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An Evaluation of Degradation in Dental Adhesive Resin Using Quantitative Light-induced Fluorescence

조선대학교 대학원

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이 논문을 치의학 석사학위신청 논문으로 제출함

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QLF를 이용한 접착레진 분해 평가

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1982년 상아질-레진 접착이 처음 이루어진 이래로, 많은 접착 레진이 접착강도를 증진하기 위해 개발되어 왔다. 물흡수, 용해도는 접착레진의 기계적 안정성에 영향을 미치고 상아질-레진접착의 급격하고 파괴적인 붕괴를 일으키기 쉽게 한다. 그래서 많은 방법들이 붕괴를 평가하기 위해 사용되었으나 이들 방법들은 사용하기 쉽지 않고 시간이 오래 걸리는 단점이 존재하였다. 이 논문의 목적은 치과용 레진 접착제의 붕괴를 평가하는데 있어 QLF를 사용하는 새로운 방법을 제시하기 위함이다. 가설은 '세 접착제간의 분해에 있어 유의적인 차이가 없을 것이다' 이다.

세종류의 접착 레진이 사용되었다; G-bond(GB), single bond(SB), D resin(DR). porphyrin I과 섞은 후 레진 디스크를 주형(5mmX0.8mm)에 넣어 제작하였다. QLF분석 후 열순환(5000cycle, 5℃ to 55℃)을 시행하였다. 열순환 후 QLF로 다시 분석을 시행하였다.

Simple Plague Score™ 에서 열순환 전후에 모든 그룹에서 유의적인 차이가 존재하였다. 열순환전에 GB가 가장 높은 값을 나타내었고 DR, SB와 유의적인 차이를 보였다. GB는 열순환후에 가장 낮은 값을 나타내었고 SB와 유의적인 차이가 존재하였다.

포피린 값은 SB의 Area △R30을 제외하고는 열순환 전후에 유의적인 감소를 보였다. GB가 모든 영역에서 많은 양의 감소를보였는데, 포피린이 거의 없어졌다. 전반적으로, DR이 가장 낮은 포피린 값 감소를 보였다. Area △R30에서는 DR이 SB보다 더 감소하였으나 SB는 DR보다 Area △R70과 Area △R120에서 더 많

은 감소를 보였다. 따라서, SB가 더 많은 양의 포피린 감소를 보였다.

이 실험의 결과에 따르면 포피린 값은 모든 그룹에서 유의하게 감소하였고 가장 높은 포피린 값 감소를 보이는 접착레진은 GB이고 가장 낮은 접착레진은 DR이었다. 따라서, 처음 가설은 기각되었다. 실험의 한계에도 불구하고, QLF 방법은 접착레진의 붕괴를 평가하는 새로운 방법으로서 사용되어질 수 있을 것이다. 더불어, 새로운 방법은 상아질-레진 계면붕괴와 연관된 기전에 대한 더 많은 정보를 제공해 줄 것이다.

Key word: Degradation, QLF-D, Porphyrin, Adhesive resin

I. Introduction

In 1982, effectiveness of 4-methacryloxyethyl trimellitate anhydride (4-META) on the adhesion of an acrylic rod with etched dentine and enamel was first studied by Nakabayashi et al._ENREF_1. This interlock was usually refered to as 'hybridization', or the formation of a 'hybrid layer' [1]. Since resin adhesion to dentin was first introduced in 1982, newly developed adhesive resins have been attempted to improve the bond strength.

The structure of resin-dentin bond depends on the type of adhesive, the various degradation phases of bond may occur after long usage within resin to dentin joint[2].

Water sorption and solubility affect the mechanical stability of composite resin and favor the rapid and catastrophic degradation of resin-dentin bonds. Therefore, a lot of methods were used for evaluating degradation of resin-dentin bonds. Use of Hoy's solubility parameters was typical method[3, 4]_ENREF_3_ENREF_3 But such methods were not easy to use and needed lots of time.

Quantitative Light-induced Fluorescence (QLF) device provided clinical tool for the quantification of mineral loss from enamel in laboratory and clinical situations for detecting early dental caries[5]. And when illuminated with blue light (405 nm), mature plaque produces red autofluorescence[6, 7]. Such red autofluorescence has been observed by porphyrin. Therefore QLF device could not only detect porphyrin but also quantizie porphyrin value.

Porphyrins were a group of organic compounds, many naturally occurring. One of the best-known porphyrins is heme, the pigment in red blood cells; heme is a cofactor of the protein hemoglobin[8]. As a photosensitizer, porphyrins were used for localization and photodynamic therapy of neoplastic

disease[9]. But porphyrin was not yet used for evaluation of restoration durability and degradation.

The principle aim of this study was to suggest new method for evaluation of the degradation in commercial dental adhesive resins using porphyrin and QLF. The null hypothesis was that the three adhesive resin would not differ in their porphyrin value before and after thermocycling

II. Materials and Methods

1. Specimen preparation

Three adhesives were selected: D/E resin (DR), Single bond (SB), G-bond (GB). D/E resin contained only bonding agent was 3 step total etching system. 2 step total etching system was Single bond included primer and bond. G-bond was 1 step self etching system. Eching, primer, bonding agent were joined together in 1 bottle. Their compositions and respective menufacturers were shown in Table 1.

Porphyrin I (Sigma, St.Louis, MO, USA) was mixed with adhesives. GB and SB were vibrated with 1 mg of porphyrin I. DR was also vibrated with porphyrin I 0.5 mg.

Twenty resin disks (n=20) of each material were produced in a mould(5 mm diameter, 0.8 mm thick). The liquid adhesive was directly dispensed to completely fill the mould. The surface of the solvated, GB and SB, was gently blown with an oil/water-free compressed air for 90 s to facilitate solvent evaporation. All visible air bubbles trapped in the adhesives were carefully removed prior to photo-activation. A glass cover slip was placed on top of the adhesives to exclude atmospheric oxygen and to displace excess solution. Photo-activation was immediately performed using a Spectrum 800 (Dentsply Caulk, Milford, DE, USA) at delivered 400 mW/cm^2 for 40 s. After removing the specimen from the mould, photoactivation was repeated on its opposite surface for another 40 s.

Control resin disk (n=20) of each non-mixing material were fabricated

Table 1. Composition of the adhesive systems used in the study

Adhesive and manufacturer	Components
1. D/E resin (DR); BISCO, Schaumburg, IL, USA	Bis-GMA, HEMA, Urathane dimethacrylates
2. Adper Single Bond (SB); 3M/ESPE, St. Paul, MN, USA	Bis-GMA, HEMA, dimethacrylates, polyalkenoic acid copolymer, initiators, water and ethanol
3. G-Bond (GB); GC, Tokyo, JAPAN	4-MET, phosphoric ester-monomer, UDMA, TEGDMA, acetone, water, stabilizer, silica filler, water, photoinitiator

Abbreviations: 4-MET: 4-methacryloxyethyltrimellitate; UDMA: urethane dimethacrylate; TEGDMA: triethyleneglycol dimethacrylate; bis-GMA: bisphenol A diglycidyl ether dimethacrylate; HEMA: 2-hyroxyethyl methacrylate. Basic composition based on manufacturers' technical profiles

with same method.

2. QLF analysis and Thermocycling

Used QLF device was QLF-D (QLF-D biluminator™, Inspektor Research systems BV, Amsterdam, Netherlands). Fluorscence image if all specimens were captured with a 'live view' -enabled digital full-sensor SLR camera (model 550D, Canon, Tokyo, Japan) at the following setting: shutter speed of 1/45 s, aperture value of 3.2, and ISO speed of 1600. Proprietary software (C3 v1.18, Inspektor Research Systems BV) was used to capture and store all digital images on a PC automatically. All fluorscence images were analysed using a computer program (QA2 v1.18, Inspektor Research Systems BV) by measuring plague patch. Measurement height was 15 cm. all analyses were performed by a single trained examiner.

Both porphyrin maturity and porphyrin quantity were calculated. A region of interest was defined by manually outlining the surface using an tnterface within the capture software. Sinse the green fluorescence of teeth is of relatively uniform color, the relatively plague-free baseline images were used to generate a function f describing this color, which expressed the value of the red channel R as a quadractic function of the value of the green channel G. Red light passed throuth the disclosed plague with very little absorption, so applying this function to the measured values of the green channel resulted in a good approximation of the red channel on clean areas of the tooth, and an underestimate elsewhere. Pixels are classified as either plague or clean by applying a threshold T, such that any pixel where $R \div f(G) - 1 \geq T$ is treated as plague. The final plague percentage coverage is the percentage of pixels whithin the tooth surface classified as plague. Different thresholds were applied on Area △R30, Area △R70, Area △R120. The increased number had high threshold. Thereby, porphyrin value were concentrated with intensity of

red fluorescence increased. Simple Plague score (SPS^{TM}) was automatically calculated from fluorescence image. A 6-point (0-5) scoring system was defined as follows: a value from 0 (no mature plague) to 5 (high amount of mature plague).

Thermocycling was performed for 5000 cycles in deionized water from 5 to 55℃ with a dwelling time of 30 s in each bath and a transfer time of 3 s.

After thermocycling, each specimen were assayed by QLF-D in the same way.

3. Statistical analysis

The mean of plaque index were calculated for each group, and all data were analyzed by one-way ANOVA. Post hoc multiple comparisons were performed using Tukey' s test. Before and after thermocycling specimen differences were analyzed by paired t-test. All statistical procedures were performed using the SPSS 12.0 for windows (IBM Corp., Armonk, NY, USA) and the level of significance was set at at p=0.05.

III. Results

The QLF-D results are presented at the plaque patch of the dental adhesives in Table 1, 2, 3 and Figure 1.

Table 1 showed the Simple Plague Score^{TM} before and after thermocycling. There is significant difference between before and after thermocyling in all groups. Before thermocycling, GB resin disk was most highest score and had significant difference with DR and SB. GB was most lowest score and had significant difference with SB, after thermocycling.

The porphyrin value was significantly decreased before and after thermocycling except SB in Area △R30 (Figure 1). GB resin disks were

extremly decreased porphyrin value in all area. Porphyrin almost disappeared. Overrall, the DR resin disk showed the lowest porphyrin value loss. In Area △R30, DR resin disk were more decreased than SB. But SB was more decreased than DR in Area △R70 and Area △R120. Therefore, SB showed more amount of porphyrin loss.

Table 2. Mean of Simple Plague Score™ before and after thermocycling

Mean ± S.D	D/E Resin (DR)	Single Bond (SB)	G Bond (GB)
Before SPS™	4.2±0.95 [a]	4.0±0 [b]	5.0±0.22 [a,b]
After SPS™	1.5±1.43	1.9±1.62 [c]	0.7±0.86 [c]

S.D = standard deviation. All groups were significantly different before and after SPS™ (Paired t-test, $p<0.05$).

Mean values with the same superscript letters are significantly difference (One-way ANOVA and Tukey's test, $p<0.05$).

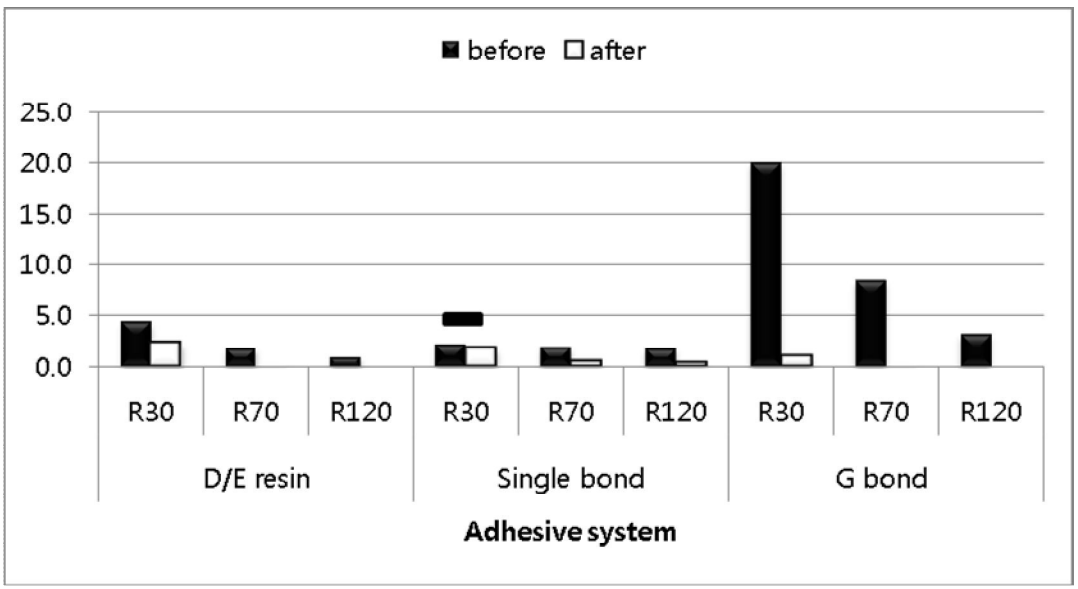


Figure 1 Graph presenting the changes in value of plague patch before and after thermocycling by 3 adhesive systems. Bar connected with a horizontal line are not statistical significantly different(Paired t-test, $p>0.05$).

Before thermocycling, GB porphyrin value was shown high percentage of all area. Especially Area △R30 level was most highest. Significant different was existed between GB and DR, GB and SB in Area △R30 and Area △R70 (Table 3).

After thermocycling, significant different was existed between SB and DR, SB and GB in Area △R30 and Area △R70 (Table 4). In area △R30, DR porphyin value was larger than others but SB porphyrin value was higher in Area △R70 and Area △R120. SB showed the highest porphyrin value and GB showed the lowest porphyrin value in all area.

Examples of the fluorescence and normal image of specimen that formed before thermocycling were given in Figure 2. Left image was photogragh image and right image was fluorescence image. All specimen had red autofluoresence. GB resin disk showed more red than other resin disk.

Figure 3 showed the resin disk fluorescence image after analysis. Analysis images showed the values of SPS^{TM},△R30, △R70, △R120. And right image showed porphyrin pixels.

After thermocyling, examples of the fluorescence and photograph image of specimen were given in Figure 4. Overall, red autofluorosecnce was decreased after thermocycling. Especially, GB resin disk was showed least red autoflurorescence.

Figure 5 also presented the resin disk ananlysis image after thermocycling.

Table 3. Mean of plague patch before thermocycling (unit: %)

Mean ± S.D	D/E Resin (DR)	Single Bond (SB)	G Bond (GB)
△R30	4.3±2.09[a]	2.2±0.1[b]	20±11.75[a,b]
△R70	1.8±1.46[c]	1.9±0.07[d]	8.5±8.84[c,d]
△R120	0.9±0.98	1.8±0.06	3.2±5.15

S.D = standard deviation.

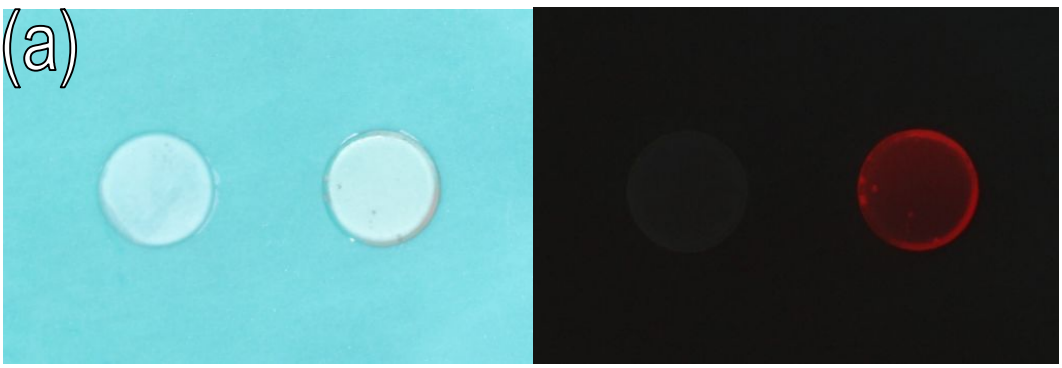
Mean values with the same superscript letters are significantly difference (One-way ANOVA and Tukey's test, $p<0.05$).

Table 4. Mean of plague patch after thermocycling (unit: %)

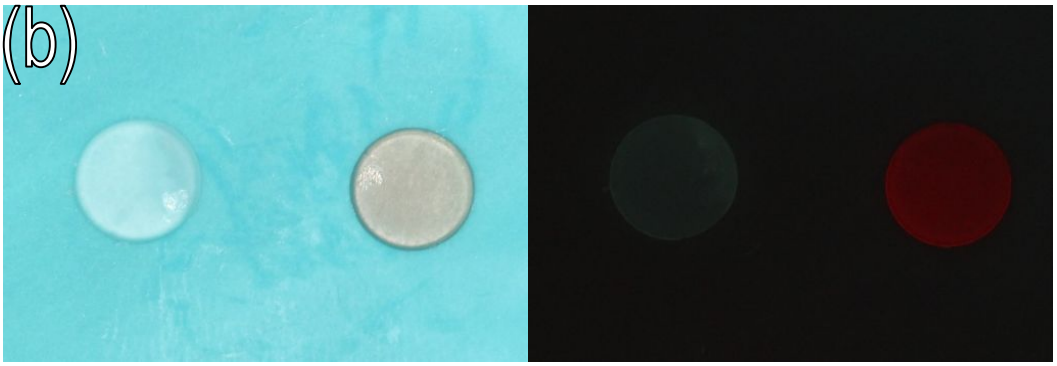
Mean ± S.D	D/E Resin (DR)	Single Bond (SB)	G Bond (GB)
△R30	2.23±2.10	1.78±1.36	1.06±1.6
△R70	0.05±0.12[a]	0.51±0.80[a,b]	0.05±0.22[b]
△R120	0±0[c]	0.40±0.71[c,d]	0±0[d]

S.D = standard deviation.

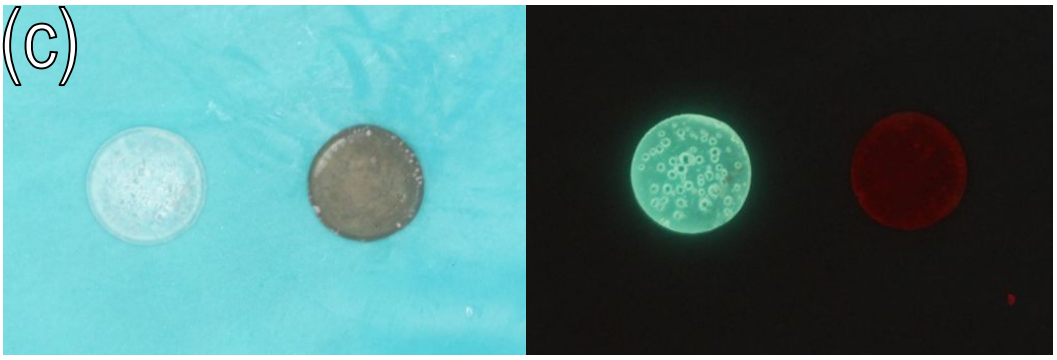
Mean values with the same superscript letters are significantly difference (One-way ANOVA and Tukey's test, $p<0.05$)



Photograph image Fluorescence image

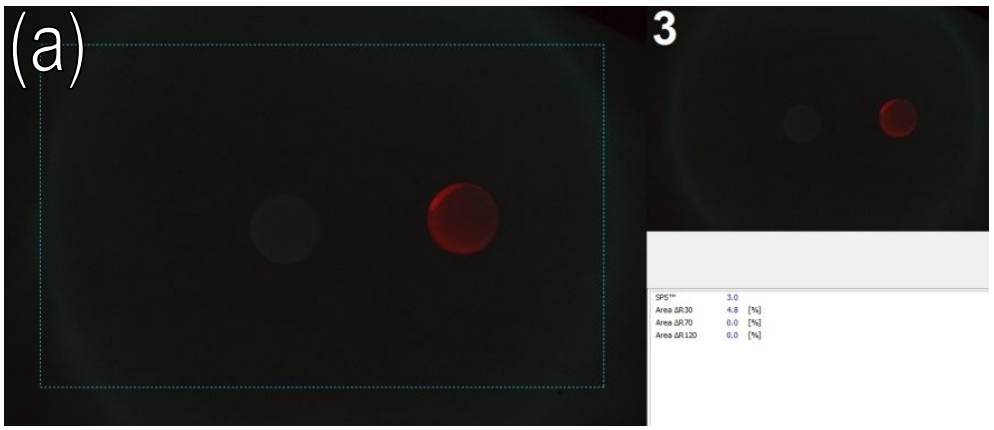


Photograph image Fluorescence image



Photograph image Fluorescence image

Figure 2 QLF-D fluorescence image and photograph image before thermocycling (X8). (a) D/E resin, (b) Single bond, (c) G bond. In photograph image, GB resin disk showed more red than other resin disk. All resin disk showed red autofluorescence.



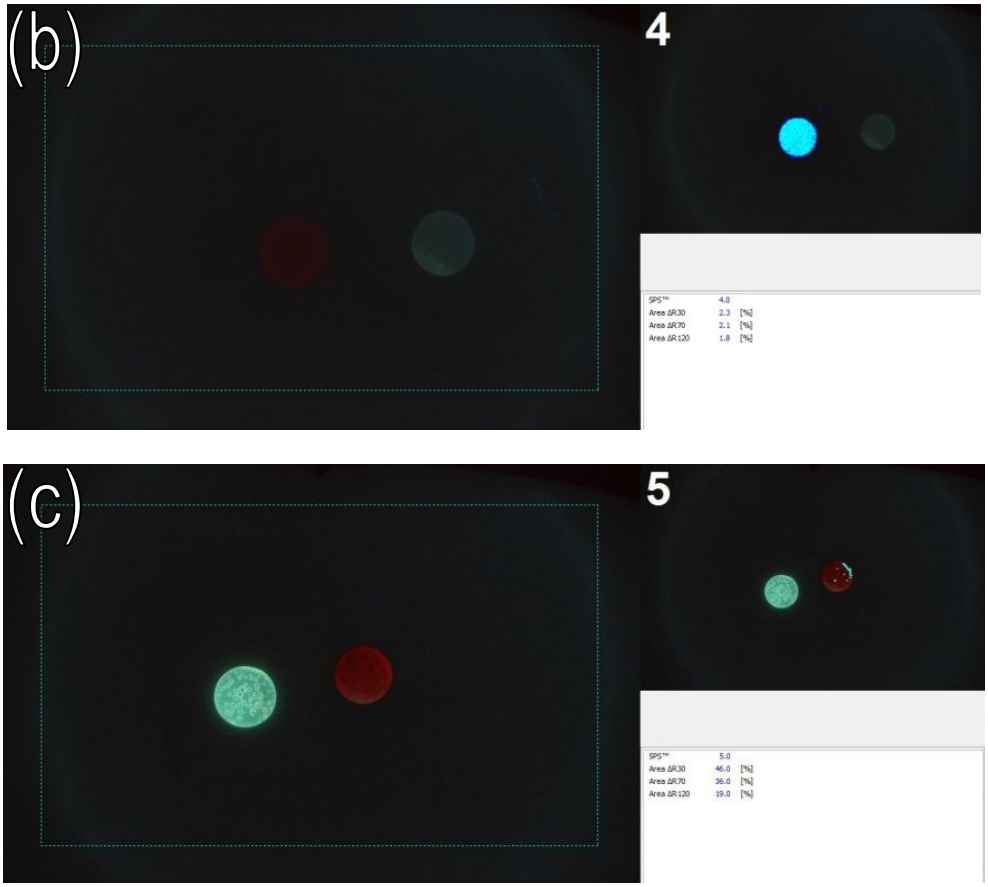
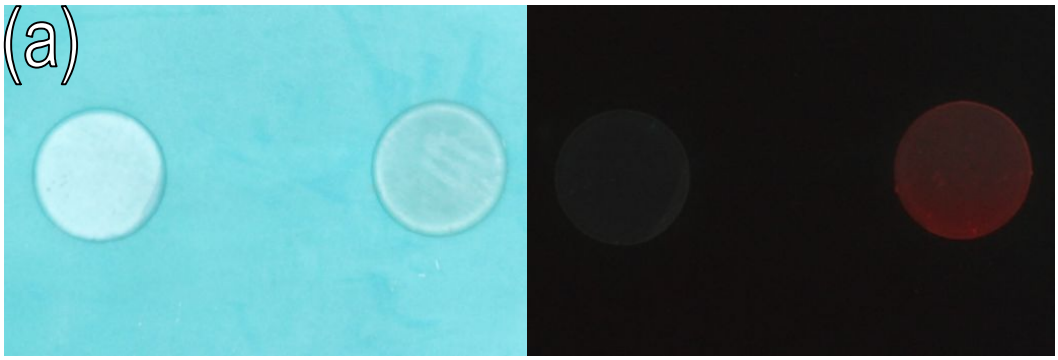
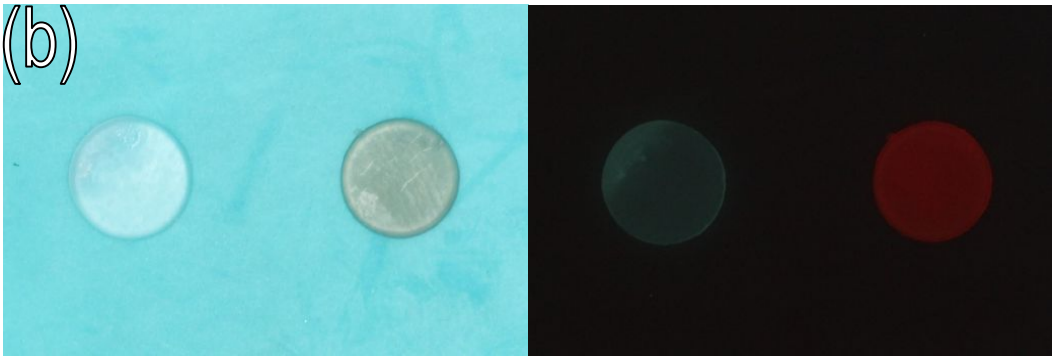


Figure 3 QA2 analysis image before thermocycling.

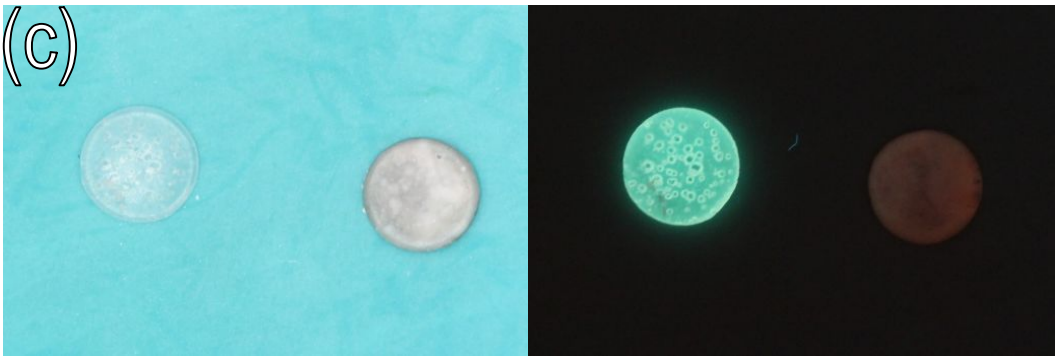
(a) D/E resin, (b) Single bond, (c) G bond. Images showed the values of SPS™, △R30, △R70, △R120.



Photograph image Fluorescence image

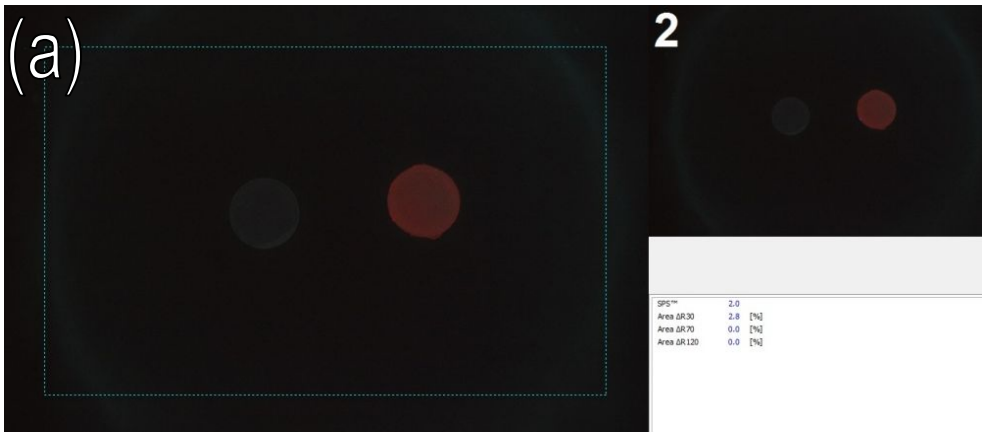


Photograph image Fluorescence image



Photograph image Fluorescence image

Figure 4 QLF-D fluorescence image and photograph image after thermocycling (X8). (a) D/E resin, (b) Single bond, (c) G bond. Overall, red autofluorosecnce was decreased after thermocycling. Especially, GB resin disk was showed least red autoflurorescence.



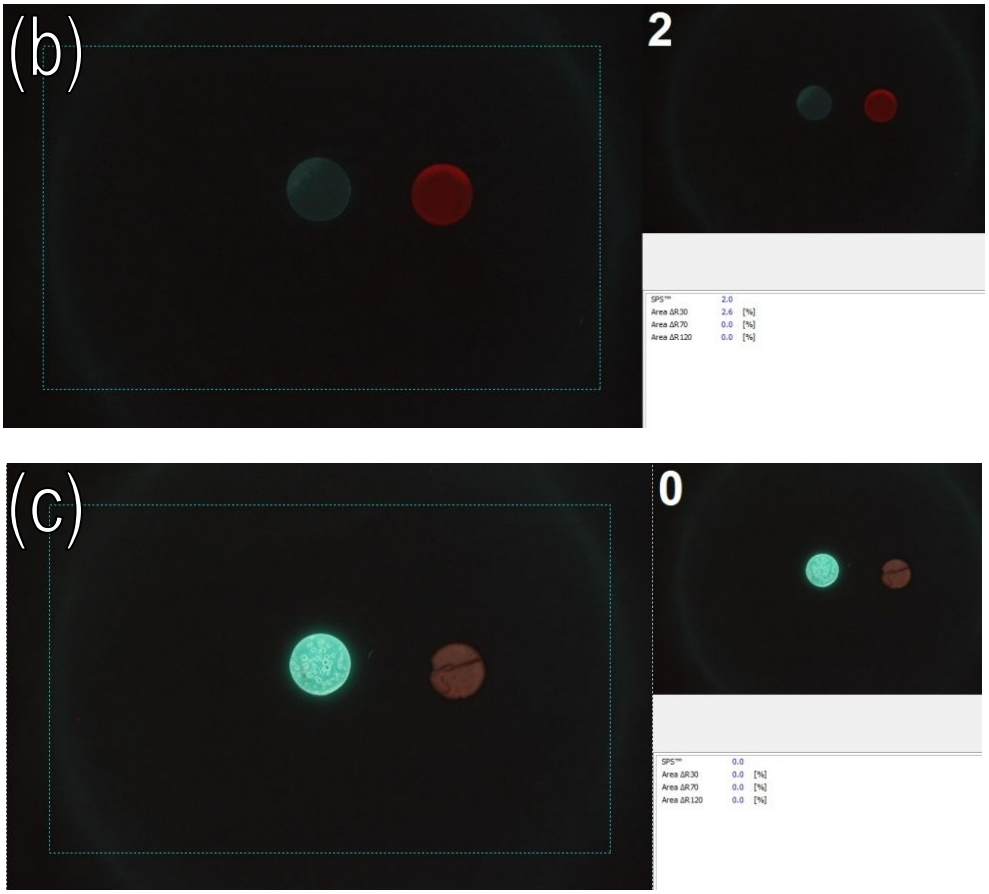


Figure 5 QA2 analysis image after thermocycling.

(a) D/E resin, (b) Single bond, (c) G bond. Image showed the value of SPS™. △R30, △R70, △R120. In particular, GB resin disk showed no score.

IV. Discussion

The durability of bonds between dentin and dental adhesive was clinical important factor for the longevity of restorations. If this resin/dentin bond interface degradation occurred, it could weaken tooth adhesion[10]. Therefore, there had been many attempts to simulate bond degradation *in vitro*[11]. A lot of results demonstrated that critical factor affecting durability *in vivo* and *in*

vitro was hydrolysis of resin/dentin interface such as collagen hydrolysis, resin elusion by hydrolysis, or the increasing amount of nanoleakage after aging[2]. Currently, the most validated method for the assessment of this degradation process *in vitro* is the storage of micro-specimens in water[12]. Previous water storage study measured their solubility and interface bond strength[13, 14]. However, there is no evidence of hydrolytic degradation in adhesive resin itself. Our study tried to evaluate the actual degradation of adhesive resin. Another attempt given in this study was to suggest more easy and valid evaluating new method.

The results of the present study indicate that the significant differences between three adhesive resins existed, after thermocycling (Figure 2). GB which is relatively highly hydrophilicity had a significantly higher porphyrin value loss than the other groups. And DR which is relatively hydrophobic had a least porphyrin value loss. Its different water solubility might contribute to the water hydrolysis after thermocycling. And that result interpreted that water hydrolysis of adhesive resin cause porphyrin value loss, so that adhesive resin was degradated. Thus, by using the data obtained with the three adhesive resin, it is suggested that the more hydrophilic resin was shown less degradation. Previous studies also demonstrated that the most hydrophilic resin showed the highest water sorption, solubility and water diffusion coefficient and generally, the extent and rate of water sorption increased with the hydrophilicity of the resin blends[13].

Containing high percentage of water caused high water sorption and solubility and highly porphyrin value. Another study also demonstrated that the one-step self-etching adhesive and self-etching primer/adhesive mixtures presented the highest water sorption and solubility values[15]. Before Thermocycling, the porphyrin value was varied according to three adhesive resin, and that result shown in Table1. The lowest porphyrin value was

appeared in DR and highest one was GB. Particulary, Area △R30 in GB was the most hishest porphyrin value compared with DR and SB. This result might be due to GB is the One-step self etching adhesive. GB contain triethylene-glycol dimethacrylate (TEGDMA) to produce a tough, highly crow linked polymer network and 15-20% water in order to ionize acidic monomers such as 4-met, phosphoric ester-monomer. The different in porphyrin value of three adhesives may be due to the differences in their composition and the degree of polymerization. SB and DR contain Bis-GMA resin as cross-linkers. Bis-GMA includes both hydrophilic and hydrophobic components. The core diphenylisopropane presents significant hydrophobicity on Bis-GMA, while the two hydroxyl groups are the major source of hydrophilicity and they can bind via hydrogen bonding so leading to absorbed water[16]. Because of poor water solubility of Bis-GMA, manufacturures usually add acetone or HEMA to facilitate Bis-GMA solubility. HEMA-based primer allow to prevent collagen collapse and rewet dry dentin[17]. HEMA is very hydrophilic and is helful for porphyrin value. Also, Even in relatively low concentrations (5% or 15%), the addition of ethanol into the experimental methacrylate-based dental adhesives tested, increased the ability of these materials to absorb and transport water[18].

Several studies had shown that water absorption is depentdent on not only the presence of residual solvent but also the degree of hydrophilicity of the materials[14-16]. When concentration of hydrophilic comonomers was high, the colligative properties of the entire mixture was changed and lowering the vapor pressure of volatile components, such as non-polymerizable solvents (i.e, acetone, ethanol, water)[17]. Even in relatively low concentrations (5% or 15%), the addition of ethanol into the experimental methacrylate-based dental adhesives tested, increased the ability of these materials to absorb and transport water[18].

The QLF-D Biluminator™ was new type of Quantitative Light-induced Fluorescence (QLF) device. It has been developed to facilitate the detection and quantification of dental plaque by representing endogenous porphyrins produced by oral bacteria species as red autofluorescence[19-21]. Specific oral bacteria could synthesize high concentrations of endogenous metal-free fluorescent porphyrin and this made the auto fluorescence phenomenon[6]. The QLF-D Biluminator™ is an upgraded version of QLF devices that examines plaque more clearly as red fluorescence by strengthening these principles, making quantification of the plaque possible[22]. This new device uses a narrow-band blue light source (centred at 405nm) obtained by modifying the filter set (D007; Inspecker Research Systems BV, Amsterdam, The Netherlands) and consists of a Biluminator™ mounted on a high-specification digital single-lens reflex (SLR) camera fitted with a 60-mm macro lens, which is equipped with an illumination tube with white and blue light-emmiting diodes positioned in a ring around the lens opening[22] (Figure 6). This device can produce high-quality photographs without any requirement for ambient light, visualize plague more clearly, and detect subtle changes in plague at a high resolution[21]. These characteristics may make the new device useful in the laboratory for the analysis of plaque[21].

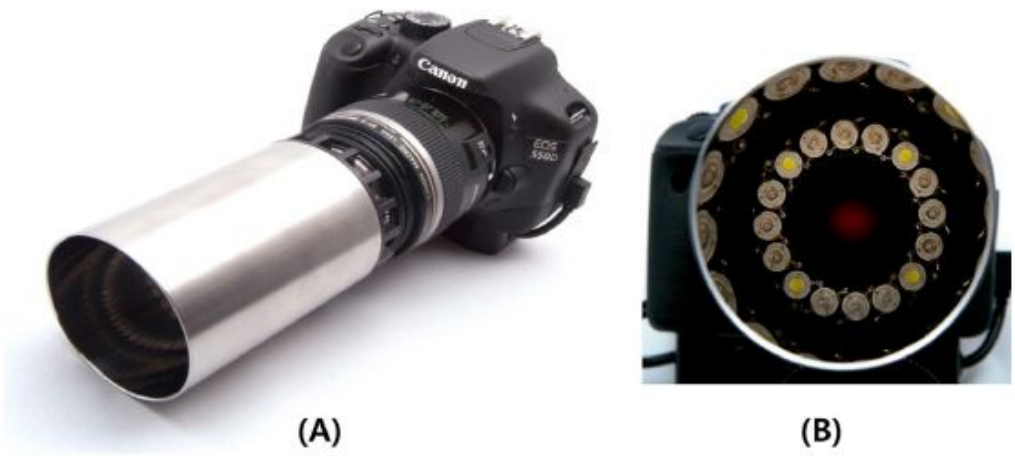


Figure 6 The QLF-D BiluminatorTM used in this study (image courtesy of Inspektor Research Systems). This device (A) is based on a full-sensor SLR camera (Canon 550D) equipped with an illumination tube with white and blue light-emitting diodes positioned in a ring around the lens opening (B).

Nevertheless, QLF-D analysis only possible on the surface of the porphyrin such as plaue. When dental caries was existed dentin under enamel, QLF blue light could make red autofluorescence. Therefore, we could recognize the dental caries. But, porphyrin value could not analyzed because porphyrin was not exist on the surface. This is critical limitation of QLF-D method.

Although the thickness of the disc may not reflect clinically same, the dimensions were selected to provide uniform homogenous samples to allow easy manipulation and avoid the risk of fracture during experiment. However, these thicker resin disk which is especially SB and GB might prevent complete solvent removal. Therefore, Lots of residual solvent in adhesive resin during polymerization would enhance water sorption though clinically solvent evaporated more effectively cause of thin film layer.

Most in vitro durability studies mimic one of the *in vivo* degradation factors

involved, to disclose its effect on the general degradation process, in contrast to the clinical situation, where all these factors are operational simultaneously[12]. Also, our results might be exaggerated, because resin disk were directly exposed to hydrothermal state. Another problem was that thermocycling process might not reflect degradation within the mouth. Therefore, this limitation should be considered.

V. Conclusion

Based on the results from this *in vitro* study, porphyrin value was significantly decreased in all groups. And the highest porphyrin value loss adhesive resin was GB, the lowest adhesive resin was DR. Therefore, the null hypothesis was rejected.

Within limits of this study, QLF method may be used to evaluate the degradation of adhesive resin as a new method. Along with, this method provide more information about the fundamental mechanisms involved in resin-dentin interface degradation.

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