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Micro-shear Bond Strength of Two All-in-one Adhesive Systems to Unground Fluorosed Enamel

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The aim of this study was to evaluate the micro-shear bond strengths of two all-in-one adhesive systems to unground fluorosed enamel.

Buccal surfaces of 24 normal and 24 moderately fluorosed teeth (Thylstrup and Fejerskov index, TFI=4-6) were bonded using G-Bond and Clearfil Tri-S Bond. The surfaces were then restored with a resin composite, stored for 24 hours in water, and tested for micro-shear bond strength (MSBS). Fracture modes, etching patterns, and adhesive interfaces were studied under a scanning electron microscope (SEM). Data were analyzed with two-way ANOVA, whereby no significant differences in MSBS were found among the four groups ($p>0.05$). Through SEM examination, it was shown that 1 μm of resin tag-like extensions had penetrated into the enamel for both adhesives at the enamel-adhesive interface. Based on the results obtained, it was concluded that MSBS was not influenced by the severity of fluorosis. Furthermore, there were no differences in enamel bond strength between G-Bond and Clearfil Tri-S Bond.

Keywords: Unground fluorosed enamel, All-in-one adhesive, Micro-shear bond strength

INTRODUCTION

Dental fluorosis is a hypomineralization of tooth enamel caused by continuous ingestion of excessive fluoride during tooth development[1)]. This results in various pathological changes in the tooth structure, ranging from opaque white patches in the enamel to striated, pitted, and discolored enamel — thus giving rise to cosmetic problems of teeth in affected individuals[2)].

Fluorosed teeth are usually restored with tooth-colored restorations, such as composite resins or ceramic veneers[3)]. When treating fluorosed enamel, it is customary to reduce a layer of enamel to remove the discolored and rough surface[4)]. However, grinding of the enamel increases the surface roughness of tooth and exposes the deeper enamel layers. From the principles of no intervention or minimal intervention, "unground" enamel may thus be better than "ground" enamel[5)]. With a definite trend toward minimal intervention, it was anticipated that enamel already affected by fluorosis might be preserved and left unground during restorative treatment.

Recently, there is a strong advocacy for simplified bonding steps and more user-friendly adhesive systems. From the clinicians' perspective, the new generation of all-in-one adhesive systems may provide a better solution for restoration of fluorosed teeth as they are uncomplicated and user-friendly. These adhesives vary in their acidity by virtue of the composition and concentration of polymerizable acids and/or acidic resin monomers[6,7)]. However, Pashley *et al.* reported that the efficacy of self-etching primers on unground enamel did not depend upon their etching aggressiveness[8)].

To date, no studies have been undertaken concerning the bond strength of all-in-one bonding systems to unground fluorosed enamel. Therefore, the purpose of this study was to evaluate the micro-shear bond strengths of two all-in-one bonding systems to unground fluorosed enamel.

MATERIALS AND METHODS

Tooth specimens

Forty-eight extracted third molars (fluorosed and non-fluorosed) from patients living in fluorosis endemic areas in Sri Lanka were collected. These teeth were cleaned and stored in distilled water in a refrigerator at 4℃. All the teeth belonged to patients aged between 20 and 40 years. Informed consent was obtained from all the patients whose teeth were used in this study. Twenty-four teeth used for this study were moderately fluorosed teeth (Thylstrup and Fejerskov index, TFI=4-6)[9)]. The

Table 1 Materials used in this study.

Product	Components
All-in-one adhesive	
G-Bond (GC Corporation, Tokyo, Japan)	4-MET, UDMA, silica, phosphoric acid ester monomer, acetone, water, photoinitiator
Clearfil Tri-S Bond (Kuraray Medical Co., Tokyo, Japan)	MDP, Bis-GMA, HEMA, hydrophobic dimethacrylate, di-camphorquinone, ethyl alcohol, water, silanated colloidal silica
Resin composite	
Clearfil ST (Kuraray Medical Co., Tokyo, Japan)	Silanated barium glass, silica, colloidal silica, Bis-GMA, TEGDMA, photoinitiator

4-MET: 4-methacryloxyethyl trimellitic acid; UDMA: urethane dimethacrylate;
MDP: 10-methacryloloxydecyl dihydrogen phosphate; Bis-GMA: bisphenol A-diglycidylmethacrylate;
HEMA: 2-hydroxyethyl methacrylate; TEGDMA; triethyleneglycol dimethacrylate

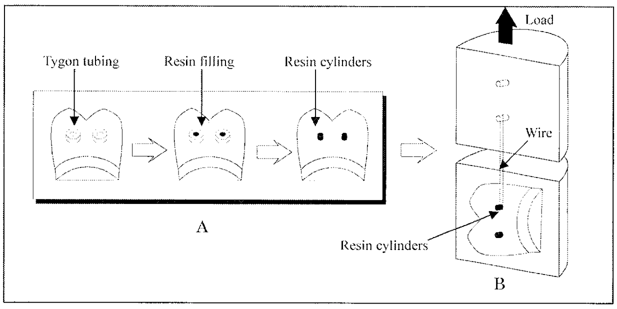

Fig. 1 Schematic illustration of specimen preparation procedure.

other 24 teeth were normal (*i.e.*, non-fluorosed) teeth.

Roots of the teeth were cut just below the cementoenamel junction. Then, the buccal and lingual surfaces of crown segments of approximately 2 mm in thickness were sliced parallel to the long axis of the tooth using a slowly rotating diamond blade (Isomet, Buehler, Lake Bluff, IL, USA). For each obtained tooth slice, two middle regions on the enamel surface — 1 mm on either side of the midline parallel to the long axis of tooth — were selected as bonding sites for micro-shear bond strength test.

Unground tooth slices were polished using a prophylactic paste (Profylax Pasta CCS, RDA 120, Dentsply, Germany) and cleaned ultrasonically in distilled water for five minutes. Subsequently, the fluorosed teeth were divided into two subgroups of twelve teeth and each group was treated with one of the two all-in-one adhesive systems, G-Bond (GC, Tokyo, Japan) or Clearfil Tri-S Bond (Kuraray Medical, Tokyo, Japan) according to manufacturer's instructions (Table 1). Twenty-four non-fluorosed (normal) teeth, twelve teeth in each group, were treated in the same manner as the fluorosed teeth.

Specimen preparation

Specimen preparation procedure is illustrated in Fig. 1. Prior to light-curing of the bonding resin, an iris of micro-bore Tygon tubing (R-3603, Norton Performance Plastic Co., Cleveland, USA) with an internal diameter of 0.8 mm and a height of 0.5 mm was mounted on enamel bonding surface to define the bonding area (Fig. 1A). Bonding site was then light-cured for 10 seconds. Following which, the cylinder was filled with a resin composite (Clearfil ST, Shade A2, Kuraray Medical, Tokyo, Japan). A plastic matrix strip was placed over the resin composite, gently pressed flat, and light-cured for 40 seconds to make very small cylinders of approximately 0.8-mm diameter and 0.5-mm height. The curing unit used was Optilux 500 (Demetron, Danbury, CT, USA) with an intensity of 700 ± 8 mW/cm^2.

Specimens were kept at room temperature (23℃) for one hour, and then the Tygon tubing was removed. Following which, specimens were stored in water at 37℃ for 24 hours. Before bond strength testing, all samples were checked for defects under an optical microscope at ×30 magnification. Samples showing air bubble inclusions, interfacial gaps, and other defects were discarded.

Micro-shear bond strength evaluation

Figure 1B shows the micro-shear test apparatus. Using cyanoacrylate glue (Zapit, DVA, Corona, CA, USA), each tooth slice was carefully bonded onto a testing device (Bencor-Multi-T, Danville Engineering Co., San Ramon, CA, USA) mounted in a universal testing machine (EZ-test-500N, Shimadzu, Kyoto, Japan) for the micro-shear bond strength test. A thin wire (0.2 mm in diameter) was looped around a resin cylinder, making contact with half of its circumference and gently held flushed against the resin-enamel interface. The resin-enamel interface, the wire loop, and the center of the load cell were aligned as straight as possible to ensure the desired orientation of the shear force. Each cylinder was then subjected to a shear force at a crosshead speed of 1 mm/min (Fig. 1B). Values of load at failure and the surface area allowed micro-shear bond strength in units of stress (MPa) to be calculated. Twenty-four sites in 12 specimens were tested for each test group.

Failure modes

Following bond strength testing, all fractured resin-enamel surfaces were examined under a confocal laser scanning microscope (1LM21-HW, Lasertec, Yokohama, Japan) to identify the failure mode. Failure modes were categorized into one of the following six types:

A – Adhesive failure in more than 95% of the bonded area between enamel, hybrid-like enamel layer or overlying adhesive resin.

B – Cohesive failure in enamel more than 95% of the bonded area.

C – Cohesive failure in adhesive resin more than 95% of the bonded area.

D – Mixed failure with adhesive failure in more than 50% of the bonded area.

E – Mixed failure with cohesive failure in enamel more than 50% of the bonded area.

F – Mixed failure with cohesive failure in resin more than 50% of the bonded area.

Scanning electron microscopic observation

Enamel slices of four groups were polished with a prophylactic paste (Merssage Fine, Shofu Inc., Kyoto, Japan), cleaned ultrasonically in distilled water for five minutes, then primed in the same manner as that employed for the bonding test samples. The slices were rinsed with acetone for 10 minutes under ultrasonic movement to remove any crystals or other residues from the primer. The specimens were dried in an incubator for 24 hours. Finally, the surfaces were sputter-coated with gold (SC-701AT, Quick Auto Coater, Sanyu Electron Inc., Tokyo, Japan) and observed under a scanning electron microscope (SEM) (JSM 5600LV, JEOL, Tokyo, Japan).

To observe the interface between enamel and adhesive resin, four bonded enamel-resin specimens were cross-sectioned at the interface and polished using lapping film sheets (Imperial, Sumitomo 3M, Tokyo, Japan) up to 3 μm. They were then etched with 0.1 N HCL for 30 seconds and washed with distilled water. The specimens were placed overnight in an incubator; gold sputter-coated, and subsequently observed using a SEM.

Statistical analysis

Two-way analysis of variance (ANOVA) with Tukey's *post hoc* test at $p<0.05$ was used to determine any significant differences among the data for micro-shear bond strength test with respect to type of enamel and adhesive system. The Chi-squared test was used for the non-parametric data analysis of failure modes. SPSS for windows (SPSS Inc., Chicago, IL) was used for data analysis.

RESULTS

Micro-shear bond strengths and failure modes

Table 2 shows the mean micro-shear bond strength values and standard deviations in MPa. Two-way ANOVA revealed no statistically significant interactions between the two types of enamel and the two adhesive systems used ($F=0.493$, $p=0.485$). Modes of failure following the micro-shear bond strength test are summarized in Table 3. Chi-squared test showed no significant association between modes of failure with the two adhesive systems (asymmetric significance, two sided: 0.136). Adhesive failure was the most prevalent type of failure for both types of enamel.

SEM observations

SEM photomicrographs of the unground enamel treated with G-Bond and Clearfil Tri-S Bond are shown in Figs. 2 and 3, respectively. Enamel surfaces conditioned with the all-in-one adhesives are shown in Figs. 2A and 3A. Clearfil Tri-S Bond and G-Bond could only partially dissolve the aprismatic surface layer. Shallow, mild coral-like etching

Table 2 Micro-shear bond strengths to unground enamel (MPa±SD)

	Normal	Fluorosed
G-Bond	22.4 ± 5.3	20.1 ± 3.8
Clearfil Tri-S Bond	21.4 ± 5.0	19.7 ± 3.9

n=24 for each group.
No statistically significant differences in micro-shear bond strength between the four groups.

Table 3 Modes of failure after micro-shear bond testing

Adhesive	Enamel	No. of specimens	Failure Mode					
			A	B	C	D	E	F
G-Bond	Normal	24	19	0	0	5	0	0
	Fluorosed	24	11	2	0	11	0	0
Clearfil Tri-S Bond	Normal	24	15	0	1	6	2	0
	Fluorosed	24	11	1	2	6	2	2

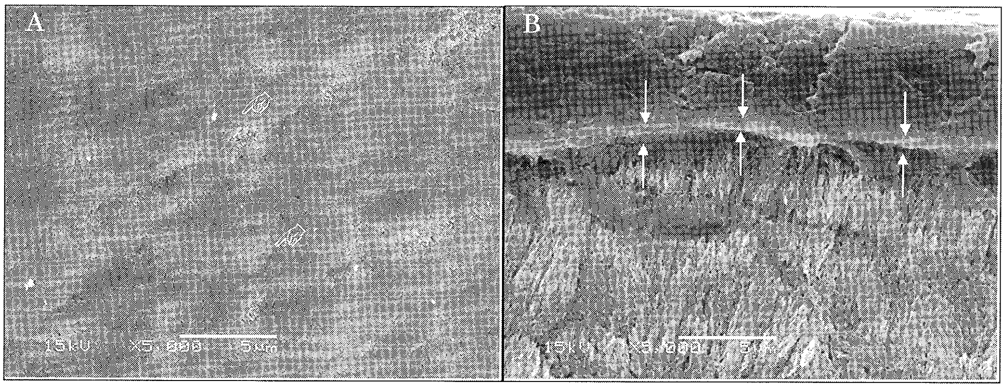

Fig. 2 SEM images of unground normal enamel conditioned with G-Bond (A). Shallow, mild coral-like etching pattern was observed in the undissolved aprismatic layer (marker). Adhesive interface of G-Bond bonded to unground fluorosed enamel (B). SEM observation showed 1 μm of resin tag-like extensions penetrating into enamel (arrows).

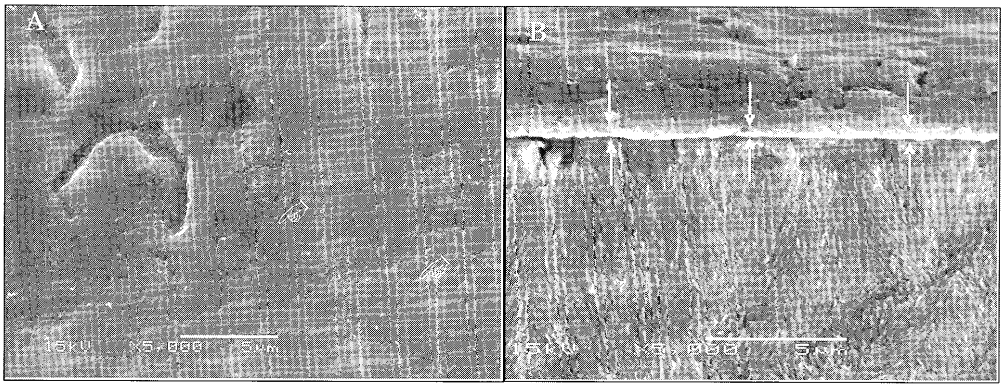

Fig. 3 SEM images of unground fluorosed enamel conditioned with Clearfil Tri-S Bond (A). Shallow, mild coral-like etching pattern was observed in the undissolved aprismatic layer (marker). Adhesive interface of Clearfil Tri-S Bond bonded to normal unground enamel (B). About 1 μm of resin tag-like extensions penetrating into enamel were observed (arrows).

pattern was observed in the undissolved aprismatic layer. SEM images of the adhesive interface between enamel and the adhesives are shown in Figs. 2B and 3B. There was no gap formation at the interfaces of both adhesives. Further, it was revealed that 1 μm of resin tag-like extensions penetrated into the enamel for both self-etching systems.

DISCUSSION

In fluorosis endemic areas in Sri Lanka, where the fluoride level in ground water exceeds 1 ppm (*versus* the recommended level of 0.6-0.8 ppm in drinking water), prevalence rate of fluorosis has been reported to range from 29 to 57%[10]. Similarly, in non-endemic areas in many parts of the world, fluorosis prevalence is on the increase. This worrisome increase in the prevalence of dental fluorosis worldwide has been attributed to high background exposure to fluoride from various sources, such as food, soft drinks, fluoride-containing dentifrices, and supplements[11,12].

For the classification of fluorosis, modified Thylstrup and Fejerskov index is very useful as it is based on clinical, fluoride-induced changes in fluorosed teeth. Besides being consistent with the histopathological changes in fluorosed enamel, the Thylstrup and Fejerskov index also boasts of high reproducibility, thus making it a very attractive evaluation method in the studies of fluorosed teeth[13]. In the present study, moderately fluorosed teeth were thus classified according to this index[9].

Moderately fluorosed teeth are characterized by a marked opacity or chalky white appearance on the affected tooth surface. Other clinical manifestations include pits of less than 2-3 mm in diameter on the smooth or occlusal surface, with attrition. In a previous study, it was found that bond strength was significantly higher in teeth for patients below 40 years old than those above 40 years old[14]. In light of this finding[14], sample teeth used in the present study were from patients of 20-40 years of age.

Methods in measuring the bond strength of adhesive systems to tooth substrates vary according to researchers[15]. Currently, many researchers prefer to use the micro-tensile bond strength method when testing bond strengths of adhesive materials to dentin[16]. However, micro-shear bond strength testing was used in the present study as enamel substrate is more brittle in nature than dentin. Further, there is an added advantage with micro-shear bond strength testing: there is no need to alter the bonding surface. With the micro-tensile method, cut slices must be obtained from specimens for bond strength evaluation. Consequently, enamel prisms may be damaged — with a possible effect on bond strength values. As for micro-shear bond testing, only a very small bonding area is needed when compared with conventional shear strength test. This should reduce the problems associated with stress distribution at bonding sites, which are inherent in conventional shear testing, and thereby give comparatively higher bond strengths[17,18].

It was reported that bonding of self-etching adhesives to ground enamel — in moderately to severely fluorosed teeth — was inferior compared to that rendered by phosphoric acid etching[19]. However, for ground enamel of mildly fluorosed teeth, self-etching adhesives might be used as alternatives to phosphoric acid conditioners[20,21]. In the present study, the all-in-one adhesive systems, G-Bond and Clearfil Tri-S Bond, were bonded to the unground enamel of both fluorosed and normal enamel. Both adhesive systems exhibited no significant differences in micro-shear bond strength between the two groups ($p>0.05$). This result thus indicated that the enamel bond strength of both adhesive systems was not affected by the mild to moderate fluorosis in unground enamel.

Presently, most commercial self-etching adhesive systems contain mildly acidic monomers, such as MDP and 4-MET, to condition and prime the underlying enamel[22]. Clearfil Tri-S Bond contained water and was an alcohol-based self-etching adhesive system. Comparably, G-Bond was an acetone-based self-etching adhesive system. As a result, resin penetration into etched enamel as indicated in the SEM micrographs of the self-etching adhesive systems was shallow. Both adhesive systems could not dissolve the outer boundaries of the individual enamel crystals on the surface. It should be mentioned that the presence of a surface aprismatic layer in unground enamel was less conducive to bonding[23,24]. The resin-enamel bond in this study was thus attributed to the underlying layer of hybrid-like enamel tissue[25,26]. Further, there were no differences between the self-etching systems in depth of penetration in both normal and fluorosed enamel.

Durable bond strength to unground enamel is of critical importance as it allows dental professionals to restore fluorosed teeth without tooth preparation. When teeth are unground, the outer hyper-mineralized, acid-resistant enamel layer may be preserved, making fluorosed teeth less vulnerable to further deterioration. Further, the predominant failure mode for both adhesive systems was adhesive failure in all groups. In other words, the enamel surface would not be damaged even if the restoration failed. This finding is important to dental clinicians as it implied that the enamel substance could be preserved when treating patients with fluorosis.

Unlike bonding to dentin, application of self-etching systems on enamel has evoked many controversial debates[27,28]. By means of micro-tensile bond strength testing, Kanemura *et al.* reported that the commercial adhesive systems evaluated produced good adhesion to ground enamel[29]. However, for bonding with intact enamel, phosphoric acid etching yielded significantly higher bond strengths than self-etching primers. This was because the etching pattern of self-etching primers was not deep enough to obtain good penetration of bonding resin when applied to intact enamel surfaces. In light of research findings like this, further studies are needed to perceive the bonding performances of contemporary all-in-one adhesive systems to fluorosed teeth as well as to compare these adhesives against phosphoric acid etching.

CONCLUSIONS

The micro-shear bond strengths of two all-in-one adhesive systems to unground fluorosed enamel were not influenced by the severity of fluorosis. Furthermore, there were no differences in enamel bonding strength between G-Bond and Clearfil Tri-S Bond.

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REFERENCES

1) Aoba T, Fejerskov O. Dental fluorosis: chemistry and biology. Crit Rev Oral Biol Med 2002; 13:155-170.
2) Fejerskov O, Richards A, DenBesten P. Fluoride in dentistry, 2nd ed, Munksgaard, Copenhagen, 1996, p.112.
3) Akpata ES. A text book of operative dentistry, Class Publishing, London, 1997, p.97.
4) Akpata ES. Occurrence and management of dental fluorosis. Int Dent J 2001; 51:325-333.
5) Tyas MJ, Anusavice KJ, Frencken JE, Mount GJ. Minimal intervention dentistry — a review. Int Dent J 2000; 50:1-12.
6) Hayakawa T, Kikutake-Sugiyama K, Fukushima T, Nemoto K. Development of self-etching primer adhesive in all-in-one bonding system. Dent Mater J 2005; 24:251-256.
7) Ikemura K, Tay FR, Hironaka T, Endo T, Pashley DH. Bonding mechanism and ultrastructural interfacial analysis of a single-step adhesive to dentin. Dent Mater 2003; 19:707-715.
8) Pashley DH, Tay FR. Aggressiveness of contemporary self-etching adhesives. Part II: Etching effects on unground enamel. Dent Mater 2001; 17:430-444.
9) Thylstrup A, Fejerskov O. Clinical appearance of dental fluorosis in permanent teeth in relation to histological changes. Community Dent Oral Epidemiol 1978; 6:315-328.
10) Ekanayake L, van der Hoek W. Prevalence and distribution of enamel defects and dental caries in a region with different concentration of fluoride in drinking water in Sri Lanka. Int Dent J 2003; 53:243-248.
11) Osuji OO, Leake JL, Chipman ML, Nikiforuk G, Locker D, Levine N. Risk factors for dental fluorosis in a fluoridated community. J Dent Res 1988; 67:1488-1492.
12) Pang DT, Phillips CL, Bawden JW. Fluoride intake from beverage consumption in a sample of North Carolina children. J Dent Res 1992; 71:1382-1388.
13) Fejerskov O, Manji F, Baelum V, Møller IJ. Dental fluorosis — a handbook for health workers, Munksgaard, Copenhagen, 1988, p.45.
14) Ateyah N, Akpata E. Factors affecting shear bond strength of composite resin to fluorosed human enamel. Oper Dent 2000; 25:216-222.
15) McDonough WG, Antonucci JM, Dunkers JP. Interfacial shear strengths of dental resin-glass fibers by the microbond test. Dent Mater 2001; 17:492-498.
16) Sudsangiam S, Van Noort R. Do dentin bond strength tests serve a useful purpose? J Adhes Dent 1999; 1:57-67.
17) Packham DE. Handbook of adhesion, Longman Group UK Ltd, London, 1992, p.35
18) McDonough WG, Antonucci JM, He J, Shimada Y, Chiang MYM, Schumacher GE, Schultheisz CR. A microshear test to measure bond strengths of dentin-polymer interfaces. Biomaterials 2002; 23:3603-3608.
19) Weerasinghe DS, Nikaido T, Wettasinghe KA, Abayakoon JB, Tagami J. Micro-shear bond strength and morphological analysis of a self-etching primer adhesive system to fluorosed enamel. J Dent 2005; 33:419-426.
20) Hara AT, Amaral CM, Pimenta LA, Sinhoreti MA. Shear bond strength of hydrophilic adhesive systems to enamel. Am J Dent 1999; 12:181-184.
21) Jayasooriya PR, Wettasinghe KA, Ogata M, Nikaido T, Tagami J. Micro-tensile bond strength of a self-etching primer bonding system to fluorosed enamel. Int Chin J Dent 2002; 2:107-115.
22) Hashimoto M, Ohno H, Yoshida E, Hori M, Sano H, Kaga M, Oguchi H. Resin-enamel bonds made with self-etching primers on ground enamel. Eur J Oral Sci 2003; 111:447-453.
23) Whittaker DK. Structural variations in the surface zone of human tooth enamel observed by scanning electron microscopy. Arch Oral Biol 1982; 27:383-392.
24) Nathanson D, Bodkin JL, Evans JR. SEM of etching patterns in surface and subsurface enamel. J Pedod 1982; 7:11-17.
25) Nakabayashi N, Nakamura M, Yasuda N. Hybrid layer as a dentin-bonding mechanism. J Esthet Dent 1991; 3:133-138.
26) Nakabayashi N, Pashley DH. Hybridization of dental hard tissue, Quintessence Publishing Co., Tokyo, 1998, pp.6-7.
27) Buonocore MG, Matsui A, Gwinnett AJ. Penetration of resin dental materials into enamel surfaces with reference to bonding. Arch Oral Biol 1968; 13:61-70.
28) Hotta K, Mogi M, Miura F, Nakabayashi N. Effect of 4-MET on bond strength and penetration of monomers into enamel. Dent Mater 1992; 8:173-175.
29) Kanemura N, Sano H, Tagami J. Tensile bond strength to and SEM evaluation of ground and intact enamel surfaces. J Dent 1999; 27:523-530.