Bisphenol A release and surface characteristics of pit and fissure sealants according to different pH conditions

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Directed by Professor Kwang-Mahn Kim

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I realized how to rely on God, perseverance, and humility in this process. I will repay and share the grace received from many people in the future. I dedicate this thesis to God and you.

Eun-deok Jo

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Abstract

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(Directed by Professor Kwang-Mahn Kim, D.D.S., Ph.D.)

Changes in pH in the oral cavity can cause changes in the chemical decomposition and surface properties of the treated resin-based pit and fissure sealants (sealant). Therefore, the purpose of this study is to evaluate the release of bisphenol A (BPA) from sealants and the changes in sealant surface properties (roughness, gloss, and color) in 3 conditions of pH over time.

Pit and fissure sealants used in this study were randomly selected from a resin-based light-cured type used commercially in clinical practice. The specimen for the BPA detection test was applied with 6 sealants 5 mg each on a glass plate (10 × 10 mm) and photopolymerized. On the other hand, a disk-shaped specimen (10 mm in diameter and 1 mm in thickness) was prepared with 4 sealants for surface characteristic tests. The specimens were immersed for 10 min, 1 h, and 24 h in solutions of pH 3.0, 6.5, and 10.0 at 37 °C. BPA release was measured using a gas chromatography-mass

spectrometer (GC-MS; Agilent Technologies 7820A GC and 5977E MSD system, Palo Alto, CA, USA). The average center line roughness (Ra) of the sealant's surface was measured with a non-contact 3D optical surface measuring machine (GT-X3, Bruker, Billerica, MA, Germany), the surface gloss was measured with a gloss meter (Novo-Curve, Rhopoint ™, East Sussex, UK), and the surface color was measured L^* a^* b^* with a spectrophotometer (CM3500d, Minolta Co., Osaka, Japan). Statistical analysis was performed by two-way ANOVA and repeated measures ANOVA to verify the interaction between pH and time on BPA release and surface characteristics. In addition, one-way ANOVA was performed to confirm the difference according to pH and time.

The BPA detection concentration in the pH 3.0 group was higher at all points than pH 6.5 and pH 10.0 ($p<0.05$) and gradually increased over time ($p<0.05$). The surface roughness of the pH 3.0 group was higher than pH 6.5 and pH 10.0 and increased over time ($p<0.05$). The surface roughness of the pH 3.0 group was higher than pH 6.5 and pH 10.0 and increased over time ($p<0.05$). On the other hand, there was no significant difference between pH groups in all surface colors. The L^* value decreased after 24 hours ($p<0.05$), the a^* value increased over time in all pH groups ($p<0.05$), and there was no significant difference in b^* values in all groups ($p>0.05$).

As a result, it was confirmed that the low pH condition was a factor negatively affecting BPA release, surface roughness, and surface gloss. Therefore, it is considered that frequent exposure to low pH due to intake of various beverages and foods after sealant treatment may negatively affect the chemical stability and surface stability of sealant in the oral cavity.

Keywords: pit and fissure sealant, pH, bisphenol A, surface roughness, surface gloss, surface color

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Ⅰ. INTRODUCTION

1.1. Pit and fissure sealant

Pit and fissure sealant (sealant) is a material used to close the pit and fissure of teeth and was introduced in the 1960s to prevent dental caries. These sealants are one of the most widely used dental materials in children because they can prevent the growth of bacteria that promote erosion in the pit and fissure of teeth (Ferracane, 1995). Sealants have been shown to effectively prevent and inhibit the progression of tooth decay in children and adolescents. In a study on the caries prevention effect of sealant, the caries reduction range was found to be 87% at 12 months old and 60% at 48-54 months old (Ahovuo-Saloranta et

al., 2008). In addition, according to the results of a follow-up study after 9 years, it was reported that the tooth surface with sealant had 27% erosion. In contrast, the tooth surface without the sealant had 77% erosion (Bravo et al., 2005). Therefore, the number of children getting sealant treatment in conjunction with the government oral prevention program increases (Dye et al., 2007).

The resin-based restorative material used in the sealant is composed of an organic matter as a monomer and a filler as an inorganic component. Organic matters such as bisphenol-A-glycidyl methacrylate (bis-GMA), urethane dimethacrylate, and trimethylene glycol dimethacrylate (TEGDMA) allow the components to be mixed and form a complicated structure after polymerization to have high mechanical properties (Chen and Suh, 2014). In particular, a Bis-GMA resin-based sealant using light polymerization is mainly used (Veiga et al., 2014). However, it is difficult to polymerize the light-curable sealant completely. There is a report that unreacted organic components may be eluted, and free organic components may have harmful effects on the human body (Hamid and Hume, 1997, Geurtsen, 1999)

1.2. Bisphenol A

Bisphenol A (BPA) has been present in many plastic polymers since the 1960s. Today, BPA is used to manufacture various products utilizing polycarbonate plastics and epoxy resins used in food packaging, toys, automobiles, detergents, and dental resin materials (Olea et al., 1996). In addition, BPA is incorporated into the food chain by leaching from polycarbonate bottles and food containers, including canned foods (Groff, 2010). However, recent studies have suggested exposure to BPA from dental materials or non-food sources (Lofroth et al., 2019).

In dentistry, monomers with BPA core are commonly used in resin-based materials such as root canal sealers, adhesives, composites, and sealants (Fleisch et al., 2010). 'Figure 1' shows the chemical structures of BPA, polycarbonate, and the BPA-derivatives commonly used in dental materials, such as BisGMA, BisEMA, and BisDMA (Chen and Suh, 2014). Although dental materials typically do not contain pure BPA, this compound can be the impurity of the manufacturing process or a by-product of the degradation of bisphenol A-glycidyl methacrylate (bis-GMA) or other components such as Ethoxylated bisphenol A dimethacrylate (BisEMA), bis-dimethylaminopropyl (BisDMA) (Olea et al., 1996, Schafer, 1999).

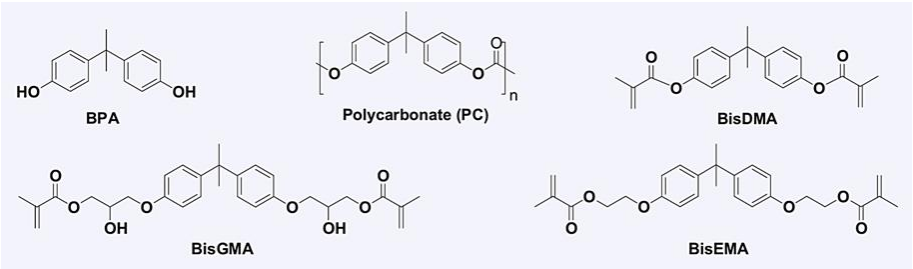

Figure 1. Chemical structures of BPA, polycarbonate, and the BPA-derivatives commonly used in dental materials, such as BisGMA, BisEMA, and BisDMA (Chen and Suh, 2014).

1.2.1. Harmful effects of BPA on the human body

BPA was already recognized as an Endocrine Disrupting Chemicals (EDC) that mimics estrogen and alters hormone function in the 1930s (Vogel, 2009). Since the 1990s, similar effects of BPA on female hormones have been reported (Maffini et al., 2006; Moriyama et al., 2002; Nagel et al., 1997)

The harmful effects of BPA include: 1) central nervous system development, differentiation, and function, 2) reproductive function (e.g., follicular development), 3) thyroid hormone function, 4) prostate and mammary gland morphology, 5) disruption of fetal oocyte meiosis 6) Immune system dysfunction, etc. (Chapin et al., 2008; Nah et al., 2011). A study has shown that early exposure to BPA accelerated the onset of puberty but reduced reproductive parameters in female rats (Nah et al., 2011). Additionally, when biologically active BPA was administered orally, a 10 times higher BPA concentration was detected in neonatal rats than adults (Taylor et al., 2008). Therefore, BPA may be harmful to children (Hunt et al., 2009). In particular, in the case of infants and young children, the sensitivity to BPA exposure is very high, so the use of products for infants and young children was completely prohibited (Braun et al., 2011).

1.2.2. Existence of BPA in sealants

BPA is present in resin-based dental sealants and composite materials used for preventive and restorative teeth. BPA is formed by the decomposition of Bis-GMA or Bis-EMA and is released in various concentrations depending on chemical or mechanical processes occurring in the oral cavity (Lopes-Rocha et al., 2021). Mass spectrometry confirmed the existence of BPA, bisphenol-A diglycidyl methacrylate (bis-GMA), bisphenol-A diglycidyl ether (BADGE), and bisphenol-A dimethacrylate (bis-DMA) in the composite material and sealant. Furthermore, after hydrolysis in alkaline (pH=13) and acidic (pH=1) by heating the composite material and sealant (100°C for 30 minutes), BPA levels increased 3-12 times compared to before hydrolysis (Olea et al., 1996). Also, previous studies have demonstrated the detection of BPA in human saliva, urine, and blood after applying the sealant (Lofroth et al., 2019). However, most previous studies on BPA leakage from sealants were *in vivo* studies. These measured the amount present in human saliva, urine, or blood (Olea et al., 1996, Arenholt-Bindslev et al., 1999; Fung et al., 2000; Sasaki et al., 2005; Joskow et al., 2006; Zimmerman-Downs et al., 2010; Han et al., 2012; Deanne Shuman, 2010). On the other hand, few previous *in vitro* studies related to BPA leakage from sealants. For example, Pulgar et al. (2000) reported BPA released from bis-GMA-based 7 composite resins and 1 sealant. And Gruninger et al. (2015) claimed that BPA was detected from 24 bis-GMA-based sealants.

Factors that can trigger BPA release from the oral environment are bacteria and salivary enzymes, and temperature changes have been reported to cause mechanical erosion and degradation (EFSA Panel on Food Contact Materials and Aids, 2015; Kamrin, 2004).

1.3. pH change in the oral cavity by beverages

The clinical performance of bis-GMA-based resin restorative materials is greatly affected by changes in the oral environment (Prakki et al., 2005). Drinking various beverages causes changes in the pH of the saliva in the mouth (Pachori et al., 2018). Recently, the consumption of carbonated drinks, fruit juices, and sports drinks by children and adolescents has increased due to improved living standards and the spread of eating out culture (Nielsen and Popkin, 2004, Storey, 2006 #17). People between 15 and 30 were more likely to drink fruit juice and soft drinks than other age groups (Mhurchu et al., 2013).

The beverages consumed on the market have different pH levels. For example, Reddy et al. (2016) classified the pH of 379 beverages in one state of the USA. As a result, 93% (354 of 379) had a pH of less than 4.0, and only the remaining 7% had a pH of 4.0 or higher (Reddy et al., 2016). On the other hand, in Korea, 90.5% of beverages on the market have a pH of 5.5 or less, and about 40 beverages have an average pH of 3.5 or less. This leads to concerns about tooth damage (Ministry of Health and Welfare, 2000). Among them, carbonated beverages showed the strongest acidity with a pH of 3.0, and milk beverages had pH 6.8. In addition, some studies have recently reported that drinking alkaline water (pH 9.5 - 10.0) is beneficial for body and blood health, and the number of people taking alkaline water has increased (Rubik, 2011; Weidman et al., 2016).

A diet that consumes a lot of acidic food and drinks can contribute to erosion by lowering the pH in the oral cavity (Reddy et al., 2016). In particular, excessive consumption of fruit juice, carbonated and refreshing beverages is continuously related to tooth corrosion (Salas et al., 2015). In addition, a high frequency of exposure to acidic foods and drinking methods causes long-term contact between low pH drinks and tooth surfaces and inevitably increases tooth corrosion risk (Kwek et al., 2015).

1.4. Influence of pH on resin-based materials

In particular, acidic food or drinks are essential factors affecting the durability and lifespan of resin restorations. Low pH may cause deterioration of physical properties and chemical degradation of restoration materials (Han et al., 2008). As a result of examining the difference in residual monomer leakage according to acidity and immersion time in the three types of composite resins, it was reported that the leakage increased significantly as the immersion time increased at pH 4 (Jeon et al., 2004). Many previous studies have reported the effect of acidic beverages on dental restoration materials. For example, Yanikoğlu et al. (2009) reported that coffee, tea, acidic foods, and low-pH beverages could reduce the surface hardness of resin-based composite materials. In addition, Erdemir et al. (2013) reported the study results that low-pH sports drinks and energy drinks accelerate the decomposition process of restorative materials and shorten the lifespan by reducing hardness (Erdemir et al., 2013).

On the other hand, when the composite resin, whose main component is bis-GMA/TEGDMA, is stored in a solvent with a different pH, the difference is that the surface microhardness is lowered. The alkali solvent increases the surface roughness (Renato et al., 2012). Further, a high color change was expected due to hydrolysis of the composite resin according to different pH conditions (Cilli et al., 2012; Moon et al., 2015; Örtengren et al., 2001).

1.5. Aims of this study

Regarding the increased consumption of beverages with different pH levels, it is essential to consider the effect of different pH levels on dental restorative materials in the oral cavity. In addition, the main targets of dental sealant treatment are major consumers of beverages. However, there are still few studies on BPA release and changes in surface properties with pH conditions and time on sealants in the laboratory. Therefore, this study measured the BPA release amount over time after immersing the sealant used in clinical practice in a solvent of different 3 pH according to time. In addition, changes in surface characteristics of surface roughness, surface gloss, and surface color were evaluated.

1.5.1. Comparison of BPA release according to pH conditions and time

In this study, an experimental method was prepared similar to the method of clinical practice for the BPA release test. The first objective of this study was to compare the differences in BPA release according to the pH 3 levels and time. Therefore, the first null hypothesis of this study is that there will be no difference in the amount of bisphenol A released according to the pH level and the immersion time.

1.5.2. Evaluation of changes in surface characteristics according to pH conditions and time

Also, the second aim of this study was to evaluate the changes in surface characteristics according to pH 3 levels and time. Therefore, the second null hypothesis was that there would be no difference in surface roughness, gloss,

and color characteristics depending on the pH levels and immersion time.

Ⅱ. MATERIALS AND METHODS

2.1. Materials

2.1.1. Sealants

The types of sealants used in this study are shown in 'Table 1'. Six sealants were randomly selected from commercially available resin-based light-curing types in the clinic.

Table 1. The composition of the sealants was tested, according to the manufacturer's information

	Sealants	Composition (% by Wt)	Manufacturer
A	Clinpro™	Bis-GMA* (40 ~ 50), TEGDMA (40 ~ 50)	3M Center, St. Paul, MN, USA
B	Eco-s®	Bis-GMA* (50 ~ 55), TEGDMA (35 ~ 40)	Vericom, Anyang, Gyeonggi, Korea
C	UltraSeal XT® plus	Bis-GMA* (not revealed), TEGDMA (10 ~ 25), DUDMA (2.5 ~ 10)	Ultradent Products, South Jordan, UT, USA
D	Charmseal®	Bis-GMA* (not revealed), TEGDMA, UDMA	DenKist, Gunpo, Gyeonggi, Korea
E	Seal-it®	Bis-EMA* (30 ~ 50), TEGDMA (20 ~30)	Spident, Namdong, Incheon, Korea
F	FORTIFY®	Bis-DMA* (5 ~ 10), UDMA (30 ~ 50)	Bisco, Schaumburg, IL, USA

*: BPA-based monomers
Bis-GMA (bisphenol A glycidyldimethacrylate), TEGDMA (triethyleneglycol dimethacrylate), DUDMA (diurethane dimethacrylate), UDMA (urethane dimethacrylate), Bis-EMA (bisphenol A ethoxylatedimethacrylate), Bis-DMA (bisphenol A dimethacrylate)

2.1.2. Solvents of pH conditions

The pH conditions of the solvent for immersing the specimens were classified into three pH groups: pH 3.0, pH 6.5, and pH 10.0. Step-by-step pH levels were measured and adjusted using a commercial pH meter (ORION™ Star A211, Thermo Scientific, Waltham, MA, USA) (Figure 2). Before all procedures, the pH meter was calibrated using pH Buffer (Thermo Scientific™ Orion™ pH 4.01, 7.00, 10.01) for accurate reproduction. This calibration was performed immediately before immersing the specimens in the solvent for each pH level. The pH 3.0 level was prepared by mixing lactic acid (CAS No. 50-21-5) in distilled water (JW-pharma. Co., Seoul, Korea), and the pH 10.0 level was prepared using sodium hydroxide solution (NaOH, CAS No. 1310-73-2). For the pH 6.5 level, sterile distilled water was used, which was opened immediately before the test and was used after checking the pH. The prepared pH solution was stored while blocking the air by packing it with a Press and Seal wrap (GLAD, OAKLAND, CA, USA).

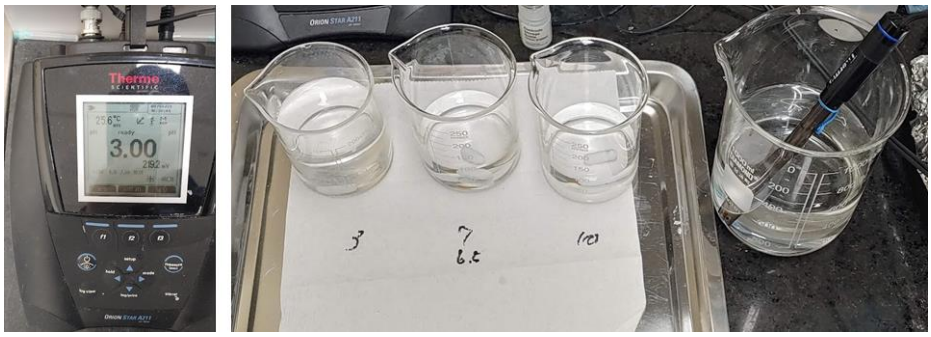

Figure 2. Preparation of pH conditions (left: a commercial pH meter, right: the solutions of pH 3.0, 6.5, 10.0 level).

2.2. Methods

2.2.1. Preparation for BPA release test

2.2.1.1. Pre-investigation on the amount of sealant

The actual amount of sealant used in the pit and fissure sealant treatment was investigated to verify the dose used in clinical practice. Five dental hygienists with more than one year of clinical experience applied sealant to the first molar artificial teeth, five each (Figure 3). In addition, the difference in mass before and after applying the sealant to the artificial teeth was analyzed, respectively.

As a result, the average amount of total sealant was 4.90 mg (Table 2). Therefore, 5 mg of sealant was used in the BPA release test in this study.

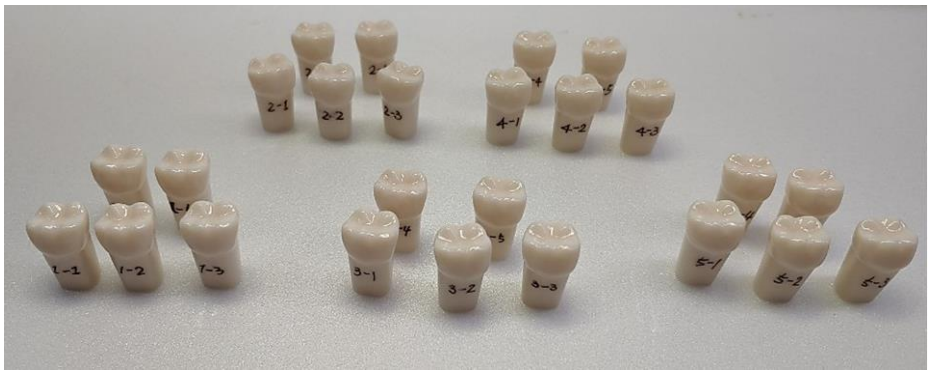

Figure 3. First molar artificial teeth with a sealant

Table 2. Amount of sealant applied to the first molar artificial teeth

No.	N	Amount of sealant (mg)	
		M ± SD	Min – Max
1	5	4.47 ± 0.30	4.11 – 4.85
2	5	5.92 ± 0.70	4.69 – 6.47
3	5	4.71 ± 0.71	4.06 – 6.01
4	5	4.38 ± 0.35	4.00 – 4.98
5	5	5.03 ± 0.63	4.07 – 5.03
Total	25	4.90 ± 0.79	4.00 – 6.47

2.2.1.2. Sealant specimens for BPA release test

The specimen's design was applied by quantifying 5 mg of sealant on a glass plate (10 mm × 10 mm) (Figure 4). According to the manufacturer's instructions, sealant specimens were light-cured at the same distance using a light-curing unit (DeepCure-S Curing Light, 76975, 230V, 3M™ Elipar™, St. Paul, MN, USA).

The total number of specimens for the BPA release test was 270, and 6 sealants were 5 each per one sealant according to the pH group and immersion time (Table 3).

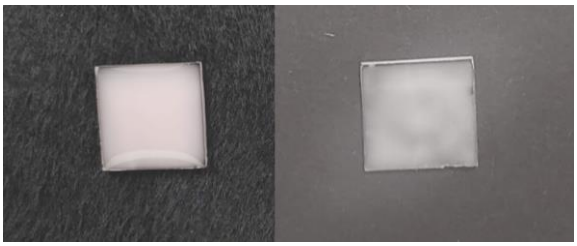

Figure 4. Design of sealant specimens for BPA release test (left: before light curing, right: after light-curing).

Table 3. Sealant specimens (pcs.) for BPA release test

Groups	Immersion time		
	10 min	1 h	24 h
pH 3.0	30 (6 S × 5 each)	30 (6 S × 5 each)	30 (6 S × 5 each)
pH 6.5	30 (6 S × 5 each)	30 (6 S × 5 each)	30 (6 S × 5 each)
pH 10.0	30 (6 S × 5 each)	30 (6 S × 5 each)	30 (6 S × 5 each)

S (Sealants)

2.2.2. Sealant specimens for surface characteristics test

Four sealants used in the surface characteristics test were Clinpro™ (3M Center, St. Paul, MN, USA), Eco-s® (Vericom, Anyang, Gyeonggi, Korea), UltraSeal XT® plus (Ultradent Products, South Jordan, UT, USA), and Charmseal® (DenKist, Gunpo, Gyeonggi, Korea). The specimen's design was a disk using a metal mold (diameter 10 mm × height 1 mm) (Figure 5). According to the manufacturer's instructions, sealant specimens were light-cured from both sides at the same distance.

The total number of specimens for the BPA release test was 120, and 4 sealants were 10 each per one sealant on the different 3 pH levels (Table 4). Therefore, the sealant specimens were repeatedly used for surface characteristics test every 4-time points (baseline, 10 min, 1 h, 24 h).

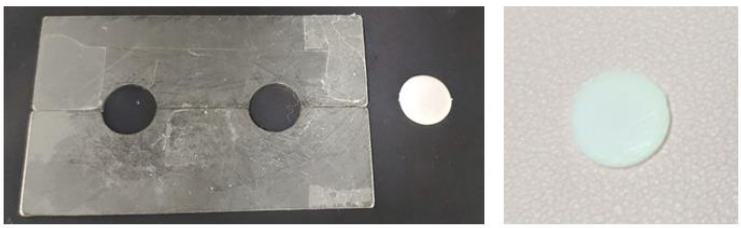

Figure 5. Design of sealant specimens for surface characteristic tests (left: a metal mold, right: a sealant specimen after light-curing).

Table 4. Sealant specimens (pcs.) for surface characteristic tests

Groups	Immersion time			
	baseline	10min	1h	24h
pH 3.0	40 (4 Sealants × 10 each), repeated measurement			
pH 6.5	40 (4 Sealants × 10 each), repeated measurement			
pH 10.0	40 (4 Sealants × 10 each), repeated measurement			

2.2.3. Procedure of BPA release test

The overall procedure for the BPA release test is shown in 'Figure 6'. First, the sealant (5 mg) was applied and photopolymerized to a glass plate (10 × 10 mm) to prepare a sealant specimen. Next, the specimens were immersed in a 15 ml conical tube with 2 ml solvent to submerge completely. These were immersed in an incubator shaker (Lab Companion SI-600, Korea) at 37 °C for each time (10 min, 1 h, 24 h). Immediately after the immersion time, the specimens were removed from the conical tube. These were cooled in a freezer and then freeze-dried for more than 12 hours using a freeze dryer (Ilshin Lab Co., Ltd., Korea). Finally, BPA detection was analyzed by gas chromatography and a mass spectrometer (GC-MS; Agilent Technologies 7820A GC and 5977E MSD system, Palo Alto, CA, USA). 2 ml pure methanol (≥ 99.99 %) was mixed in the conical tubes. 1.5 ml of them were taken and put in 1.5 ml vials (Agilent, Palo Alto, CA, USA) and used for BPA detection analysis.

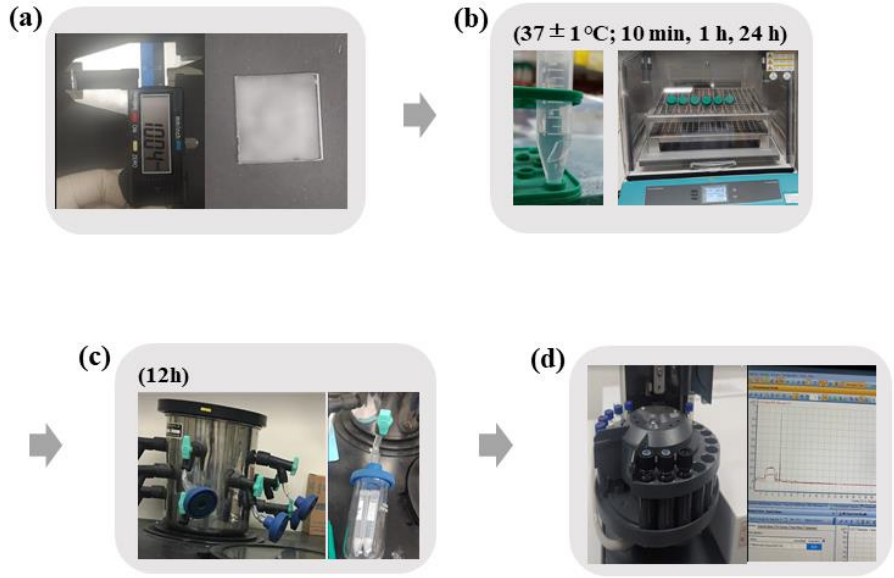

Figure 6. The procedure of BPA release test (a: preparation of sealant specimens, b: immersion in solvent (pH 3.0, pH 6.5, pH 10.0), c: cooling and freeze-drying, d: GC-MS analysis of BPA detection).

2.2.3.1. Conditions of GC/MS

The conditions of the GC-MS instrument for BPA detection are shown in 'Table 5'. A column of analysis was used HP-5ms Ultra Inert (Agilent 19091S-433UI, Santa Clara, CA, USA) with Length 30 m, Diameter 250 μm, Film Thickness 0.25 μm. The carrier gas was helium, and its flow rate was 0.7 mL/min. The sample was 1 μL by splitless injection, and the inlet temperature was 250 ℃. The oven temperature was gradually increased in the following order, 40 ℃ → 5 ℃/min → 50 ℃ (0 min) → 5 ℃/min → 80 ℃ (2 min) → 10 ℃/min → 120 ℃ (5 min) → 10 ℃/min → 280 ℃ (1 min) → 10 ℃/min → 320 ℃.

Table 5. Instrument conditions of GC-MS for BPA detection

	Conditions			
Column	HP-5ms Ultra Inert (30 m 250 μm 0.25 μm)			
	Unit	Rate (℃/min)	Temp. (℃)	Hold (min)
Oven temp.	Initial	-	40	0
	Ramp 1	5	50	0
	Ramp 2	5	80	2
	Ramp 3	10	120	5
	Ramp 4	10	280	1
	Ramp 5	10	320	0
Inlet Temp.	250 ℃			
Injection mode	splitless			
Injection vol.	1 μL			
Carrier gas	Helium			
Carrier flow	0.7 mL/min			
Scan Parameters	40 ~ 615			
Sim Parameters	Bisphenol A: 213.3, 119, 228, 214			

2.2.3.2. Calibration after GC-MS measurement

First, the molecular weight of BPA was confirmed by performing a qualitative analysis of standards (SCAN) according to the four BPA standard concentrations (10, 20, 50, 100 ppm). The standard material of BPA was prepared by mixing 1 g of Bisphenol A (CAS: 80-05-7) and 1,000 mg/L of methanol (≥ 99.99 %) and then diluted with the same solvent to 10, 20, 50, and 100 ppm each. These solutions were stored at -18 °C. After GC-MS measurement of the samples in this study, calibration was performed based on the standard concentration to obtain accurate BPA detection data. 'Figure 7' shows the calibration after GC-MS measurement of the standard samples. The resultant calibration functions had correlation coefficients (R^2) ranging from 0.998 to 1.000.

Then, to evaluate the amount of BPA detected for each sample, a quantitative analysis of selecting and measuring specific ions (selected ion monitoring, SIM) was performed.

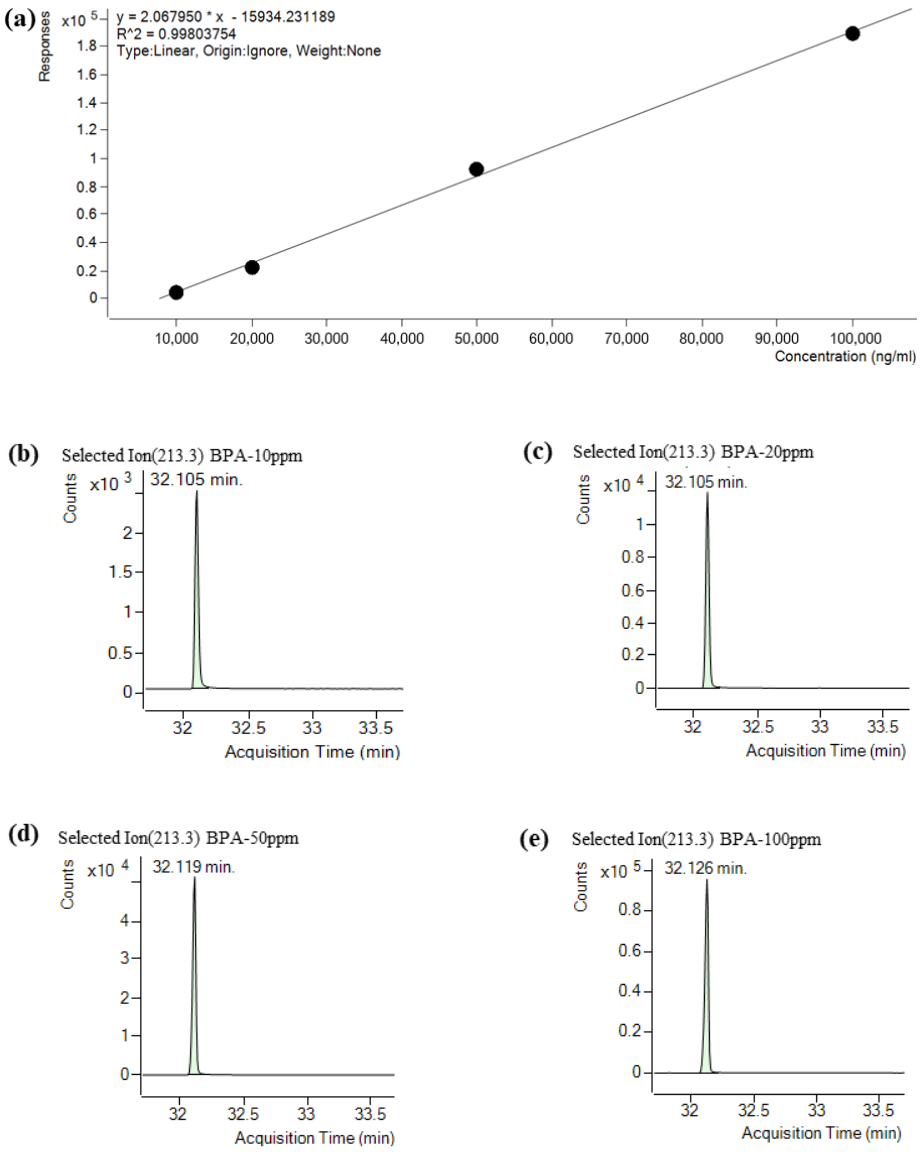

Figure 7. Calibration after GC-MS measurement of the standard samples: (a) Linear calibration of 4 samples, (b) Selected Ion (213.3) bisphenol A 10 ppm, (c) Selected Ion (213.3) bisphenol A 20 ppm, (d) Selected Ion (213.3) bisphenol A 50 ppm, (e) Selected Ion (213.3) bisphenol A 100 ppm.

2.2.4. Procedure of surface characteristics test

The overall procedure for the surface characteristics test is shown in 'Figure 8'. First, disk-shaped sealant specimens were prepared using a metal mold (diameter 10 mm × height 1 mm). According to the manufacturer's instructions, sealant specimens were light-cured from both sides at the same distance. The surface of the original specimen was evaluated before immersion in the pH solution. Next, the specimens were immersed in a 15 ml conical tube with 2 ml solvent to submerge completely. Next, these were immersed in an incubator shaker (Lab Companion SI-600, Korea) at 37 °C for each time (10 min, 1 h, 24 h). Immediately after the immersion time, the specimens were removed from the conical tube. The surfaces of the disks were evaluated in the order of roughness (Optical surface profiler Contour GT-X3, Bruker, Billerica, MA, Germany), gloss (Gloss meter, Novo - Curve, Rhopoint ™, East Sussex, UK), and color (Spectrophotometer, CM3500d, Minolta Co. Ltd., Osaka, Japan).

The surface roughness was measured using the 50x objective lens of the GT-X3. The surface gloss was measured at a constant diameter angle of 60° in all cases. And the reference color was measured according to the CIE (Commission Internationale de I' Eclairage) L^* a^* b^* color scale for the standard light source D65 in the white background (Schulze et al., 2003). The CIE $L^*a^*b^*$ color system is a 3D color measurement. L^* represents the brightness coordinates, and the range of values is 0 for complete black and 100 for complete white. a^* and b^* are color coordinates of the axes of green-red (-a^*=green, +a^*=red) and blue-yellow (-b^*=blue, +b^*=yellow).

The roughness, gloss, and color values on the surface characteristics test were measured randomly three times on one side per specimen, and then the average of these was used.

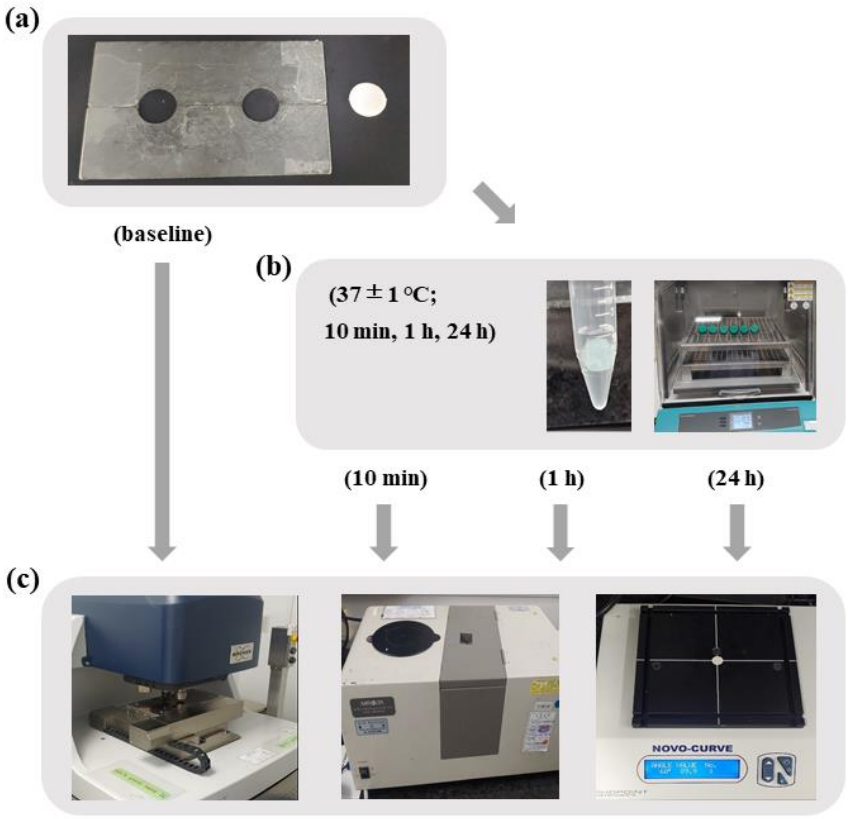

Figure 8. The procedure of surface characteristic tests (a: preparation of sealant specimens, b: immersion in solvent (pH 3.0, pH 6.5, pH 10.0), c: measurement of surface characteristics (top: surface gloss, middle: surface color, bottom: surface roughness).

2.3. Statistical analysis

First, data on BPA release were separately subjected to a two-way ANOVA (pH level × immersion time) and Tukey's test. Additionally, one-way ANOVA was performed to compare the difference in BPA concentration according to the pH group and time.

Next, data on surface gloss, surface color, and surface roughness of surface characteristics were separately subjected to two-way repeated-measures ANOVA (pH level × immersion time) and Tukey's test. If the p-value of the Mauchly sphericity test result for the two-way repeated-measures ANOVA analysis was less than 0.05, it was confirmed using the Greenhouse and Geisser correction result (Howell, 2002, Field, 2013). Additionally, one-way ANOVA was performed to compare the differences in surface gloss, color, and roughness over time with the pH group.

Statistical analysis was performed using IBM SPSS Statistics 18 (IBM, Armonk, NY, USA) for Windows; significance was determined at the p=0.05 level.

III. RESULTS

3.1. BPA release

3.1.1. BPA release according to pH levels and time

The results of two-way ANOVA to evaluate the interaction between pH and time on BPA release are as follows. First, the null hypothesis was rejected because there was a significant difference in the interaction between pH and time on BPA release (p<0.05). Also, there was a significant difference in BPA release between the pH groups (p<0.05). Moreover, there was a significant difference in BPA release according to the time factor within-group (p<0.05).

The difference in BPA concentration according to pH levels and time is ‘Table 6’. First, as a result of comparing within pH levels, in the ‘pH 3.0’ group, 24 h was higher than 10 min and 1 h (p<0.05). Similarly, in the ‘pH 6.5’ and ‘pH 10.0’ groups, 24 h was higher than 10 min and 1h (p<0.05). Next, as results of the comparison within time points, there were significant differences that ‘pH 3.0’ was higher than the ‘pH 6.5’ and ‘pH 10.0’ at all time points of '10 min', '1 h', and '24 h’. At ‘10 min’, pH 3.0 was higher than pH 6.5 and pH 10.0 (p<0.05). Similarly, at ‘1 h’ and ’24 h’, pH 3.0 was higher than pH 6.5 and pH 10.0 (p<0.05).

Table 6. Mean values (± SD) of BPA concentration (ppm) according to pH levels and time points

Group	BPA (ppm)		
	10 min	1 h	24 h
pH 3.0	0.35 ± 0.30 ^{Ab}	0.72 ± 0.73 ^{Ab}	2.14 ± 2.55 ^{Aa}
pH 6.5	0.09 ± 0.14 ^{Bb}	0.18 ± 0.23 ^{Bab}	0.28 ± 0.28 ^{Ba}
pH 10.0	0.09 ± 0.13 ^{Bb}	0.25 ± 0.31 ^{Bb}	0.52 ± 0.60 ^{Ba}

The capital letters indicate post-analysis by Tukey in a time point (p<0.05).
The small letters indicate post-analysis by Tukey within a group (p<0.05).
The same letters indicate no differences in BPA concentration among the experimental groups (p>0.05).

The difference in BPA concentration (ppm) according to each factor is shown in 'Figure 9'. First, as a result of comparing BPA release according to pH levels, the pH 3.0 group was 5.7 times and 3.7 times higher than the pH 6.5 group and pH 10.0 group, respectively (p<0.05). Next, as a result of comparing the release of BPA over time, 24 hours were 5.5 times and 2.6 times higher than 10 min and 1 h, respectively (p<0.05).

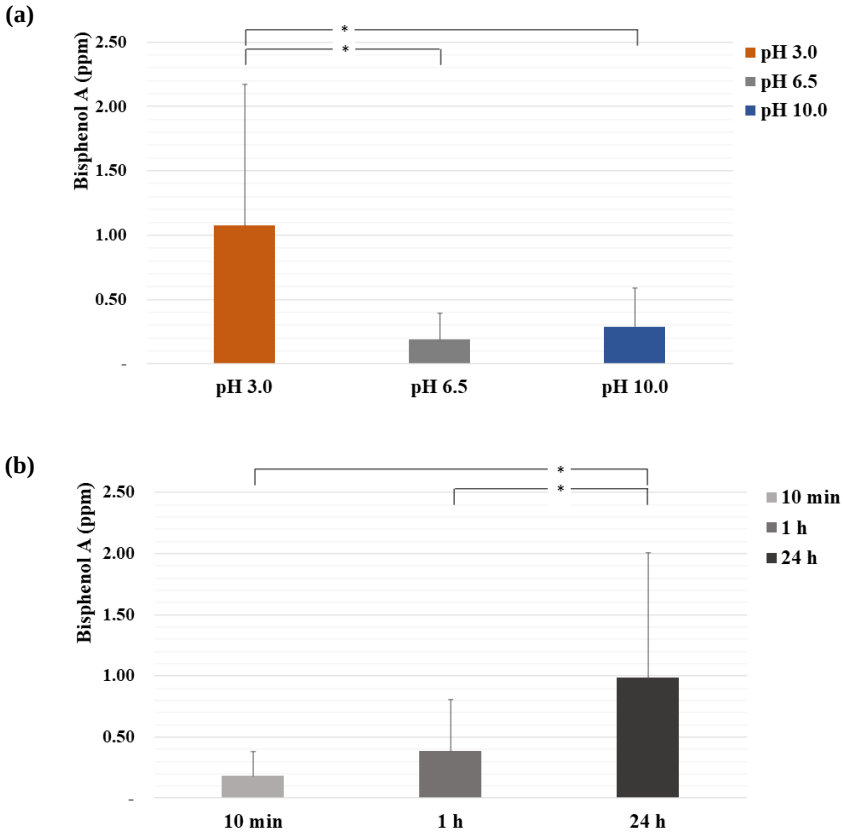

Figure 9. The difference in BPA concentration (ppm) to each factor: (a) BPA release according to pH levels (*: p<0.05), (b) BPA release according to time (*: p<0.05).

‘Figure 10’ shows the comparison of BPA concentrations according to pH levels and time of each sealant product. BPA was detected from all sealants under all conditions. In addition, in all sealants, BPA was higher in ‘pH 3.0’ conditions than ‘pH 6.5’ and ‘pH 10.0’ over time (p<0.05).

First, in the mean value of BPA detected from Clinpro™ was 0.58 ± 0.10 ppm (10 min), 1.65 ± 0.47 ppm (1 h), and 2.09 ± 0.48 ppm (24 h) in the 'pH 3.0' group, which increased over time (p<0.05). In addition, it was higher in the order of 0.26 ± 0.05 ppm (10 min), 0.40 ± 0.07 ppm (1 h), 0.62 ± 0.08 ppm (24 h) in the 'pH 6.5' group (p<0.05), 0.35 ± 0.09 ppm (10 min), 0.61 ± 0.22 ppm (1 h), and 1.36 ± 0.87 ppm (24 h) in the ‘pH 10.0’ group (p<0.05). Second, the average value of BPA detected from Eco-s® was 0.09 ± 0.07 ppm (10 min), 1.67 ± 0.42 ppm (1 h), and 6.93 ± 0.95 ppm (24 h) in the 'pH 3.0' group, which increased over time (p<0.05). Also, it was higher in the order of 0.22 ± 0.12 ppm (10 min), 0.52 ± 0.3 ppm (1 h), 0.67 ± 0.24 ppm (24 h) in the 'pH 6.5' group (p<0.05), 0.11 ± 0.05 ppm (10 min), 0.65 ± 0.3 ppm (1 h), and 0.90 ± 0.24 ppm (24 h) in the 'pH 10.0 group' (p<0.05). Third, the average value of BPA detected in UltraSeal XT® was 0.11 ± 0.01 ppm (10 min), 0.16 ± 0.01 ppm (1 h), and 0.28 ± 0.05 ppm (24 h) in the 'pH 3.0' group, which increased over time (p<0.05). It was higher in the order of 0.03 ± <0.01 ppm (10 min), 0.06 ± <0.01 ppm (1 h), 0.11 ± 0.01 ppm (24 h) in the 'pH 6.5' group (p<0.05), 0.04 ± 0.01 ppm (10 min), 0.07 ± 0.02 ppm (1 h), and 0.12 ± 0.01 ppm (24 h) in the 'pH 10.0 group' (p<0.05). Fourth, the average value of BPA detected in Charmseal® was 0.21 ± 0.02 ppm (10 min), 0.38 ± 0.25 ppm (1 h), and 0.75 ± 0.37 ppm (24 h) in the 'pH 3.0' group, which increased over time (p<0.05). It was higher in the order of 0.03 ± <0.04 ppm (10 min), 0.04 ± <0.01 ppm (1 h), 0.19 ± 0.12 ppm (24 h) in the 'pH 6.5' group (p<0.05), 0.03 ± 0.01 ppm (10 min), 0.04 ± 0.04 ppm (1 h), and 0.33 ± 0.06 ppm (24 h) in the 'pH 10.0 group' (p<0.05). Fifth, the average value of BPA detected in Seal-it® was 0.25 ± 0.1 ppm (10 min), 0.25 ± 0.1 ppm (1 h), and 2.52 ± 0.98 ppm (24 h) in the 'pH 3.0'

group (p<0.05). It was higher in the order of 0.02 ± 0.02 ppm (10 min), 0.02 ± 0.02 ppm (1 h), 0.05 ± 0.04 ppm (24 h) in the 'pH 6.5' group, 0.02 ± <0.01 ppm (10 min), 0.04 ± 0.02 ppm (1 h), and 0.34 ± 0.3 ppm (24 h) in the 'pH 10.0 group'. Lastly, the average value of BPA detected in FORTIFY® was 0.08 ± 0.01 ppm (10 min), 0.22 ± 0.09 ppm (1 h), and 0.27 ± 0.08 ppm (24 h) in the 'pH 3.0' group, which increased over time (p<0.05). Also was higher in the order of 0.02 ± <0.01 ppm (10 min), 0.04 ± <0.01 ppm (1 h), 0.07 ± <0.01 ppm (24 h) in the 'pH 6.5' group (p<0.05), 0.02 ± 0.01 ppm (10 min), 0.05 ± 0.02 ppm (1 h), and 0.09 ± 0.01 ppm (24 h) in the 'pH 10.0 group' (p<0.05).

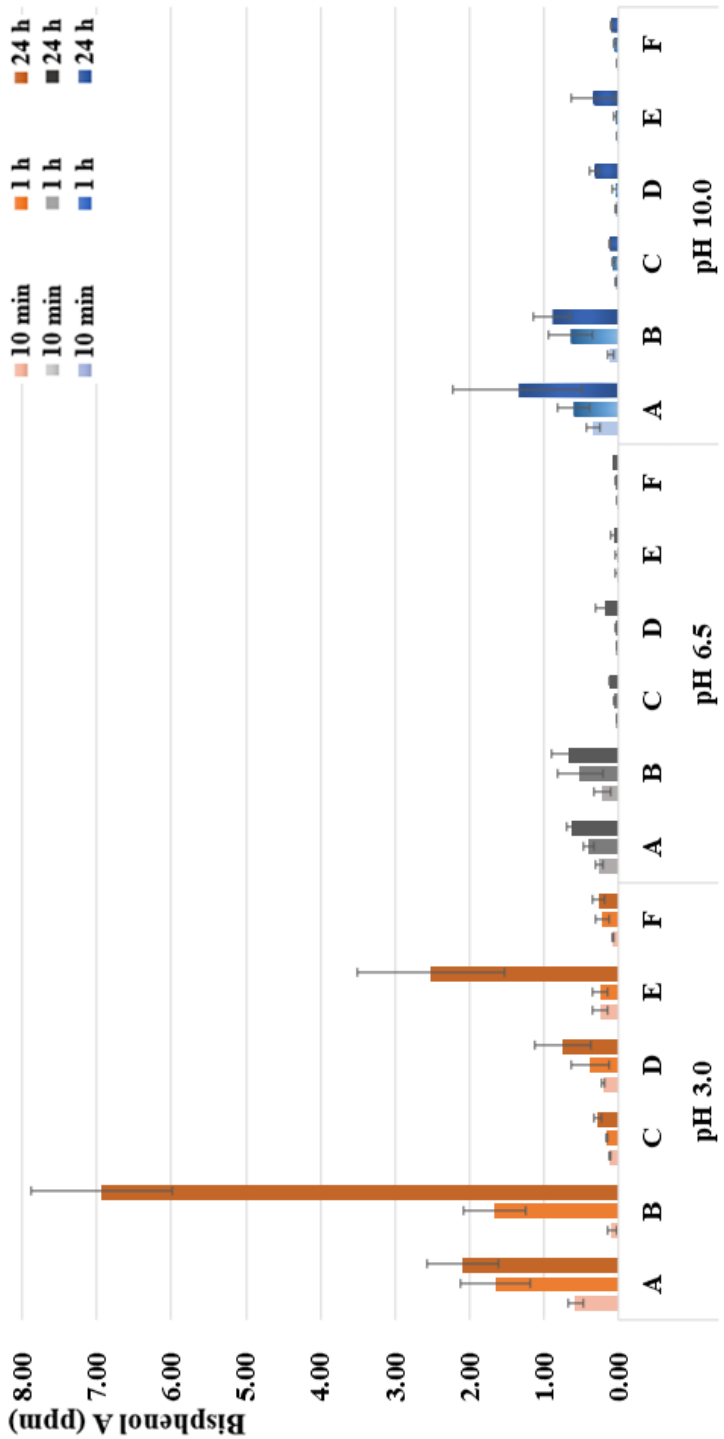

Figure 10. Comparison of BPA concentration according to pH levels ($p<0.05$) and time ($p<0.05$) of each sealant (A: Clinpro™, B: Eco-S®, C: UltraSeal XT® plus, D: Charmseal®, E: Seal-it®, F: FORTIFY®).

3.2. Surface characteristics

3.2.1. Surface roughness according to pH levels and time

Repeated measures ANOVA to verify the interaction between pH and time on surface roughness are as follows. First, the null hypothesis was rejected because there was a significant difference in the interaction between pH and time on surface roughness (p<0.05). Also, there was a significant difference in surface roughness between pH groups (p<0.05). Moreover, surface roughness was significantly different according to the time factor within-group (p<0.05).

The difference in surface roughness among pH groups by time is shown in ‘Table 8’. At the '1 h', pH 3.0 was significantly higher than pH 6.5 and pH 10.0 groups (p<0.05). Similarly, at the '24 h', pH 3.0 was significantly higher than pH 6.5 and pH 10.0 groups (p<0.05).

Table 7. Mean values (± SD) of surface roughness (Ra) among pH groups by time

Group	Surface roughness (Ra, μm)			
	baseline	10 min	1 h	24 h
pH 3.0	0.062 ± 0.054 ^{Ad}	0.089 ± 0.067 ^{Ac}	0.142 ± 0.092 ^{Ab}	2.384 ± 4.607 ^{Aa}
pH 6.5	0.074 ± 0.044 ^{Aa}	0.086 ± 0.051 ^{Aa}	0.087 ± 0.046 ^{Ba}	0.113 ± 0.060 ^{Ba}
pH 10.0	0.065 ± 0.034 ^{Ad}	0.084 ± 0.048 ^{Ac}	0.101 ± 0.052 ^{Bb}	0.174 ± 0.074 ^{Ba}

The capital letters indicate post-analysis by Tukey in a time point (p<0.05).
The small letters indicate post-analysis by Tukey within a group (p<0.05).
The same letters indicate no differences in surface roughness among the experimental groups (p>0.05).

The difference in surface roughness (Ra) according to each factor is shown in

'Figure 11'. First, as a result of comparing surface roughness according to pH levels, the pH 3.0 group was 1.8 times and 1.6 times higher than the pH 6.5 group and pH 10.0 group, respectively (p<0.05). Next, as a result of comparing surface roughness over time, 24 hours were 2.5 times, 1.9 times, and 1.5 times higher than baseline, 10 min, and 1 h, respectively (p<0.05).

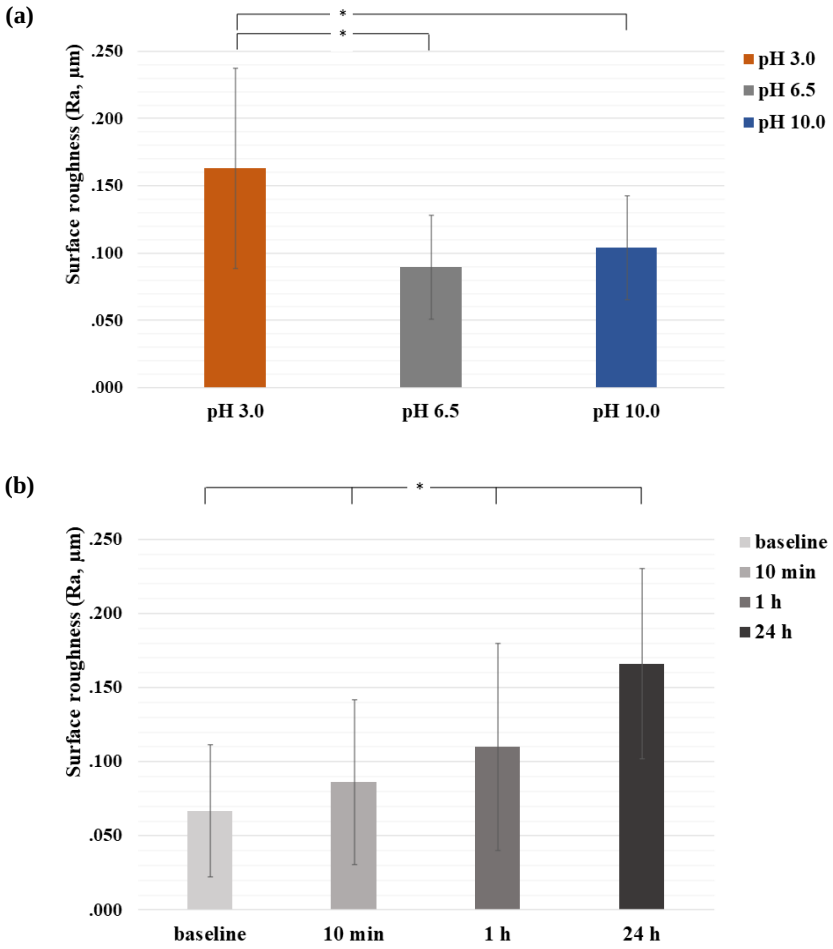

Figure 11. The difference in surface roughness (Ra) according to each factor: (a) surface roughness according to pH levels (*: p<0.05), (b) surface roughness according to time (*: p<0.05).

'Figure 12' shows the surface roughness (Ra) according to pH levels and time of each sealant product. In all sealants, surface roughness was higher than pH 3.0 conditions at pH 6.5 and 10.0 over time (p<0.05). Additionally, in all sealants, at pH 3.0 conditions, the surface roughness was increased over time (p<0.05).

First, at Clinpro™, the mean surface roughness values of the 'pH 3.0' group was 0.075 ± 0.10 μm (baseline), 0.08 ± 0.06 μm (10 min), 0.165 ± 0.09 μm (1 h), and 0.611 ± 0.08 μm (24 h), which increased over time. Second, at Eco-s®, the mean surface roughness values of the 'pH 3.0' group was 0.048 ± 0.03 μm (baseline), 0.051 ± 0.03 μm (10 min), 0.067 ± 0.05 μm (1 h), and 0.172 ± 0.07 μm (24 h), which increased over time. Third, at UltraSeal XT®, the mean surface roughness values of the 'pH 3.0' group was 0.059 ± 0.03 μm (baseline), 0.095 ± 0.08 μm (10 min), 0.155 ± 0.10 μm (1 h), and 0.287 ± 0.17 μm (24 h), which increased over time. Lastly, at Charmseal®, the mean surface roughness values of the 'pH 3.0' group was 0.065 ± 0.04 μm (baseline), 0.091 ± 0.06 μm (10 min), 0.182 ± 0.08 μm (1 h), and 0.364 ± 0.09 μm (24 h), which increased over time.

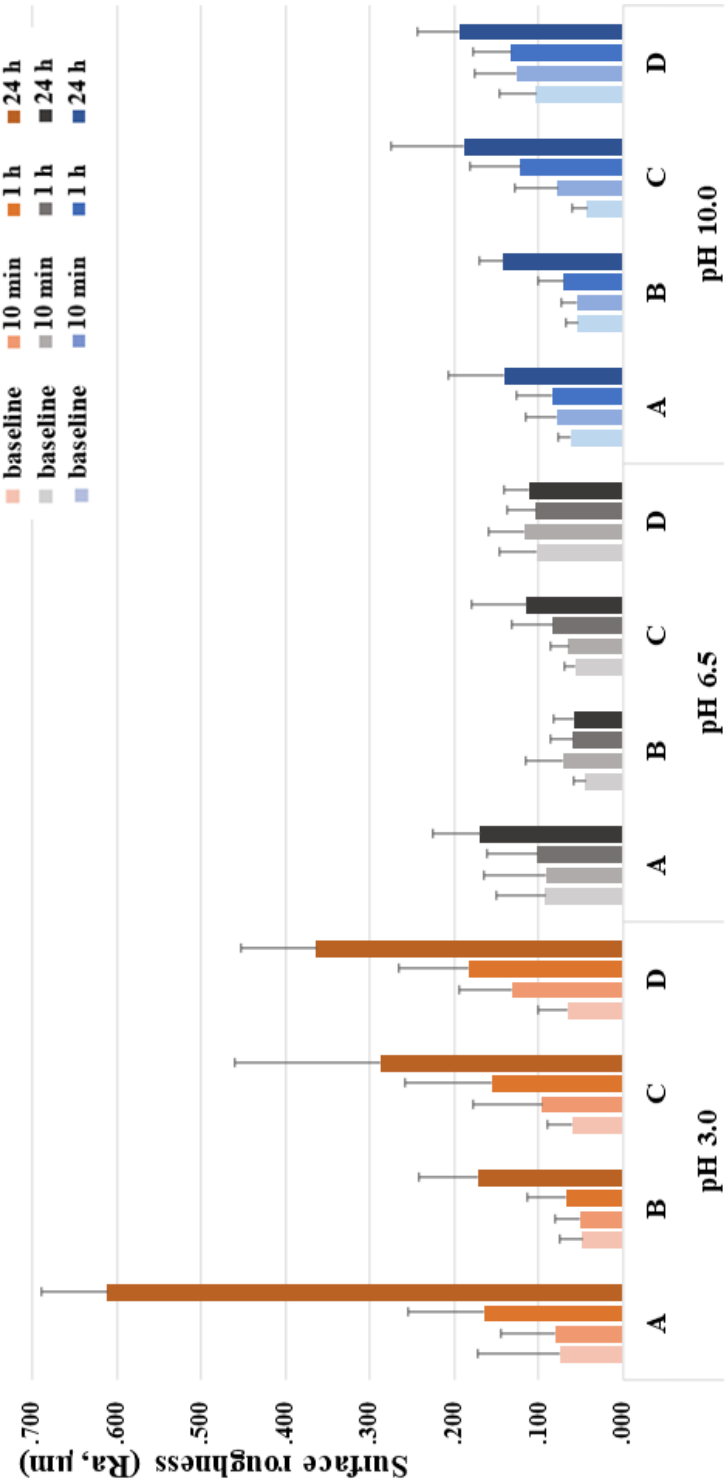

Figure 12. Comparison of surface roughness (Ra) according to pH levels and time ($p<0.05$) of each sealant (A: Clinpro™, B: Eco-S®, C: UltraSeal XT® plus, D: Charmseal®).

3.2.2. Surface gloss unit according to pH levels and time

Repeated measures ANOVA to verify the interaction between pH and time on the surface gloss unit are as follows. First, the null hypothesis was rejected because there was a significant difference in the interaction between pH and time on the surface gloss unit (p<0.05). In addition, the surface gloss unit changed significantly over time within the group (p<0.05). However, there was no significant difference in surface gloss unit between pH groups, only between some groups. (p=0.055).

The difference in surface gloss unit among pH groups by time is shown in 'Table 8'. At the '24 h', pH 3.0 was lower than the pH 6.5 and pH 10.0 groups (p<0.05).

Table 8. Mean values (± SD) of surface gloss unit (GU) among pH groups by time

Group	Surface gloss unit (GU)			
	baseline	10 min	1 h	24 h
pH 3.0	84.74 ± 9.36 ^{Aa}	72.15 ± 15.54 ^{Ab}	66.59 ± 16.01^{Ac}	34.43 ± 18.49 ^{Bd}
pH 6.5	80.59 ± 11.37 ^{Aa}	66.21 ± 17.63 ^{Ab}	58.72 ± 20.55 ^{Ac}	60.53 ± 16.84 ^{Ac}
pH 10.0	84.38 ± 11.56 ^{Aa}	72.25 ± 15.51 ^{Ab}	64.04 ± 16.37 ^{Ac}	64.08 ± 14.42 ^{Ac}

The capital letters indicate post-analysis by Tukey in a time point (p<0.05).
The small letters indicate post-analysis by Tukey within a group (p<0.05).
The same letters indicate no differences in surface gloss unit among the experimental groups (p>0.05).

'Figure 13' shows the difference in surface gloss unit (GU) according to each factor. First, comparing the surface gloss unit according to pH levels, the pH 3.0 group was about 10 % lower than the pH 10.0 group (p<0.05). Next, in comparing surface gloss over time, surface gloss gradually decreased over time, and '24 h' was about 40 % lower than the 'baseline' (p<0.05).

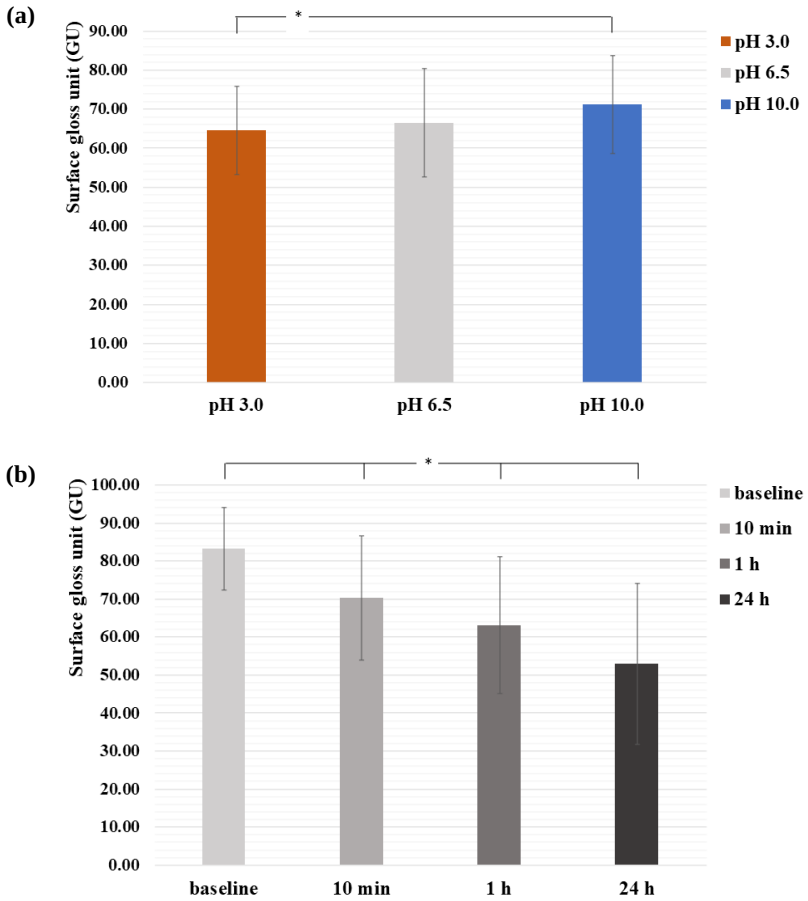

Figure 13. The difference in surface gloss unit (GU) according to each factor: (a) surface gloss according to pH levels (*: p<0.05), (b) surface gloss according to time (*: p<0.05).

'Figure 14' shows the surface gloss unit (GU) according to pH levels and time of each sealant product. In all sealants, at pH 3.0 condition, the surface gloss was decreased over time ($p<0.05$).

First, at Clinpro™, the mean surface gloss values of the 'pH 3.0' group was 81.41 ± 6.30 (baseline), 69.18 ± 11.92 (10 min), 59.24 ± 19.22 (1 h), and 16.90 ± 2.02 (24 h), which decreased over time. Second, at Eco-s®, the mean surface gloss values of the 'pH 3.0' group were 92.44 ± 7.77 (baseline), 88.63 ± 10.47 (10 min), 79.26 ± 12.11 (1 h), and 43.17 ± 21.95 (24 h), which decreased over time. Third, at UltraSeal XT®, the mean surface gloss values of the 'pH 3.0' group were 85.78 ± 7.47 (baseline), 70.95 ± 9.40 (10 min), 63.70 ± 15.27 (1 h), and 41.66 ± 19.74 (24 h), which decreased over time. Lastly, at Charmseal®, the mean surface gloss values of the 'pH 3.0' group were 79.34 ± 10.60 (baseline), 59.82 ± 14.96 (10 min), 64.17 ± 10.39 (1 h), and 36.01 ± 10.91 (24 h), which decreased over time.

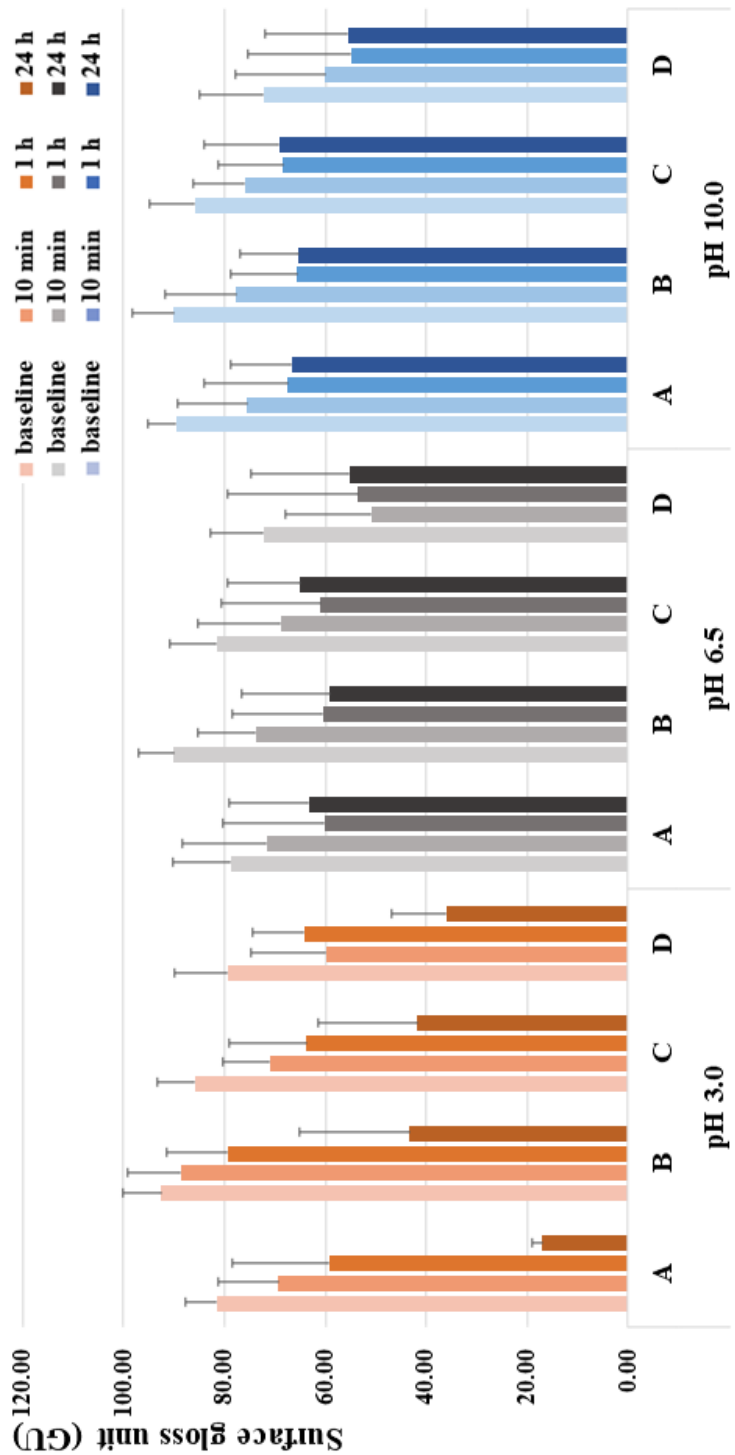

Figure 14. Comparison of surface gloss unit (GU) according to pH levels and time (p<0.05) of each sealant (A: Clinpro™, B: Eco-s®, C: UltraSeal XT® plus, D: Charmseal®).

3.2.3. Surface color

3.2.3.1. *L** value according to pH levels and time

Repeated measures ANOVA to verify the interaction between pH and time on the *L** value are as follows. First, the null hypothesis was rejected because there was a significant difference in the interaction between pH and time on the *L** value ($p<0.05$). Second, there was no significant difference between the pH groups ($p=0.315$), but there was a significant difference by time within the group ($p<0.05$).

The difference in *L** value among pH groups by time is shown in 'Table 9'. In the 'pH 3.0' group, '24 h' was significantly lower than 'baseline', '10 min', and '1 h' ($p<0.05$). However, there was no significant change according to time in the 'pH 6.5' and 'pH 10.0' groups. At 24 hours, the pH 3.0 was lower than the pH 6.5 and pH 10.0 groups ($p<0.05$).

Table 9. Mean values (± SD) of *L** value among pH groups by time

Group	*L** value			
	baseline	10 min	1 h	24 h
pH 3.0	68.94 ± 5.40 ^{Aa}	68.61 ± 5.58 ^{Aa}	68.44 ± 5.49 ^{Aa}	63.94 ± 4.59 ^{Bb}
pH 6.5	68.78 ± 5.81 ^{Aa}	69.06 ± 5.81 ^{Aa}	69.29 ± 5.54 ^{Aa}	69.52 ± 5.29 ^{Aa}
pH 10.0	68.91 ± 5.03 ^{Aa}	68.74 ± 5.11 ^{Aa}	68.29 ± 5.63 ^{Aa}	68.90 ± 5.08^{Aa}

The capital letters indicate post-analysis by Tukey in a time point ($p<0.05$).
The small letters indicate post-analysis by Tukey within a group ($p<0.05$).
The same letters indicate no differences in *L** value among the experimental groups ($p>0.05$).

'Figure 15' shows the difference in L^* value according to each factor. First, as a result of comparing the L^* value according to pH levels, the pH 3.0 group was about 2 % lower than the pH 6.5 group (p=0.315). Next, in comparing L* value over time, L^* value gradually decreased over time, and '24 h' was about 2 % lower than the 'baseline' (p<0.05).

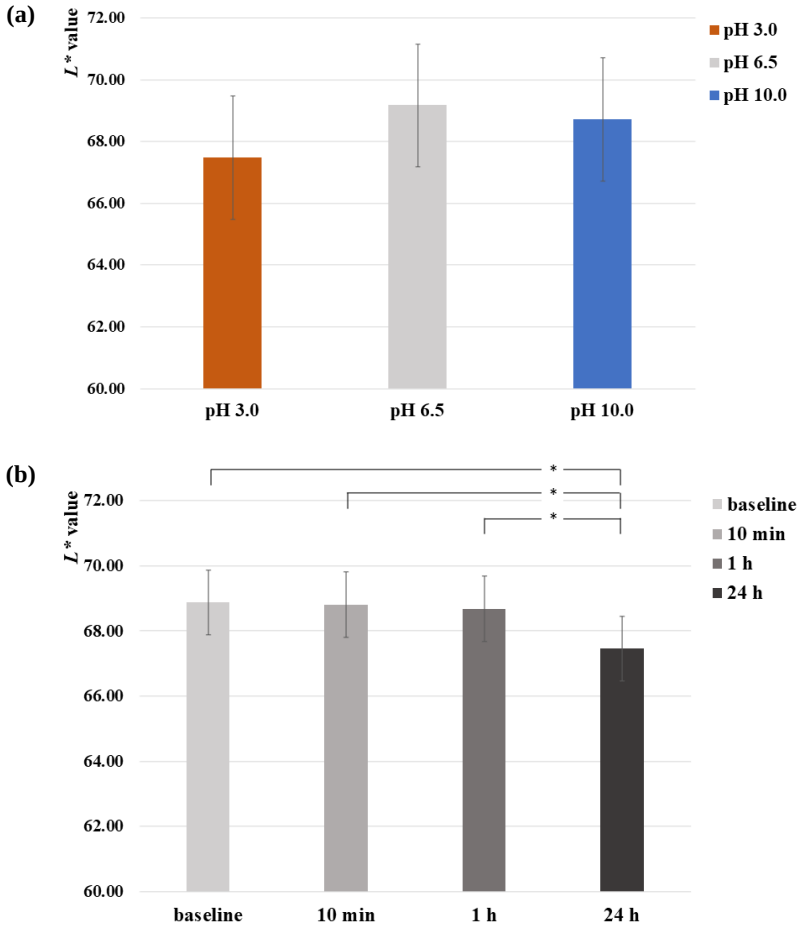

Figure 15. The difference in L^* value according to each factor: (a) L^* value according to pH levels (*: p<0.05)**,** (b) L^* value according to time (*: p<0.05).

'Figure 16' shows the L^* value according to pH levels and time of each sealant product. In most products except UltraSeal XT®, the L^* value at pH 3.0 decreased over time ($p<0.05$).

First, at Clinpro™, the mean L^* values of the 'pH 3.0' group was 74.38 ± 1.72 (baseline), 74.48 ± 1.85 (10 min), 74.16 ± 1.70 (1 h), and 68.32 ± 2.58 (24 h), which decreased over time. Second, at Eco-s®, the mean L^* color values of the 'pH 3.0' group was 72.22 ± 0.77 (baseline), 72.22 ± 0.92 (10 min), 72.19 ± 0.80 (1 h), and 65.76 ± 1.10 (24 h), which decreased over time. Lastly, at Charmseal®, the mean L^* values of the 'pH 3.0' group was 66.81 ± 4.87 (baseline), 65.21 ± 4.78 (10 min), 65.22 ± 4.32 (1 h), and 58.25 ± 2.08 (24 h), which decreased over time.

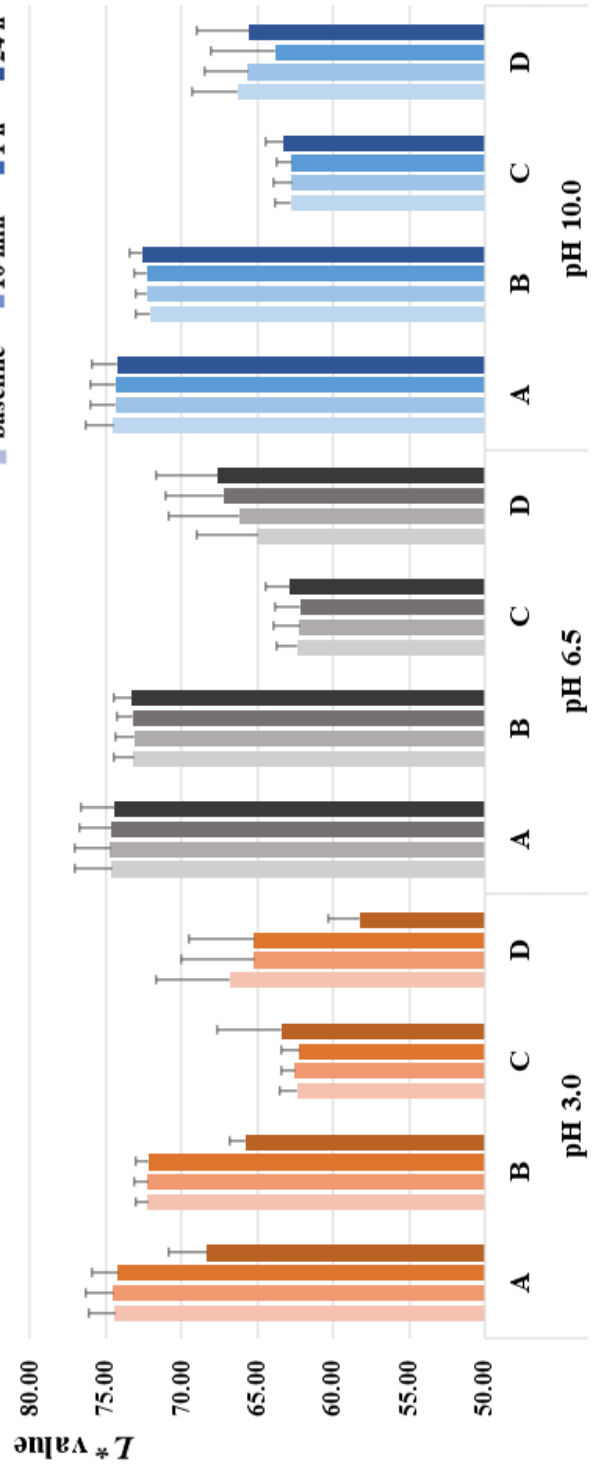

Figure 16. Comparison of L^* value according to pH levels and time ($p<0.05$) of each sealant (A: Clinpro™, B: Eco-s®, C: UltraSeal XT® plus, D: Charmseal®)

3.2.3.2. *a** value according to pH levels and time

Repeated measures ANOVA to verify the interaction between pH and time on *a** value are as follows. First, the null hypothesis is not rejected because there was no significant difference in the interaction between pH and time in a* value (p=0.165). Also, there were no significant differences between the pH groups (p=0.941). However, there was a significant difference with time within the group (p<0.05).

The difference in *a** value among pH groups by time is shown in 'Table 10'. The *a** value was significantly changed according to time in all groups of 'pH 3.0', 'pH 6.5', and 'pH 10.0'. First, in the pH3.0 group, *a** value increased over time in the order of baseline, 10min, 1h, and 24h. Next, in the 'pH 6.5' group, 10 min and 1h were lower than baseline and higher than 24h. Finally, in the pH 10.0 group, 1h and 24h were higher than baseline and 10min, respectively.

Table 10. Mean values (± SD) of *a** among pH groups by time

Group	*a** value			
	baseline	10 min	1 h	24 h
pH 3.0	-1.90 ± 0.75 ^{Ad}	-1.86 ± 0.75 ^{Ac}	-1.82 ± 0.73 ^{Ab}	-1.64 ± 0.64 ^{Aa}
pH 6.5	-1.93 ± 0.87 ^{Ac}	-1.88 ± 0.86 ^{Ab}	-1.85 ± 0.82 ^{Ab}	-1.77 ± 0.81 ^{Aa}
pH 10.0	-1.88 ± 0.75 ^{Ac}	-1.85 ± 0.75 ^{Ab}	-1.77 ± 0.75 ^{Aa}	-1.70 ± 0.75 ^{Aa}

The capital letters indicate post-analysis by Tukey in a time point (p<0.05).
The small letters indicate post-analysis by Tukey within a group (p<0.05).
The same letters indicate no differences in a* value among the experimental groups (p>0.05).

'Figure 17' shows the difference in *a** value according to each factor. First, as a result of comparing *a** value according to pH levels, there is little difference between the pH groups (p=0.941). Next, in comparing a* value over time, a* value gradually increased over time, and '24 h' was about 10 % higher than the 'baseline' (p<0.05).

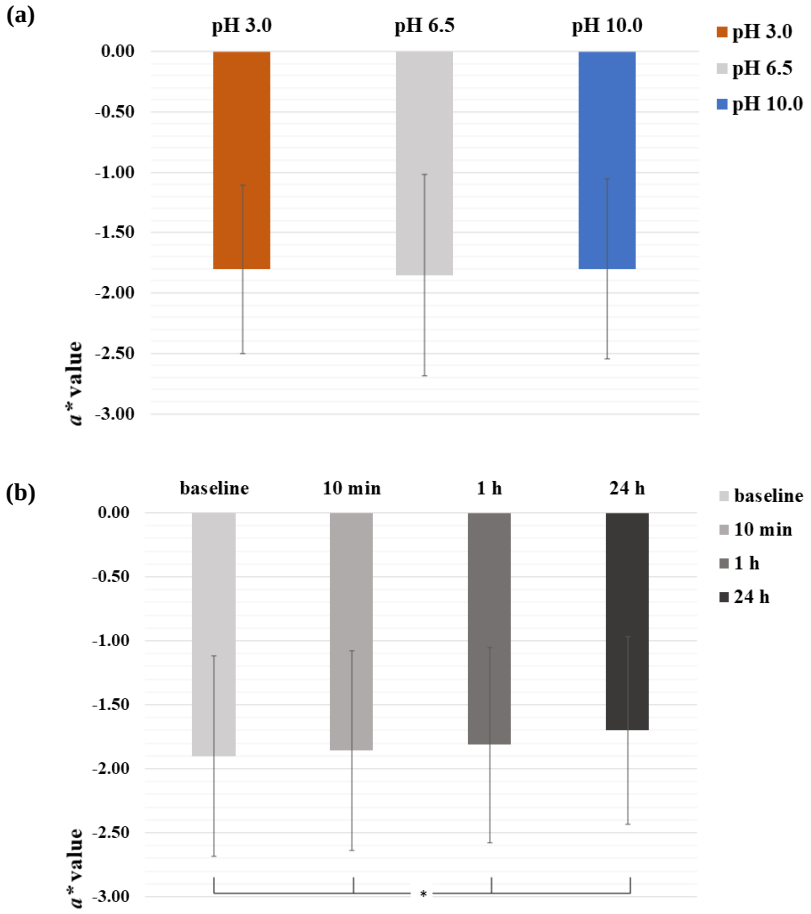

Figure 17. The difference in *a** value according to each factor: (a) *a** value according to pH levels, (b) *a** value according to time (*: p<0.05).

'Figure 18' shows the a^* value according to pH levels and time of each sealant product. In most products except UltraSeal XT®, the a^* value at pH 3.0 increased over time ($p<0.05$).

First, at Clinpro™, the mean a^* values of the 'pH 3.0' group was -0.90 ± 0.08 (baseline), -0.84 ± 0.10 (10 min), -0.83 ± 0.08 (1 h), and -0.66 ± 0.09 (24 h), which increased over time. Second, at Eco-s®, the mean a^* value values of the 'pH 3.0' group was -2.94 ± 0.16 (baseline), -2.87 ± 0.20 (10 min), -2.81 ± 0.18 (1 h), and -2.30 ± 0.16 (24 h), which increased over time. Lastly, at Charmseal®, the mean a^* value values of the 'pH 3.0' group was -2.05 ± 0.11 (baseline), -2.03 ± 0.08 (10 min), -2.00 ± 0.09 (1 h), and -1.55 ± 0.04 (24 h), which increased over time.

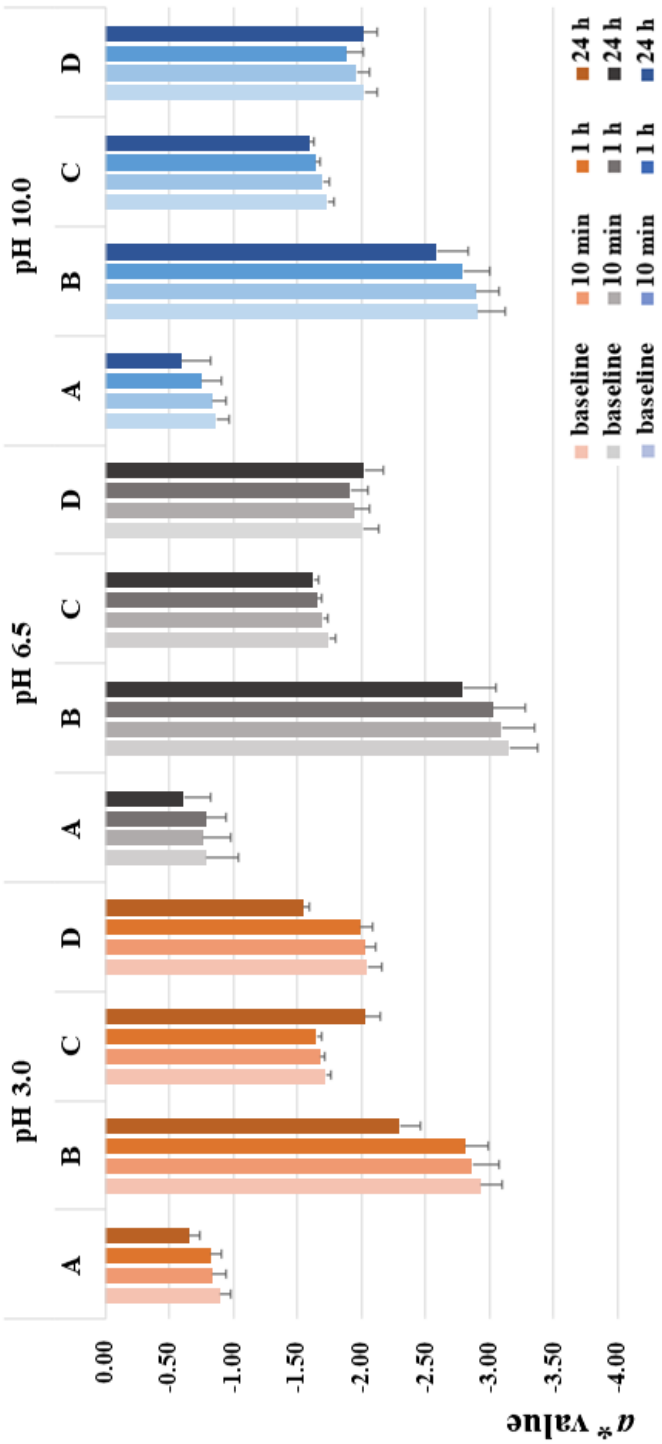

Figure 18. Comparison of a^* value according to pH levels and time ($p<0.05$) of each sealant (A: Clinpro™, B: Eco-s®, C: UltraSeal XT® plus, D: Charmseal®).

3.2.3.3. *b** value according to pH levels and time

Repeated measures ANOVA to verify the interaction between pH and time on *b** value are as follows. First, the null hypothesis is not rejected because there was no significant difference in the interaction between pH and time in the *b** value (p=0.896). Also, there was no significant difference between the pH groups (p=0.936), and there was no significant difference with time within the pH group (p=0.456).

The difference in *b** value among pH groups by time is shown in 'Table 11'. First, in the 'pH 3.0' group, the baseline of *b** value decreased at 10 min and 1 h. Moreover, in the pH 6.5 group, the baseline was lowered at 10 min.

Table 11. Mean values (± SD) of *b** among pH groups by time

Group	*b** value			
	baseline	10 min	1 h	24 h
pH 3.0	-2.46 ± 3.92 ^{Aa}	-2.58 ± 3.90 ^{Ab}	-2.70 ± 3.80 ^{Ab}	-2.33 ± 3.33 ^{Aab}
pH 6.5	-2.23 ± 4.02 ^{Aa}	-2.33 ± 4.18 ^{Ab}	-2.39 ± 4.06 ^{Aab}	-2.28 ± 3.84 ^{Aab}
pH 10.0	-2.51 ± 3.96 ^{Aa}	-2.60 ± 3.95 ^{Aa}	-2.68 ± 3.90 ^{Aa}	-2.60 ± 3.70 ^{Aa}

The capital letters indicate post-analysis by Tukey in a time point (p<0.05).
The small letters indicate post-analysis by Tukey within a group (p<0.05).
The same letters indicate no differences in b* value among the experimental groups (p>0.05).

'Figure 19' shows the difference in b^* value according to each factor. First, comparing the b^* value according to pH levels, there is little difference between the pH groups (p=0.936). Next, there is little difference by time points when comparing the b^* value over time(p=0.456).

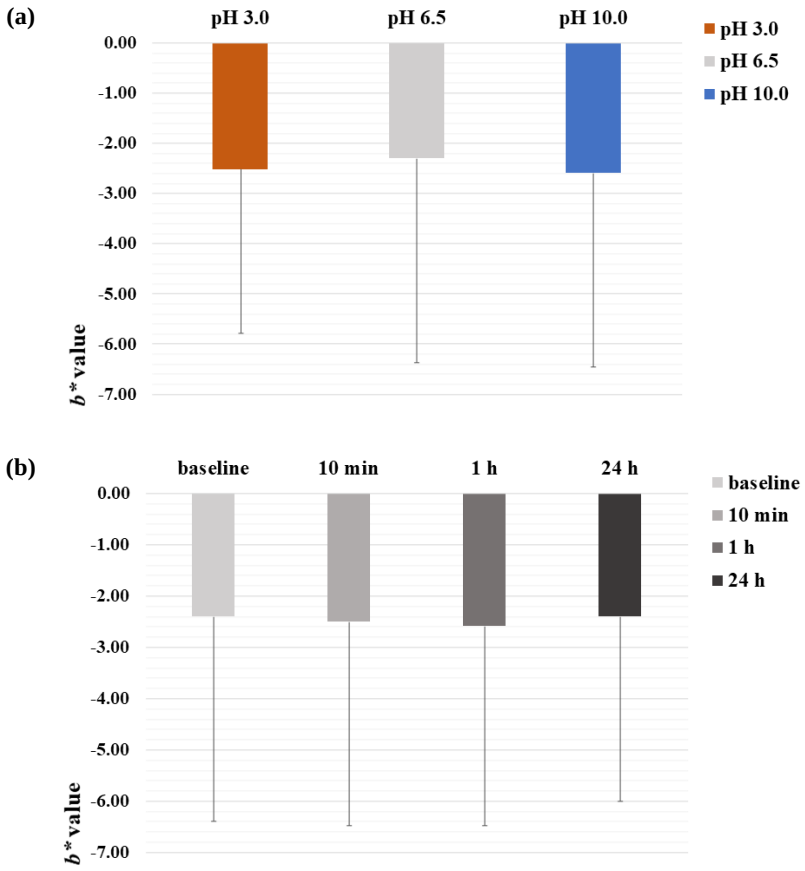

Figure 19. The difference in b^* value according to each factor: (a) b^* value according to pH levels, (b) b^* value according to time.

‘Figure 20’ shows the *b** value according to pH levels and time of each sealant product. In most products except UltraSeal XT®, the *b** value at pH 3.0 decreased over time.

First, at Clinpro™, the mean *b** value of the 'pH 3.0' group was 0.92 ± 0.74 (baseline), 0.90 ± 0.82 (10 min), 0.60 ± 0.81 (1 h), and 0.55 ± 0.71 (24 h), which decreased over time. Second, at Eco-s®, the mean *b** value of the 'pH 3.0' group was 0.43 ± 0.45 (baseline), 0.24 ± 0.55 (10 min), 0.12 ± 0.50 (1 h), and 0.01 ± 0.55 (24 h), which decreased over time. Lastly, at Charmseal®, the mean *b** values of the 'pH 3.0' group was -2.46 ± 0.23 (baseline), -2.70 ± 0.30 (10 min), -2.75 ± 0.29 (1 h), and -7.69 ± 0.31 (24 h), which decreased over time.

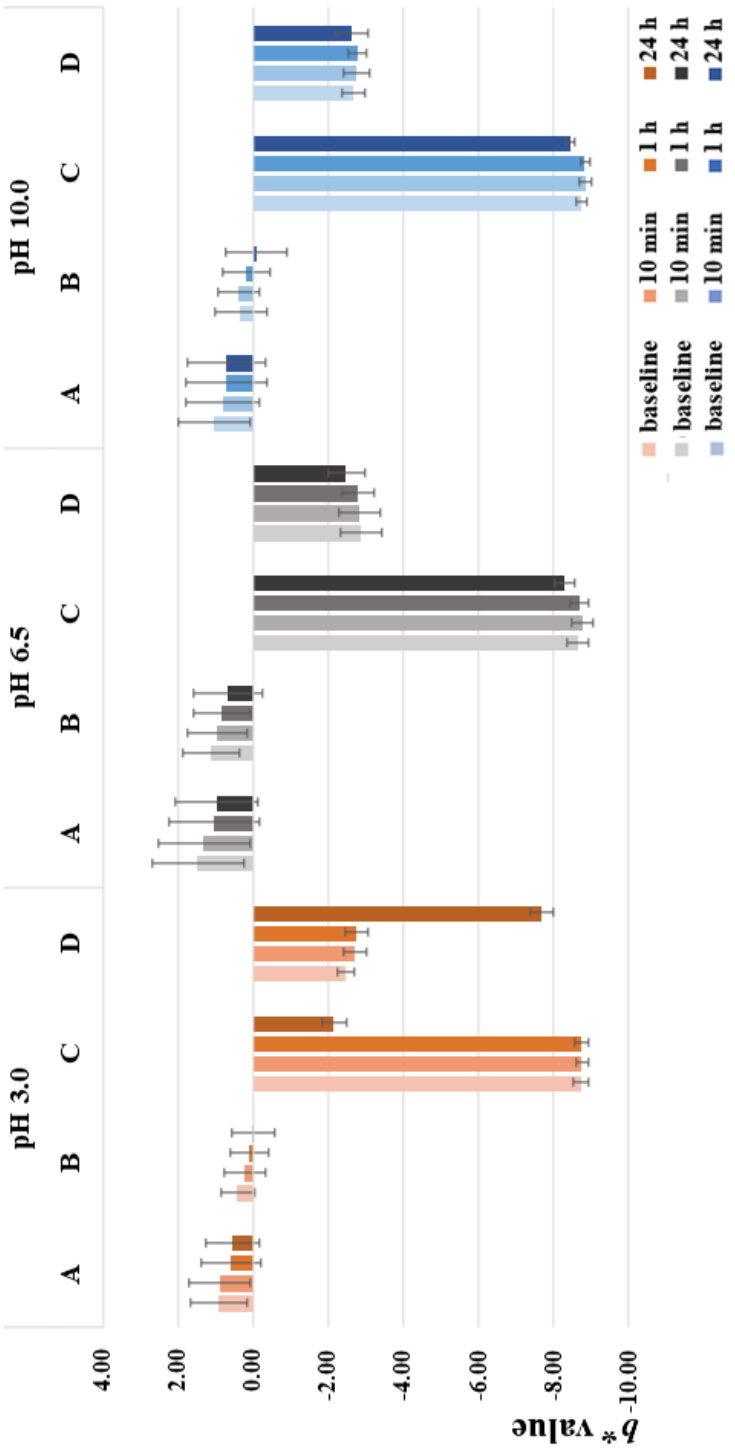

Figure 20. Comparison of *b** value according to pH levels and time of each sealant (A: Clinpro™, B: Eco-s®, C: UltraSeal XT® plus, D: Charmseal®).

Ⅳ. DISCUSSION

The sealant is an essential dental material to prevent occlusal caries of teeth today. However, there was a concern that BPA, an environmental hormone-disrupting substance, is detected from these restorative materials. In addition, the beverages we consume have different pH levels, which alter the pH conditions in our mouths (Pachori et al., 2018). Therefore, there was concern that chemical and physical changes would occur to the restorative materials if the oral pH were changed after dental sealant treatment. Therefore, this study evaluated the difference in BPA release and surface properties according to different 3 pH conditions and immersion time after polymerized resin-based light-curing type dental sealant.

There are several important points in the research method for evaluating BPA emissions in this study. First, the amount of sealant used in the BPA release test was investigated. The reason is that the range of amounts of sealant placed in previous laboratory studies varied widely, and even the amount used in actual clinical practice was different (Arenholt-Bindslev et al., 1999; Gruninger et al., 2015; Olea et al., 1996; Pulgar et al., 2000). Therefore, 5 dental hygienists with more than 1 year of clinical experience applied 5 of the first molar model sealants. As a result, it was confirmed that an average of 5 mg of sealant was used per tooth ($p<0.05$). Next, a certain amount (5 mg) was applied using a 10 × 10 mm glass plate to control the same exposure amount of the treated sealant surface. Therefore, it may be considered very reasonable to compare the BPA emission concentrations under different pH conditions within the results of this study.

The results of this study confirmed that there was a significant difference in BPA emission according to pH level and immersion time ($p<0.05$). First, in the release of BPA by pH groups, the detected BPA concentration of the pH 3.0 group was 5.7 times and 3.7 times higher than the pH 6.5 group and pH 10.0 group, respectively ($p<0.05$). The pattern of these results was similarly

confirmed in all 6 sealants. Similarly, several studies have reported that low pH beverages caused surface decomposition in resin composite materials (ABU-BAKR et al., 2000; Han et al., 2008). In addition, in a survey of the effect of acidity on the chemical dissolution of composite resins, the outflow of monomers from pH 4 solution was significantly increased compared to pH 7 solution (Jeon et al., 2004). Sealants are continuously exposed to various types of accommodation environments in the mouth. Hydrolysis reaction by water and expansion of the matrix surface by water absorption are the leading causes of the chemical decomposition of resin-based restorations (Øysæd and Ruyter, 1986). Most composite resins are composed of non-polymer compound monomers, but polymers can also cause hydrolysis. Figure 21 (a) shows the hydrolysis step of Bis-GMA (Finer and Santerre, 2003). Hydrolysis occurs when the OC=O bond between the acyl group of resin molecules and oxygen is broken (Göpferich, 1996). At this time, since pores are generated, a decomposition product appears, and the types of decomposition products are bisphenol A dimethacrylate (BADGE), 2,2-bis[4 (2,3-hydroxypropoxy)phenyl] -propane (bis-HPPP), BPA, etc. (Finer, 2004; Atkinson, 2002) (Figure 21-(b)).

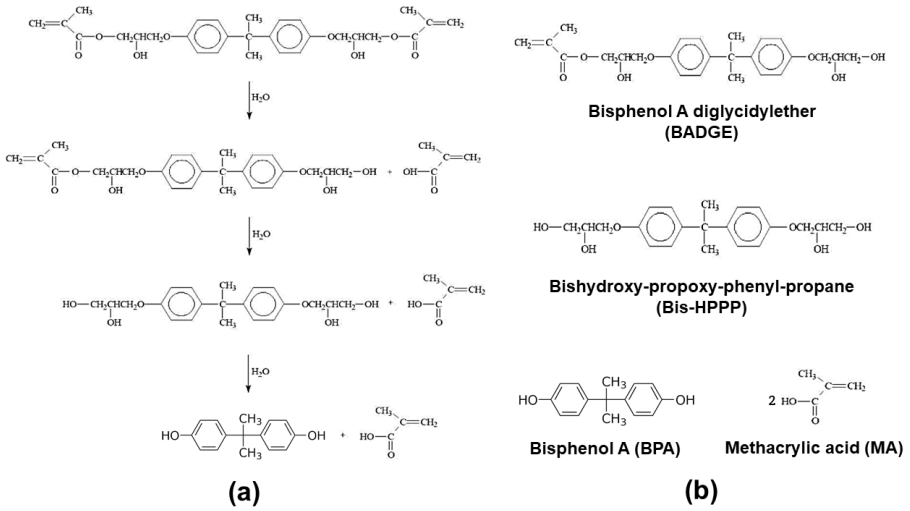

Figure 21. Hydrolysis of resin component: (**a**) the hydrolysis step of Bis-GMA (Finer and Santerre, 2003); (**b**) the types of decomposition products.

Perhaps the reason is that BPA is hardly soluble in water but generally dissolves well in acetic acid, benzene, ethanol, etc.; Log Kow (Octanol-Water Partition Coefficient) = 3.32. (Hoekman, 1996). Therefore, when these are put together, acidity can accelerate the decomposition of the sealant of the resin substrate and destroy chemical stability.

Second, in the release of BPA over time in the results of this study, the detected BPA concentration after 24 hours were 5.5 times and 2.6 times higher than 10 min and 1 h, respectively ($p<0.05$). At pH 3.0, the BPA detection concentration was 0.35 ppm (10 min), 0.75 ppm (1 h), and 2.14 ppm (24 h), showing higher values over time. Similar to the effect of pH, the pattern of these results was similarly confirmed in all 6 sealants. In other words, the longer undervalued drinks stay in our mouths, the more BPA is detected within 24 hours. In addition, BPA was detected at pH 6.5, pH 10.0, but it was lower than pH 3.0, increasing in the order of 10 minutes (0.09 ppm, 0.09 ppm), 1 hour (0.18 ppm, 0.24 ppm), and 24 hours (0.28 ppm). After 24 hours reported in this study, the BPA concentration (pH 0.3 = 2.14 ppm, pH 6.5 = 0.28 ppm, pH 10.0 = 0.52) was lower than Pulgar and colleagues (pH 1.0 = 6.5 ppm, Ph 7.0 = 7.8 ppm) (Pulgar et al., 2000). It is also similar to the values reported by Arenholt-Bindslev and colleagues (0.3–2.8 ppm) (Arenholt-Bindslev et al., 1999). In 2000, MANABE and colleagues reported that when sealant (1 mg) was immersed in water for 24 hours, BPA was detected at 0.02–0.09 ppm, lower than 0.28 ppm (pH 6.5, 24 hours) under similar conditions. (Manabe et al., 2000). The reason is expected to be different research methods from 20 years ago, such as how to use sealant, type of sealant, type of GC-MS equipment, and function improvement.

Lastly, BPA was detected from all silanes under all conditions. Since various BPA concentrations were detected for each sealant, the minimum-maximum range of BPA according to pH levels in the total sealant was in the order of pH 3 (0.07 ~ 7.74 ppm), pH 10.0 (<0.01 ~ 2.81 ppm), and pH 6.5 (<0.01 ~ 1.06

ppm). The standard for BPA elution of plastic food containers is 0.05 ppm in the EU, 0.6 ppm in South Korea, and 2.5 ppm in Japan (Chang Yeob et al., 2017; Safety, 2016; Tarja Laaninen, 2018). In addition, the use of BPA was prohibited in the manufacture of some baby products, including baby bottles, in the EU and South Korea. In addition, the use of BPA in manufacturing raw materials for cosmetics in the EU and South Korea was prohibited. Comparing the range of BPA concentrations by pH group in this study, there may be concerns about the stability of dental sealant harmful to the human body. However, there are two limitations when determining the amount of BPA detected in this study. First, it is impossible to evaluate the harmfulness caused by only the BPA detection amount in this study. This is because BPA quickly converts into metabolites without estrogen action in the body and is released through the kidneys. BPA is administered orally quickly absorbed in the gastrointestinal tract, and BPA is converted into BPA glucuronide in the liver. It is transferred into the bloodstream before reaching the kidneys or excreted quickly into the urine (Völkel et al., 2008). Next, it was impossible to compare and analyze the amount of BPA detected by sealant type in this study. Previous research on the solubility of synthetic resins to acidity and time said that the solubility of resin was influenced by the kind of resin monomer and the composition of filler. (Örtengren et al., 2001). Perhaps the reason is that hydrophilicity varies depending on the type of resin monomer. The sealant used in this study was randomly selected as used in clinical practice. The types and configurations of resin substrates for each sealant cannot be accurately classified according to the manufacturer's confidentiality. To gain more insight into BPA release, it may be necessary to evaluate the difference in which BPA release is affected by the resin matrix after controlling the resin composition of the experimental material.

In this study's results of surface properties, first, both surface roughness and surface gloss had negative results over time in the pH 3.0 group than the pH 6.5

group and the pH 10.0 group, and similar results in all sealants. The surface roughness of the pH 3.0 group was 1.8 times and 1.6 times higher than the pH 6.5 group and pH 10.0 group, respectively ($p<0.05$). Moreover, Surface roughness after 24 hours was 2.5 times, 1.9 times, and 1.5 times higher than baseline, 10 min, and 1 h, respectively. The surface gloss of the pH 3.0 group was about 10 % lower than the pH 10.0 group ($p<0.05$). Surface gloss gradually decreased over time, and '24 h' was about 40 % lower than the 'baseline' ($p<0.05$). The reason why the surface roughness and gloss continue to change over time within 24 hours may be due to the hydrophilicity of the sealant monomer. Because in a previous study on acidity and surface properties, the surface roughness of all three types of composite resins increased over time in acid beverages. However, the composite resin containing more hydrophobic monomers showed stronger resistance to chemical decomposition and surface roughness than the conventional composite resin (Camilotti et al., 2021). The bis-GMA molecule has an OH hydroxyl group in the side chain, responsible for the composite resin's moisture absorption and solubility properties (Finer and Santerre, 2003). Therefore, it is presumed that moisture absorption by the hydroxyl group led to hydrolysis of the polymer, resulting in a degradation in the chemical and mechanical properties of the sealant surface.

Increasing surface roughness and decreasing gloss can increase the adhesion of the bacteria film on the sealant surface. Previous studies have argued that surface roughness exceeding the threshold Ra value of 0.2 μm increases plaque accumulation and stain (Bollen et al., 1997, Quirynen, 1995). This is less than 2.384 μm of the surface roughness after '24 hours' in the pH 3.0 group in this study. In addition, the decrease in gloss due to the increase in surface roughness leads to plaque accumulation, causing color instability of the restoration and increasing the failure rate of restoration over time (Furuse et al., 2008).

Finally, as a result of the surface color change in this study, there was no significant difference between the pH groups, and there was little change within

1 hour, but there was a significant change after 24 hours. *L** value gradually decreased over time, and '24 hours' was 2% lower than the 'baseline' (p<0.05). *a** value gradually increased over time, and '24 hours' was 10% upper than the 'baseline' (p<0.05). In previous studies on the hydrolysis influence of resin complexes, color changes were expected due to hydrolysis in acidic (pH 3.0) or alkaline (pH 9.0) solutions (Örtengren, 2001; Cilli, 2012). However, in the study of Moon and colleagues, the pH 6.0 group significantly changed the *L** value compared to the pH 3.0 and pH 9.0 groups, which was different as a result of this study (Moon et al., 2015). Also, in the results of this study, in most products except UltraSeal XT®, the *L** value decreased (p<0.05) and the *a** value increased (p<0.05) at pH 3.0 condition over time. UltraSeal XT® products showed different patterns of results from the other three sealant products. Comparing its components, there is a difference in the addition of DUDMA (2.5 ~ 10 % by Wt). The reason cannot be determined since the sealant manufacturer does not disclose all product compositions in commercial confidentiality. The matrix of composite resins most commonly used in dental clinical trials is bis-GMA, but urethane UDMA is also used as the matrix of composite resins. The bis-GMA-based matrix of the composite resin has low wear resistance and may cause discoloration due to water absorption. In previous studies, low Ph softens the matrix, causing loss of ionic structure and affecting wear resistance of dental materials, which negatively affects surface integrity (Bagheri et al., 2005; Patel et al., 2004; Villalta et al., 2006). Moisture absorption of bis-GMA-based resins increased proportion to TEGDMA concentration and decreased with partial replacement of TEGDMA with UDMA (Sarafianou et al., 2007). UDMA with the -NH group is less sensitive to color change because it has lower moisture absorption than Bis-GMA with the -OH group (Ertas et al., 2006). From these results, the type and content of the sealant substrate will influence surface properties such as surface roughness, surface properties, and surface color. Therefore, to accurately analyze the

change in the surface properties of the sealant under various Ph conditions in the oral cavity, further research is considered necessary in consideration of the type and content of the monomer.

The limitation of this study is the static effect of the pH conditions. The pH in the oral cavity can be recovered and changed by saliva buffer, depending on the various foods consumed. Likewise, the pH in the oral cavity can be recovered and changed by saliva buffering capacity depending on the various foods consumed (Pachori et al., 2018). In future studies, a complementary experiment is needed considering the pH recovery time of intraoral saliva. Nevertheless, this study is significant because it evaluated changes in sealants' chemical stability and surface stability according to different pH conditions and time factors.

Ⅴ. CONCLUSION

In this laboratory study, confirming the changes of BPA release and surface characteristics of the resin-based pit and fissure sealant according to the pH conditions confirmed that pH 3.0 conditions were more important than pH 6.5, 10.0 as follows.

1) BPA was eluted from all six tested sealants. BPA release varies depending on the sealant's products but was affected by acidity and time.
2) The released BPA concentration in the pH 3.0 group was higher at all points than pH 6.5 and pH 10.0 and gradually increased over time.
3) The surface roughness of the pH 3.0 group was higher than pH 6.5 and pH 10.0 and increased over time.
4) The surface gloss of the pH 3.0 group was lower than pH 10.0 and decreased over time.
5) The L^* value decreased after 24 hours in the pH 3.0. There was no significant difference in pH groups in a^* and b^* values.

As a result of the study, it was confirmed that the null hypothesis of this experiment was almost rejected except for some surface colors. Furthermore, low pH was a factor that negatively influenced BPA release, surface roughness, and surface gloss. Therefore, frequent exposure to low pH due to consumption of various beverages after sealant treatment can negatively affect the sealant's chemical and surface stability in the oral cavity.

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ABSTRACT (IN KOREAN)

다양한 pH 조건에 따른 치면열구전색재의 비스페놀 A 방출 및 표면 특성

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구강 내 pH의 변화는 처치된 레진계 치면열구전색재(pit and fissure sealant; 이하 실란트)의 화학적 분해 및 표면 특성에 변화를 일으킬 수 있다. 그러므로 본 연구의 목적은 3가지 수준의 pH 조건(3.0, 6.5, 10.0)에서 시간 경과에 따라 실란트로부터의 bisphenol A(이하 BPA) 방출과 실란트 표면 특성(거칠기, 광택, 색상)의 변화를 평가하는 것이다.

실란트는 레진계 광중합 유형을 무작위로 선정하였다. BPA 검출시험을 위한 시편은 유리판(10 × 10 mm)에 6 종의 실란트를 각 5 mg씩 도포 한 후 광중합 하였다. 한편, 표면 특성(표면 거칠기,

표면 광택, 표면 색상) 시험을 위한 시편은 4종의 실란트를 원판형(지름 10 mm, 두께 1 mm)의 금속 몰드에 충전 후 광중합하였다. 모든 시험에서 시료를 37 ℃에서 pH 3.0, 6.5, 10.0 용액에 10분, 1시간, 24시간 동안 침지시켰다. 실란트로부터 방출된 BPA는 기체 크로마토그래피 질량분석기(GC-MS; 7820A GC and 5977E MSD system, Agilent Technology, Palo Alto, CA, USA)를 사용하여 농도를 측정하였다. 실란트의 표면의 중심선 평균 거칠기(Ra)는 비접촉식 3차원 광학 표면 측정기(GT-X3, Bruker, Billerica, MA, Germany), 표면 광택도는 광택계(Novo-Curve, Rhopoint TM, East Sussex, UK), 표면의 색상은 분광측색계(CM3500d, Minolta Co. Ltd., Osaka, Japan)를 이용하여 L^*, a^*, b^*를 측정하였다. 통계 분석은 pH 수준과 침지 시간에 따라 BPA 방출량의 차이를 분석하기 위해 이원분산분석을 사용하였고, pH 수준별 침지 시간에 따른 표면 특성 변화의 차이는 반복측정 분산분석을 사용하였다.

pH 3.0 그룹의 BPA 검출 농도는 pH 6.5와 pH 10.0 보다 모든 시점에서 높았고($p<0.05$), 시간이 지날수록 점차 증가하였다($p<0.05$). pH 3.0 그룹의 표면 거칠기는 pH 6.5와 pH 10.0 보다 높았고, 시간이 지날수록 증가하였다($p<0.05$). pH 3.0 그룹의 표면 광택은 pH 10.0 보다 낮았고($p<0.05$), 시간이 지날수록 감소하였다($p<0.05$). 반면 모든 표면 색상에서는 pH 그룹간 유의한 차이는 없었다. L^* 값은 24시간 후 감소하였고 ($p<0.05$), a^* 값은 모든 pH 그룹에서 시간이 지남에 따라 증가하였으며($p<0.05$), b^* 값은 모든 그룹에서 유의한 차이가 없었다($p>0.05$).

낮은 pH는 BPA 방출, 표면 거칠기, 표면 광택에 부정적인 영향을 미치는 요인임을 확인하였다. 따라서 실란트 처리 후 다양한 음료 및 음식 섭취로 낮은 pH에 빈번하게 노출되는 경우, 구강 내 실란트의 화학적 안정성과 표면 안정성에 부정적인 영향을 미칠 가능성이 있다고 사료된다.

핵심 되는 말: 치면열구전색재, pH, 비스페놀 A, 표면거칠기, 표면 광택, 표면색상