phosphopeptides-amorphous calcium phosphate fluoride (CPP-ACPF), the combination of CPP-ACP with



Figure 1. Bracket attachment.

fluoride, is proven to have a stronger remineralization effect compared to CPP-ACP.⁹

It was stated that 3% and 5% CPP-ACP addition to the GIC bonding agent attains a lower fluoride-releasing property than pure GIC bonding agent, although there was an increased release of calcium ions and inorganic phosphates.¹⁰ Demineralization lesions found on the enamel around the GIC bonding agent are also fewer than those found in the GIC bonding control group. However, the effect of using CPP-ACPF paste before orthodontic treatment towards GIC orthodontic bracket bonding agent is still unknown.

One of the methods utilized to determine the bonding strength of orthodontic bracket bonding agents is performing shear strength tests. The shear strength value is the maximum force attained as the bracket shifted parallelly on dental surface.² A low shear strength can mean that the bonding strength is also low. Based on the idea, this study was done to measure the effect of CPP-ACPF pretreatment to GIC orthodontic bonding agent shear strength. This study was expected to widen the knowledge of dentists and dental students about the effect of remineralization prior to orthodontic treatment.



Figure 2. Shear strength test aid appliance (A and B).

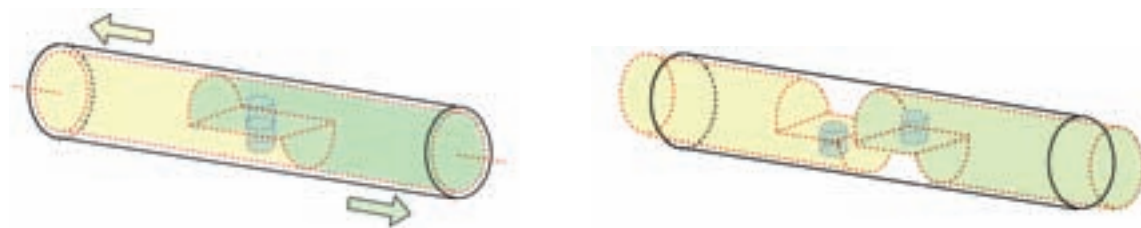


Figure 3. The diagram of shear strength test aid appliance. Yellow and green area indicates the smaller two halves of bronze cylinder; each blue area indicates acrylic from plastic tube I and II.

MATERIALS AND METHODS

Fifty samples of post extracted human maxilla and mandibular premolars with normal enamel. Samples were obtained from The Department of Oral and Maxillofacial Surgery in Faculty of Dentistry, Universitas Airlangga. The teeth samples were divided randomly into 2 groups: the treatment group which received CPP-ACPF paste (Tooth Mousse Plus, GC) application, and the control group which did not receive CPP-ACPF paste application. Artificial saliva was made by mixing all following ingredients to attain a homogenic mixture. The ingredients are: Na_2HPO_4 (0,426 grams), NaHCO_3 (1,68 grams) CaCl_2 (0,147 grams), extraction H_2O (800 ml), and HCl -1M (2,5 ml).¹¹

After extraction the teeth were cleaned in flowing water and stored in a moist condition by wrapping in a piece of cloth drabbed with distilled water, then placed in a closed container and frozen in the temperature of -4°C until the experiment was done to preserve the condition and humidity the teeth as it would be within the mouth cavity.¹² The tooth surface was cleaned using pumice on a low-speed handpiece brush to remove debris, and dried using cotton pellets.

The teeth were cut on the crown with the same thickness as plastic tube I using a separating disc attached to a straight handpiece while holding the samples around the root by hand. The teeth were cut on the cervical section to separate the crown and the root. Plastic tube I was placed on top a glass plate oiled with vaseline. Then the acrylic admixture was filled into the plastic tube layer by layer up to the dough stage before placing the cut tooth inside the tube with its buccal surface facing upwards. Mesio-distal and occluso-gingival surfaces were arranged to be balanced to the most convex part at the same height as the top of the plastic tube. Excessive acrylic was then cleaned using a modelling knife without changing the position of the attached tooth.

The CPP-ACPF paste was applied on the enamel surface of the tooth for 2 minutes. Afterwards the tooth was then irrigated using 10 ml of distilled water twice to ensure that the tooth enamel is clean, then it was dried using sterile cotton pellet and stored in a glass bottle filled with artificial saliva. This treatment was given twice a day (once every 12 hours) for 14 days.¹³ Samples were stored within glass bottles filled with artificial saliva when the samples do not receive any treatment.

Polyacrylate acid (20%) conditioner (Fuji Ortho LC, GC) was applied using cotton pellets on cleaned tooth surface. Application was done on the area of the bracket bonding (20 mm^2). Conditioner was applied for 20 seconds, then flushed using water spray for 30 seconds. GIC powder and liquid (Fuji Ortho LC, GC) were mixed according to manufacturer direction, which is 3:1 in weight (1 spoon of powder to 2 drops of the liquid). The powder was separated into 2 equal parts, one part was mixed with the whole liquid for 10 seconds, and the second part was mixed into the admixture in the following 10-15 seconds.

GIC adhesive was applied on bracket (Ortho Organizer) using an applicator stick, pressed on the bracket to allow mechanical tension on the back of the bracket. Bracket was then attached onto the tooth to obtain the thinnest layer of adhesive as possible. Excessive adhesive agent was removed using a probe. The bracket was then cured using a light curing unit from four directions, each for 10 seconds. All specimens were stored in normal saline for 3 days before performing the attachment into plastic tube II using acrylic (Vertex).

Area around the bracket was given blue wax (Cavex) at the height of tube I to eliminate undercut areas before the application of acrylic in plastic tube II. Then, teeth attached in acrylic were removed from plastic tube I. The remaining height of plastic tube II was filled with acrylic by inserting a layer of powder (3 mm), and then dropped with monomer. This was done repeatedly until the tube was full and then flattened using a glass plate. The excessive acrylic was removed using a modelling knife. After the acrylic hardens, the tooth and bracket attached in the acrylic were removed from plastic tube II and soaked in distilled water for 24 hours before further testing was performed.

The cast, tooth along with the bonding agent and bracket in a linear arrangement was then placed within a bronze cylinder opposite to the two halves of the bronze cylinder. To secure the movement that will occur, the two bronze halves were inserted into larger tube and fastened with a screw. The bronze aid appliance was placed on the Autograph Shimatzu from Japan with its lower hook clasped on the Autograph's holder and the upper hook is attached to the Autograph's hook which will later move upwards. The speed of the cross head was set on 0.5 mm/minute on the Autograph's control panel and the load recorded on the monitor from the release of the bracket was

then recorded. Afterwards, obtained data were analysed using an Independent t-test.

RESULTS

The shear strength (MPa) was obtained by dividing shear force value (kN) read on the Shimatzu Autograph device by the bracket surface area attached to tooth enamel, which was 12 mm². The shear strength of the treatment group which received an application of CPP-ACPF paste was greater than that of the control group. Kolmogorov Smirnov normality testing showed that data from both groups were normally distributed ($p > 0.05$). Homogeneity testing using Levene's test showed that data from both groups were homogen ($p > 0.05$). On a difference testing using Independent t-test, it was found that there was a significant difference between shear strength of the treatment group and the control group ($p < 0.05$).

DISCUSSION

Initial caries or white spot lesion is formed by the combination of carbohydrate diet and bacterial infection mediated by saliva. It is caused by the imbalance between enamel demineralization and remineralization. White spot lesion is a precursor to enamel caries. White discoloration of the early caries lesion is caused by an optical phenomenon due to the loss of minerals on the enamel surface or subsurface. The solubility of the enamel crystal started from demineralization at the enamel subsurface creating pores between enamel rods. This will cause alterations on enamel refraction index and coarseness.¹⁴

White spot lesion is a problem during orthodontic treatments, especially in fixed appliances treatments. The usage of fixed appliances will increase plaque retention. Furthermore, in a bad oral hygiene condition, plaque and acidogenic bacteria accumulation will cause demineralization. A study stated that 38% of patients have white spot lesion within 6 months after the attachment of fixed appliances and 50% of patients has white spot lesion by the end of their orthodontic treatment.¹⁵

Minimizing white spot lesions before fixed orthodontic treatment has become an important issue to clinicians. White spot lesion may exist on patients who have or have never received prior orthodontic treatment, or those who have received the first phase of orthodontic treatment before using fixed appliances, for instance: functional appliances, rapid maxillary expansion, or other treatments. Clinicians can help patients to overcome white spot lesions using some pretreatment procedures on the enamel.¹⁶

Besides, mineral content on enamel may also affect the attachment of orthodontic brackets. On demineralized enamel, for instance because of the presence of caries lesions or post bleaching, the bonding strength of the orthodontic bracket tends to be lower compared to the bracket bonding

Table 1. Descriptive analytic mean and standard deviation shear strength (MPa) and shear force (kN) results on each group

Group	Shear force		Shear strength	
	Mean	Standard deviation	Mean	Standard deviation
Treatment	0.23	0.05	19.22	4.04
Control	0.16	0.05	12.97	3.97

on normal enamel.^{17,18} Therefore, a procedure is needed to remineralize the enamel to improve the bonding strength of the bonding agent.

Several mechanisms that can be applied to minimize the enamel demineralization process during orthodontic treatment are by using topical remineralization agent, maintaining oral hygiene and controlling diet. The use of CPP-ACP as a remineralization agent showed a synergistic effect with topical fluoride, which has been widely known as a remineralization agent. Topical anticariotic effect of milk is the initial idea of the production of CPP which has the ability to stabilize calcium and phosphate in amorphous form. CPP molecule contains residual phosphoseryl group which increases calcium phosphate solubility by stabilizing ACP in neutral and alkaline condition. Multiple phosphoseryl residue in CPP binds with ACP nanoclusters in a supersaturated condition. This will hamper ACP molecule growth towards critical size which will allow ACP to change phase.¹⁴

In this study, the remineralization agent used is CPP-ACPF paste, which is CPP-ACP added with 0.2% of fluoride. The anticariotic activity of CPP-ACPF has been proven in vitro and in vivo on humans and animals. CPP-ACPF nanocomplexes binds with plaque and tooth surface, then acts as calcium and phosphate reservoir, and cause an increase in the concentration of calcium and phosphate ions in plaques. This will keep the supersaturated condition of ACP on enamel mineral, hamper demineralization and increase enamel remineralization. An immunolocation study states that CPP-ACPF fuses with supragingival plaque by binding to the bacteria's cell surface, intercellular matrix components of plaque and adsorbed macromolecules of the tooth surface. This condition will reduce the formation of cariogenic plaque.¹⁹

Recently, the most commonly used orthodontic bonding agent is the composite resin. Composite resin possesses the highest bonding strength of other types of bonding agents.²⁰ However, resin-based bonding agents have many disadvantages, such as the occurrence of more demineralization lesions in areas adjacent to the bracket.²¹ Acid etch is also required in the bonding procedure. The usage of phosphoric acid as acid etch can increase decalcification due to the loss of enamel minerals. This loss can also occur during debonding procedure. In addition, resin tags which penetrated the enamel surface during the bonding procedure will stay within the enamel even after debonding, which in turn causes the increase of staining on

tooth surface after orthodontic treatment is completed.²²

Another alternative to resin-based bonding agent is matrix-based bonding agent, which is commonly known as GIC bonding agent. GIC has a number of advantages compared to resin-based ones. GIC does not need absolute moisture control, which is hard to achieve because despite of isolation, the teeth in oral cavity will still be moistened by saliva.²³

GIC also possesses the ability to prevent enamel demineralization around the bracket because it contains fluoride that can be released to adjacent enamels in a relatively long period of time, other than capable to absorb fluoride from other sources, such as fluoride from toothpaste or mouthwash. In other words, this agent serves as a slow-release and rechargeable fluoride media. It will cause a decrease in the incidence of decalcification and formation of early caries lesion adjacent to the orthodontic bracket. Besides, GIC does not require acid etch which damages enamel surface and increase vulnerability to caries.²⁴

In spite of its advantages, GIC orthodontic bonding agent still has a limited use due to its lower bonding strength than other bonding agents like composite resin.²⁵ The bonding strength can be known by measuring the shear strength between GIC-bonded orthodontic bracket and the enamel surface it is attached to. Based on past studies, it has been known that the average shear strength of GIC bonding agent after CPP-ACPF paste treatment (19.22 MPa) was greater than that of control group which did not receive CPP-ACPF paste treatment (12.97 MPa). This number far exceeds the required bonding strength for orthodontic treatment, which is 6-8 MPa.^{26,27} This is probably caused by an increase in the level of calcium ion on the enamel due to the CPP-ACPF paste application. GIC bonding agent adheres to the enamel through chemical bonding with the calcium ions. In this case, GIC is capable to bond with more ion calcium on the enamel, resulting in a greater shear strength.

This study was done with in vitro approach so that it cannot prove the remineralization influence of CPP-ACPF paste towards the bonding strength of GIC bonding agent in the oral cavity. There are a few limitations of an in vitro study which is not similar to the biological conditions of the oral cavity such as: the absence of caries bacteria which can cause demineralization and create biofilm or plaque on tooth surface.²⁸ This plaque may inhibit CPP-ACPF activity to caries bacteria or biofilm.

To create an experimental condition as close to the condition within the oral cavity, freshly extracted teeth were used in this study. The teeth were stored and frozen right after extraction to avoid dehydration and loss of teeth minerals. The storage is intended so that all teeth have the same moisture during the experiment because the samples were not obtained at the same time. This moisture needs to be maintained because normal enamel in the oral cavity is comprised of 96 percent inorganic material, 1 percent organic material and 3 percent water²⁹ and the loss of water molecules may cause enamel alteration which will influence

the remineralization.

Artificial saliva was used in this study, however, it may never completely replace the function of saliva in the oral cavity. The composition of saliva in the oral cavity is different with those of the artificial saliva, thus the bonding of calcium and other molecules contained in the saliva to the tooth surface can not happen.²⁸ The composition and flow of saliva in the oral cavity is also influenced by the diet and activity of the person, causing it to be different in each people.³⁰ The volume of the artificial saliva also can not be the same to that of the saliva in the oral cavity. Tooth surface in different position in the oral cavity, for instance on the maxilla or mandible, will be moistured by a different volume and source of saliva. An increase in remineralization agent concentration given also may not be equalized as the volume increase done in vivo, causing the results of this study to less represent the effect of remineralization in vivo.²⁸

As a conclusion, the use of CPP-ACPF paste pretreatment could significantly increase the shear strength of orthodontic bracket bonding using GIC bonding agent.

REFERENCES

- Willmot D. White spot lesion after orthodontic treatment. *Seminars in Orthodontics*, 2008; 14(3): 209–19.
- Powers JM, Messersmith ML. Enamel etching and bond strength. In: Brantley WA, Eliades T, editors. *Orthodontic materials: Scientific and clinical aspects*. Stuttgart: Thieme; 2001. p. 111–3.
- Bishara SE, Soliman M, Laffoon JF, Warren J. Shear bond strength of a new high fluoride release glass ionomer adhesive. *Angle Orthod* 2008; 78(1): 125–8.
- Valletta R, Prisco D, De Santis R, Ambrosio L, Martina R. Evaluation of the debonding strength of orthodontic brackets using three different bonding systems. *Eur J Orthod* 2007; 29: 571–7.
- Watts DC. Orthodontic adhesive resins and composites: principles of adhesion. In: Brantley WA, Eliades T, editors. *Orthodontic materials: Scientific and clinical aspects*. Stuttgart: Thieme; 2001. p. 190–200.
- Zachrisson BU, Buyukyilmaz T. Bonding in orthodontics. In: Graber LW, Vanarsdall RL, Vig KWL, editors. *Orthodontics: Current principles and techniques*. 5th ed. Philadelphia: Elsevier; 2012. p. 727–37.
- Oshiro M, Yamaguchi K, Takamizawa T, Inage H, Watanabe T, Irokawa A, Ando S, Miyazaki M. Effect of CPP-ACP paste on tooth mineralization: an FE-SEM study. *J Oral Sci* 2007; 49(2): 115–20.
- Moule CA, Angelis F, Kim GH, Malipaoil S, Foo MS, Burrow MF, Thomas D. Resin bonding using an all-etch or self-etch adhesive to enamel after carbamide peroxide and/or cpp-acp treatment. *Australian Dent J* 2007; 52: 2.
- Jayarajan J, Janardhanam P, Jayakumar P, Deepika. Efficacy of CPP-ACP and CPP-ACPF on enamel remineralization—an in vitro study using scanning electron microscope and DIAGNodont. *Indian J Dent Res* 2011; 22(1): 77–82.
- Al Zraikat H, Palamara JEA, Messer HH, Burrow MF, Reynolds EC. The Incorporation of casein phosphopeptide-amorphous calcium phosphate into a glass ionomer cement. *Dent Mater* 2010; 27(3): 235–43.
- Jarpa P. Medición del pH de 12 preparaciones distintas de pasta de tabaco de mascar, relacionándolas con la adición a la nicotina. *Revista de la Facultad de Farmacia*. 2003; 45(2): 7–11.
- Budipramana ES. Pengaruh amine fluorida, sodium monofluorophosphate, dan sodium fluorida sebagai bahan remineralisasi pada permukaan enamel gigi dengan karies buatan.

- Thesis. Surabaya: Universitas Airlangga; 1987.
13. Rachmawati D. Permeabilitas enamel gigi permanen muda setelah aplikasi pasta casein phosphopeptide amorphous calcium phosphate (CPP-ACP). Thesis. Surabaya: Universitas Airlangga; 2009.
 14. Sudjalim TR, Woods MG, Manton DJ. Prevention of white spot lesions in orthodontic practice: a contemporary review. *Australian Dent J* 2006; 51(4): 284–9.
 15. Tufekci E, Dixon JS, Gunsolley JC, Lindauer SJ. Prevalence of white spot lesions during orthodontic treatment with fixed appliances. *Angle Orthod* 2011; 81(2): 206–10.
 16. Baysal A, Uysal T. Do enamel microabrasion and casein phosphopeptide-amorphous calcium phosphate affect shear bond strength of orthodontic brackets bonded to a demineralized enamel surface?. *Angle Orthod* 2012; 82(1): 36–41.
 17. Gonzalez-Lopes S, de Madeiros CLSG, Defren CA, Bolarios-Carmona MV, Sanchez-Sanches P, Menendez-Nunez M. Demineralization effects of hydrogen peroxide on bovine enamel and relation of shear bond strength of brackets. *J Adhes Dent* 2009; 11(3): 1–7.
 18. Attin R, Stawarczuk B, Kecik D, Knosel M, Wiechmann D, Attin T. Shear bond strength of brackets to demineralize enamel after different pretreatment methods. *Angle Orthod* 2012; 82(1): 56–61.
 19. Ekizer A, Zorba YO, Uysal T, Ayrikcil S. Effects of demineralization-inhibitor procedures on the bond strength of brackets bonded to demineralized enamel surface. *Korean J Orthod* 2012; 42(1): 17–22.
 20. Mandall NA, Millett DT, Mattic CR, Hickman J, Worthington HV, Macfarlane TV. Orthodontic adhesives: a systematic review. *J Orthod* 2002; 29(3): 205–10.
 21. Benson PE, Shah AA, Millett DT, Dyer F, Parkin N, Vine RS. Fluoridas, orthodontics and demineralization: a systematic review. *J Orthod* 2005; 32(2): 102–44.
 22. Komori A, Ishikawa H. Evaluation of a resin-reinforced glass ionomer cement for use as an orthodontic bonding agent. *Angle Orthod* 1997; 67(3): 189–96.
 23. Ali H, Maroli S. Glass ionomer cement as an orthodontic bonding agent. *J Contemp Dent Pract* 2012; 13(3): 650–4.
 24. Godoy-Bezerra J, Vieira S, Oliveira JHG, Lara F. Bond strength of resin-modified glass ionomer cement with saliva present and different enamel pretreatments. *Angle Orthod* 2006; 76(3): 470–4.
 25. Cook PA, Luther F, Youngson CC. An in vitro study of the bond strength of light-cured glass ionomer cement in the bonding of orthodontic brackets. *European J Orthod* 1996; 18: 199–204.
 26. Reynolds IR. A review of direct orthodontic bonding. *Br J Orthod* 1975; 2: 171–8.
 27. Whitlock BO III, Elck JD, Ackerman RJ Jr, Glaros AG, Chappell RP. Shear strength of ceramic brackets bonded to porcelain. *Am J Orthod Dentofacial Orthop* 1994; 106(4): 358–64.
 28. White DJ. The application of in vitro models to research on demineralization and remineralization of the teeth. *Adv Dent Res* 1995; 9(3): 175–97.
 29. Bath-Balogh M, Fehrenbach MJ. *Illustrated dental embryology, histology, and anatomy*. St. Louis: Elsevier Saunders; 2006; p. 180.
 30. Almeida PDV, Gregio AMT, Machado MAN, Lima AAS, Azevedo LR. Saliva composition and functions: a comprehensive review. *J Contemp Dent Pract* 2008; 9(3): 1–11.