# Inhibition of dental caries around composite resin containing ursolic acid

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# Inhibition of dental caries around composite resin containing ursolic acid

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December 2012

# 감사의 글

3년의 길고도 짧은 시간이 흘러 어느새 이렇게 논문을 작성하는 시간까지 오게 되었습니다. 부족하지만 이렇게 하나의 결실을 맺게 되어 도움과 의지가 되어 주신 많은 분들께 감사의 말씀을 전하고자 합니다.

좋은 스승님을 만나 학문과 덕을 배우고 좋은 인연을 맺는 경험을 할 수 있는 것은 한 사람의 인생에 있어서 큰 행운이라고 생각합니다. 논문이 나오기까지 좋은 가르침 주시고 방향을 잡아주신 이찬영 선생님께 진심으로 감사드립니다. 모든 과정에서 꼼꼼하고 지혜로운 조언, 따뜻한 격려 아끼지 않고 주신 박정원 선생님과 박성호 선생님께도 깊은 감사의 말씀을 드립니다. 초보 의사였던 저를 3년의 수련기간 동안 잘 이끌어 주시고 많은 것을 가르쳐주신 신수정 선생님, 송민주 선생님께도 감사를 드립니다. 바쁜 일정 중에서도 정성 들여 가르쳐 주시고 도와주신 이승종 선생님, 노병덕 선생님, 정일영 선생님, 김의성 선생님, 신유석 선생님, 장지현 선생님께 감사의 말씀 드립니다.

아울러, 실험의 전반적인 과정 동안 정말 많은 도움을 주신 예방치과학 교실 김백일 교수님, 강시묵 선생님께도 진심으로 감사드립니다. 3년이란 시간 동안 힘들 때, 즐거울 때 함께하며 힘이 되었던 소중한 수련동기들과 의국원들에게도 고마운 마음을 전합니다.

언제나 변함없이 큰 사랑을 주고 지켜보며 응원해 주는 나의 든든한 버팀목 부모님과 언니, 동생에게 무한한 감사와 사랑을 전하며 글을 마칩니다.

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원 유 경

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# Abstract

## Inhibition of dental caries around composite resin containing ursolic acid

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1. Objective

Recurrent caries formation around existing restorations represents the primary reason for replacement of composite resin restorations. Use of the antimicrobial restorative material to inhibit the bacterial biofilm formation can be one way to prevent the recurrent caries around restoration. The aim of this study is to compare the degree of caries-like lesion formation around experimental composite containing different concentrations of ursolic acid.

2. Materials and methods

Four different concentrations of ursolic acid (0, 0.1, 0.2, 0.5 wt%) were added to Filtek Z350 (3M ESPE, St Paul, MN, U.S.A.). Standard cavities (2mm x 3.5mm x 2mm) were prepared on the buccal and lingual surfaces of twelve extracted human molars and restored with these composite resins (n=6)

containing antibacterial substances. Caries-like lesions were produced on the experimental teeth using *S. mutans*-induced artificial caries system. Specimens were incubated in a mixture of saliva and medium containing 1% carbohydrate at 37℃ for 5 days. Demineralization of dental enamel around the margins of restorations were analyzed with quantitative light-induced fluorescence(QLF). The demineralized specimens were photographed with QLF-D Biluminator™ 2 (Inspektor Research Systems bv, Amsterdam, Netherlands) and the fluorescence images of white spot area 1mm around the restorations were analyzed using image analysis software QA2 (Inspektor Research Systems bv, Amsterdam, Netherlands).

3. Results

After 5 days of incubation, all the specimens showed the production of white spot lesions. For QLF analysis results, the total loss of fluorescence radiance of dental enamel around the margins of restorations was significantly less in the 0.5% ursolic acid-containing composite group compared with 0.1% ursolic acid-containing composite group and control group ($p<0.05$).

4. Conclusion

Following the limitation of this experiment, composite resin containing 0.5% ursolic acid might inhibit the secondary caries induced by *S. mutans* around restoration.

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Key words : ursolic acid, composite resin, artificial caries

# Inhibition of dental caries around composite resin containing ursolic acid

Directed by Prof. Chan-Young Lee

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Department of Dental Science

Yu-Kyoung Won

## I. Introduction

Recurrent caries formation around existing restorations represents the primary reason for the replacement of composite resin restoration(Kidd et al., 1992; Mjor, 2005). Use of the antimicrobial restorative material to inhibit the bacterial biofilm formation can be one way to prevent the recurrent caries around restoration. There is a considerable interest in efforts to develop the materials having anticariogenic properties like the release of OH-, calcium and

fluoride which are able to inhibit bacterial growth. Investigation about composite resin containing a low concentration (0.1−0.5 wt%) of ursolic acid showed an antibacterial effect of the materials inhibiting the growth of *Streptococcus mutans* in vitro(Kim, 2011). Ursolic acid (Fig. 1) is one of the triterpenoid compound which exists widely in natural plants having anticancer, anti−wrinkle, and antibacterial effects(Fontanay et al., 2008; Liu, 1995). It has an advantage having little toxicity on the normal cells(Liu, 1995) compared with other materials incorporated to composite resin for antibacterial effect. Moreover, due to its hydrophobic nature, it can be dissolved in the composite resin matrix. As the effect of ursolic acid−containing composite on inhibition of the growth of *S. mutans* was proved(Kim, 2011), it needs to be proved that this material actually have a potential of inhibiting dental caries formation by *S. mutans*. Quality evaluation about the anticariogenic effect of ursolic acid−containing composite is required before clinical trials or applications.

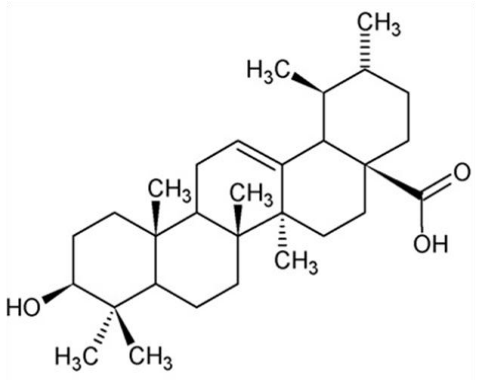


Fig. 1. Structure of ursolic acid [3β−hydroxy−urs−12−en−28−oic acid; C30H48O3; molecular weight 456.71].

Various methods are used to induce artificial caries to the tooth structure. To investigate the effect of antimicrobial material, artificial caries model induced by bacteria should be used. Guggenheim et al used the artificial caries model using *S. mutans* biofilm(Guggenheim et al., 2004) to study primary and secondary caries with regard to the antimicrobial properties of restorative materials. This model is a reproducible and reliable method used to achieve demineralization of enamel and investigate the performance of antimicrobials.

Quantitative light-induced fluorescence(QLF) is a nondestructive diagnostic method for quantifying severity of caries lesions. It uses the natural fluorescence of teeth to discriminate between caries and sound enamel(Van Der Veen and de Jong, 2000). QLF has been successfully applied in a number of studies testing products designed to inhibit demineralization and promote remineralization of enamel(Al-Khateeb et al., 1998; al-Khateeb, Oliveby, et al., 1997; al-Khateeb, ten Cate, Angmar-Mansson, et al., 1997). This method was also applied in this study to evaluate the degree of caries-like lesion formation around experimental composite resin. When a tooth becomes carious, the fluorescence radiance at the location of the caries lesion decreases. The amount of fluorescence radiance loss is related to the mineral loss in the lesion(Hafstrom-Bjorkman et al., 1992).

QLF has some advantages over traditional methods quantitatively assessing the degree of demineralization such as PLM(polarized light microscopy), LMR(longitudinal microradiography). Most traditional techniques are destructive and consequently permit single measurements only, as the tooth substrate itself is altered during the procedure(Ten Bosch and Angmar-Månsson, 1991). On the

other hand, QLF is simple, nondestructive and sensitive method. Moreover, it showed higher sensitivity and a lower discrimination threshold than conventional methods(Ando et al., 1997; Hafstrom-Bjorkman et al., 1992; Ten Bosch and Angmar-Månsson, 1991).

The aim of this in vitro study was to evaluate the effect of ursolic acid-containing composite resins on inhibition of artificial caries around the restorations formed by *S. mutans* biofilm.

# II. Materials and Methods

## 1. Preparation of experimental teeth

Twelve extracted human molars, free of caries and structural defects, were selected for the study. Immediately following extraction, the teeth were placed in saline and the teeth were not allowed to be dried during any stage of the experiment. After the roots of each teeth were cut off, the crown portions were cut in halves mesiodistally and the pulp tissues were removed. Rectangular cavities were prepared in the middle third of the buccal and lingual surfaces of all specimens. The approximate dimensions of the formed cavities were 3.5 ± 0.5 mm width, 2 ± 0.5 mm height and 2 ± 0.5 mm depth. In total, there were 24 specimens from 12 teeth. Following preparation, the specimens were soaked in 70% ethyl alcohol for 24 hours(Dummer et al., 1982) and ultrasonicated for 30seconds with chlorhexidine, washed with distilled water and restored with four groups of composite resin (n=6).

Three different concentrations (0.1, 0.2, 0.5 wt%) of ursolic acid (U6753, Sigma Aldrich, St. Louis, MO, U.S.A.) were added to Filtek Z350 A2 shade (3M ESPE, St Paul, MN, U.S.A.) using acetone as a dissolvent. Filtek Z350 only dissolved with acetone without ursolic acid was used as a control group. These experimental composite resins were manufactured according to the method described by Kim(Kim, 2011).

All cavities were cleaned with water and dried with air. Cavity margins were etched with 37% phosphoric acid gel for 15 seconds, taking care to limit the

etching to the prepared enamel walls. The cavities were then washed for 30 seconds and dried thoroughly. Clearfil SE bond (Kuraray Medical, Inc., Tokyo, Japan) was applied on the cavities of the specimens. According to the manufacturer's instructions, the primer agent was applied to the cavity for 20 seconds and gently air-dried. The bonding agent was then applied, gently air-dried, and light-cured for 10 seconds.

Afterwards, the specimens were randomly divided into four groups (n=6) and restored with composite resins described above. Resin composite material was inserted in one increment and light-cured for 40 seconds. The excess composite overlying the unprepared enamel surface was removed and polished.

## 2. Lesion production

Each tooth surface was coated with acid-resistant nail varnish except for the restoration and 1~2mm peripheral zone around the restoration. Before starting lesion production, the specimens were ultrasonicated with chlorhexidine for 30 seconds and washed with distilled water.

*S. mutans* biofilm induced artificial caries model was used to create carious-like lesions around composite restorations of the experimental teeth(Guggenheim et al., 2001; Guggenheim et al., 2004). The specimens were incubated in vitro with the cariogenic bacteria *S. mutans* in a mixture of saliva and medium containing 1% carbohydrate for 5 days. The microorganism used for this study was *Streptococcus mutans* ATCC 25175.

Prior to start the artificial caries model, medium and artificial saliva for the

incubation were prepared. 37g of BHI and 10g of sucrose were dissolved into the distilled water to manufacture one liter of culture medium of which carbohydrate concentration to be 1%. The composition of manufactured artificial saliva was as follows. One liter of distilled water contained 2.2g Gastric musin, 0.381g NaCl, 0.213g $CaCl_2$, 0.738g $KH_2PO_4$, 1.114g KCl and was adjusted to a pH of 6.8(Hae-Sun Kim, 2011). Manufactured media and saliva were sterilized by autoclaving and kept in cold storage.

The experiment, including the preparatory phase, lasted from Monday to Friday. The summarized diagram indicating the experimental steps are shown in Figure 2.

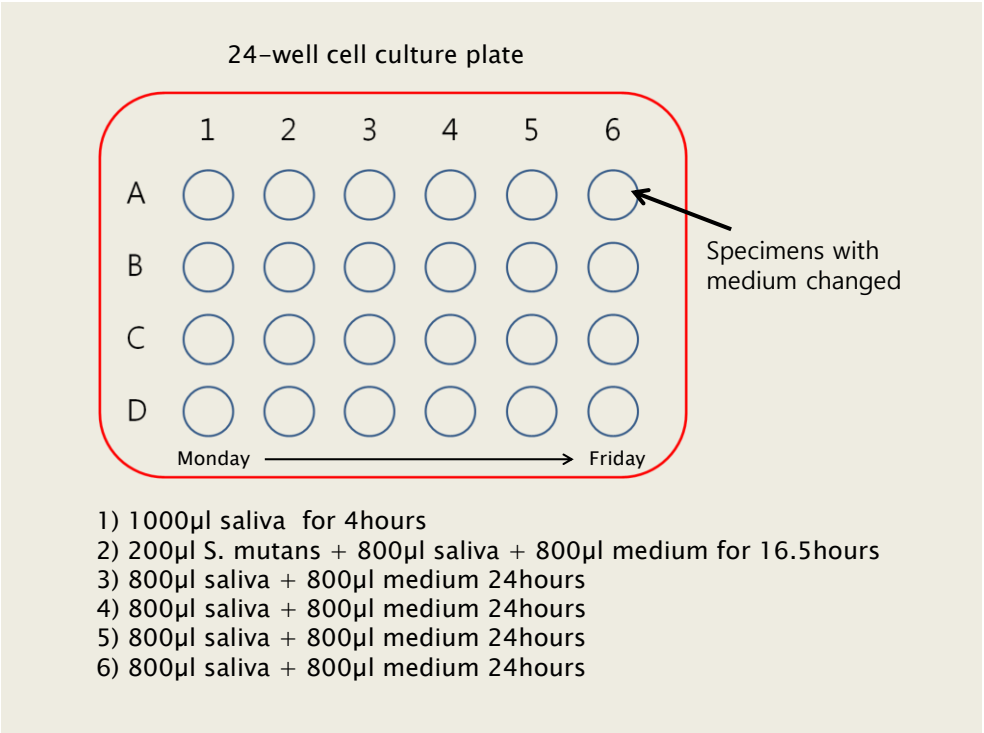


Fig. 2. Schematic presentation of experimental procedures used for biofilm induced artificial caries model.

24-well cell culture plates were used for incubating the specimens. For 4 hours, the specimens were preconditioned with 1000μL of artificial saliva at 37℃ and then they were transferred to new wells containing a fresh mixture of saliva (800μL) + medium (800μL). At this time, the wells were also inoculated with the pooled *S. mutans* species (200μL) and incubated in $CO_2$ chamber at 37°C for 16.5 hours. After 16.5 hours, specimens were transferred to new wells containing a fresh mixture of saliva (800μL) + medium (800μL) and again incubated in CO2 chamber at 37℃ for 24hours. This process was repeated a further three times, renewing saliva and medium at 24-hour intervals, so that by the end of the experimental period the teeth had been subjected to four days of challenge. Following replacement, plates were returned to the $CO_2$ incubator.

Above procedure were conducted twice for each 12 specimens to confirm the reproducibility of the model.

## 3. Lesion examination and measurement

All the specimens were examined and recorded by quantitative light-induced fluorescence (QLF) before and after the lesion formation. All the specimens were previously confirmed that are free of any subclinical lesions or defects using QLF examination. During the intervals for examination, the specimens were individually mounted on wet sponge zig that enabled the specimens to be stored in a humidified environment preventing air-drying.

At the end of the experiment for artificial caries formation, the teeth were removed from the well plates and cleaned of adherent microbial deposit.

Macroscopic examination of the exposed enamel border around each restoration revealed a white, opaque region resembling that observed in early natural white spot caries lesions. For QLF analysis, the demineralized specimens were photographed with QLF-D Biluminator™ 2 (Inspektor Research Systems bv, Amsterdam, Netherlands) (Fig. 3) on a black background. The distance between the tooth surface and camera sensor was fixed equally for all specimens.

A PC program image analysis software QA2 (Inspektor Research Systems bv, Amsterdam, Netherlands) was used for display, storage, and subsequent analysis of images. To quantify the fluorescence losses of white spot lesions 1mm around the restorations, the area of interest was defined as 1mm outside the restorations excluding composite restorations(Van Der Veen and de Jong, 2000). The fluorescence of sound tissue at the lesion site is reconstructed from the radiances of sound tissue bordering the lesion and the decrease in fluorescence is determined by calculating the percentage difference between actual and reconstructed fluorescence surface.

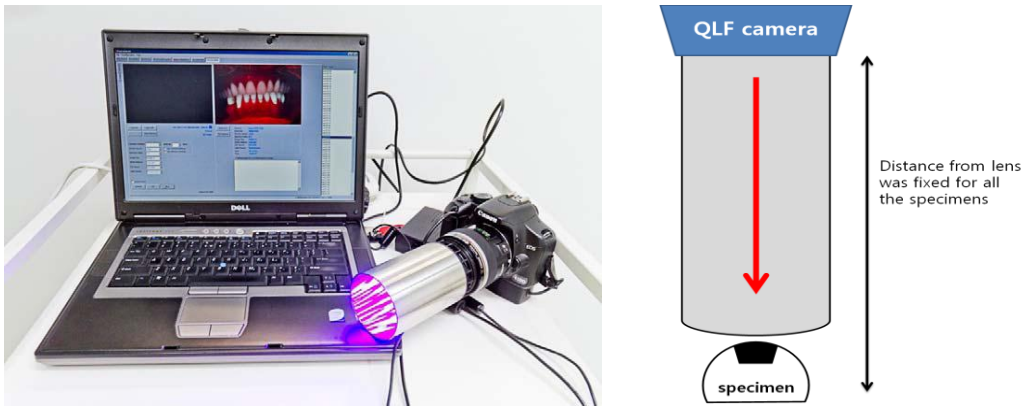


Fig. 3. QLF-D Biluminator™ 2.

## 4. Statistical analysis

One-way ANOVA followed by the Bonferroni correction were used to statistically analyze the data. The adjusted P value less than 0.05 was considered to be statistically significant.

# III. Result

After 5 days of incubation, all the specimens showed the production of white, opaque regions on enamel around the restorations resembling natural white spot lesions. Figure 4a shows a typical lesion produced after 5 days process of artificial caries model. A representative QLF image of the specimen after demineralization is shown in Figure 4B.

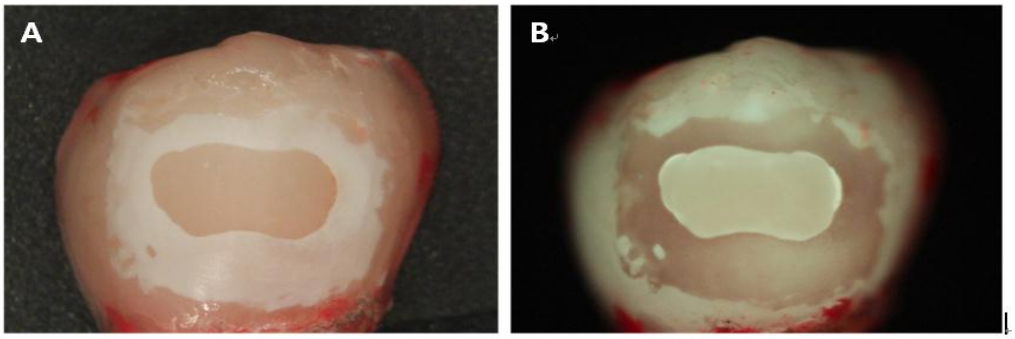


Fig. 4. Formation of early caries lesion.

(A) normal white light image. (B) QLF image.

The mean fluorescence loss of the specimens are summarized in Figure 5 and 6. QLF measures fluorescence radiance loss (−%) and lesion size (pixel) as well as total fluorescence loss (−%·Px) to describe lesion severity. ΔF(−%) represents the mean loss of fluorescence radiance. ΔQ(−%·Px) represents total fluorescence loss integrated over the lesion area and calculated as multiplication of the lesion area (pixel) and the mean change in fluorescence radiance (−%). In this study, there is one thing that should be considered in analyzing ΔQ result. After the area of interest was defined on software program as 1mm outside the

restorations, the lesion area (pixel) was determined as demineralized area inside the area of interest. However, the areas of interest are not consistent between the specimens because there are small differences between cavity sizes and window sizes of the specimens. To solve this problem, determined area of interest was measured in all the specimens, and the differences between them were calculated as relative ratio. The smallest value of specimen A in 0.5% group was set as a standard, and the ratio compared with the standard was shown in Table 1 as relative ratio. The calculated ΔQ values considering the relative ratio of area between all the specimens are also shown.

Table 1. Relative ΔQ after the differences of area were considered

| Group | Specimen | ΔQ (original value) | Relative ratio of area | Relative ΔQ | Mean | SD |
|---|---|---|---|---|---|---|
| Control | A | 186391 | 1.316 | 141585.5 | 176249.6[a] | 38081.49 |
| | B | 296120 | 1.296 | 228565.9 | | |
| | C | 211574 | 1.373 | 154138.1 | | |
| | D | 177523 | 1.202 | 147729.7 | | |
| | E | 282697 | 1.286 | 219815.9 | | |
| | F | 195971 | 1.183 | 165662.8 | | |
| 0.1% | A | 201926 | 1.199 | 168434.5 | 189330.1[b] | 41378.76 |
| | B | 201129 | 1.371 | 146648.3 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | C | 201118 | 1.234 | 163035.5 | | |
| | D | 231048 | 1.085 | 212989.9 | | |
| | E | 297172 | 1.143 | 260083.4 | | |
| | F | 266292 | 1.441 | 184789 | | |
| 0.2% | A | 29157 | 1.549 | 18819.29 | 123492.2 | 86569.01 |
| | B | 41351 | 1.205 | 34328.28 | | |
| | C | 317434 | 1.386 | 229103.5 | | |
| | D | 220737 | 1.180 | 187026 | | |
| | E | 126470 | 1.308 | 96663.99 | | |
| | F | 219596 | 1.255 | 175012.2 | | |
| 0.5% | A | 33093 | 1 | 33093 | 75796.97[a b] | 41167.38 |
| | B | 74366 | 1.112 | 66855.35 | | |
| | C | 156040 | 1.283 | 121574.6 | | |
| | D | 62983 | 1.231 | 51163.57 | | |
| | E | 199567 | 1.509 | 132294.6 | | |
| | F | 61302 | 1.231 | 49800.69 | | |

Relative ΔQ = ΔQ(original value) / relative ratio of area

a, b indicate significant differences in relative ΔQ ($p<0.05$).

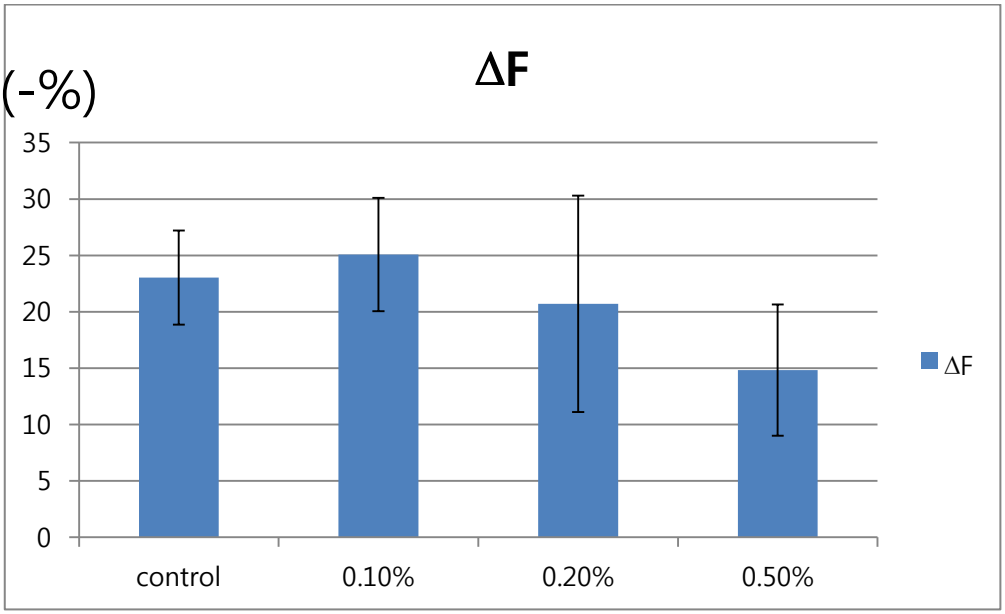


Fig. 5. Mean fluorescence loss (−%).

No significant differences between groups.

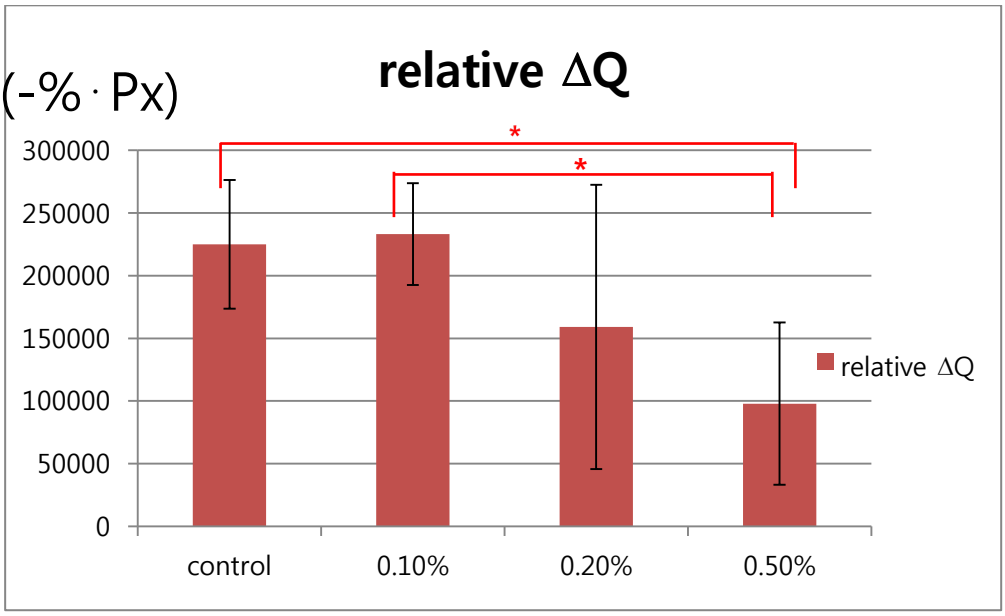


Fig 6. The total fluorescence loss over the selected lesion area (−%·Px).

Significant differences between groups at the 95% level are indicated by *.

As shown in figure 5 and 6, mean fluorescence loss(ΔF) of 0.5% ursolic acid group was less than control group, but there was no significant difference among groups. However, the total fluorescence loss(ΔQ) was significantly less in 0.5% ursolic acid group compared with control group and 0.1% ursolic acid group ($p<0.05$). Based on ΔQ results, the degree of demineralization was decreased around 0.5% ursolic acid-containing composite groups compared with control group. It can be thought that composite resin containing 0.5% ursolic acid may inhibit the development of experimental secondary caries lesions induced by *S. mutans* biofilm.

Figure 7 shows QLF images after loss of fluorescence area was analyzed with Image Analysis Software QA2 program. After the area of interest was defined (Fig 7A, C), the demineralized area was displayed as shades of gray corresponding to fluorescence loss (Fig. 7B, D). The red shift of the fluorescence spectrum is another indicator of a caries lesion(Sundstrom et al., 1985) and presents more pronounced loss of fluorescence than gray shift (Fig. 7B). The difference between control group and 0.5% ursolic acid group is clearly visible in Figure 7B and D.

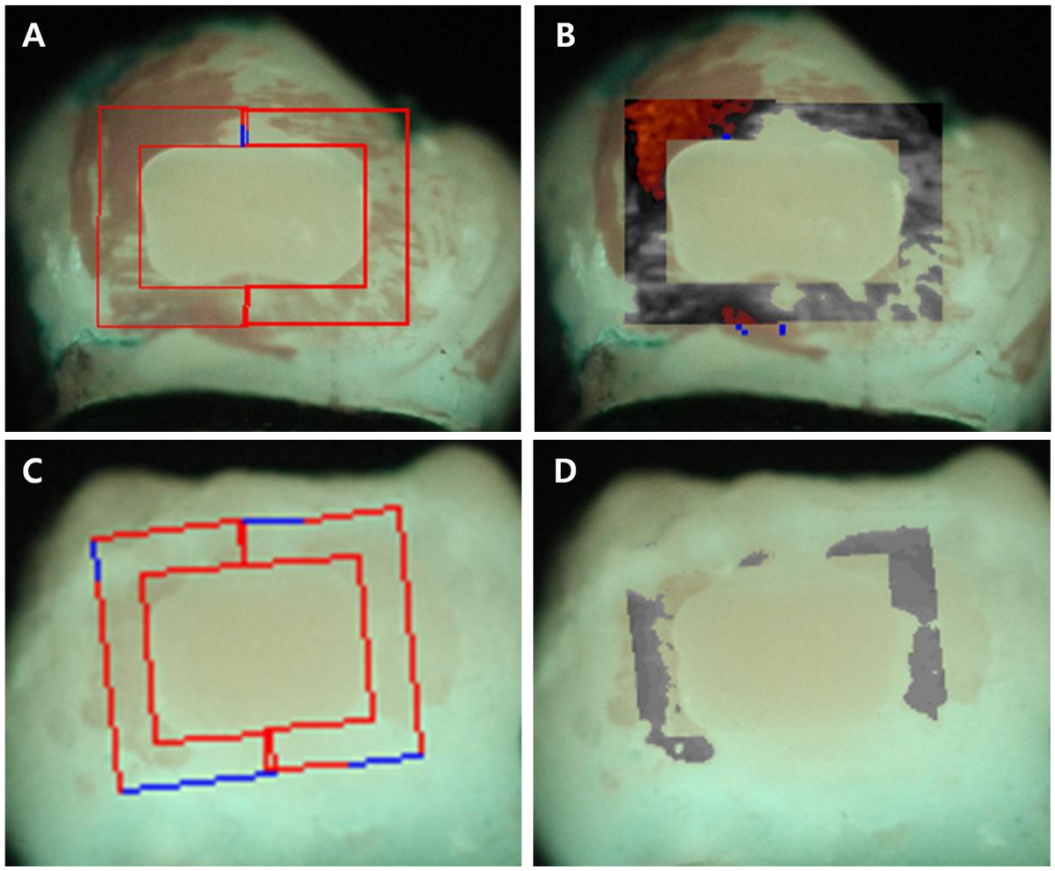


Fig. 7. Representative QLF images after the loss of fluorescence was analyzed.

(A) control. (The area of interest was determined.)

(B) control (Fluorescence loss was analyzed and displayed as gray and red shades. Red shift presents more pronounced loss of fluorescence.)

(C) 0.5% ursolic acid-containing composite resin. (The area of interest was determined.)

(D) 0.5% ursolic acid-containing composite resin. (Less fluorescence loss is clearly visible compared with control group.)

# IV. Discussion

Antimicrobial activity of ursolic acid against *S. mutans*(M. J. Kim et al., 2011) and its effect incorporated in composite resin were previously demonstrated(S. Kim, 2011). However, this is the first study to prove that ursolic acid, when incorporated in composite resin, have a possibility of preventing the recurrent caries around the restorations. For this purpose, artificial caries lesions were induced on teeth specimens by *S. mutans* biofilm.

Two basic methods exist at present for providing an artificial cariogenic challenge to the tooth structure: purely chemical systems(Silverstone et al., 1988), which use an acidic environment to demineralize the tooth, and bacterial systems, in which a specific bacterial culture causes demineralization. Although in vitro chemical caries systems allow strict control of the experimental environment and are relatively simple and cheap, they do not simulate the in vivo situation as accurately as a bacterial system. Bacterial systems where the mixed natural flora are controlled by in vitro environmental and nutrient conditions provide a means for studying complex microbial ecosystems such as dental plaque and its effect on the development of dental caries. Antimicrobial properties of the restorations have been successfully investigated by bacterial artificial caries system and cannot be evaluated by chemical caries system. Therefore, in this study, the biofilm model developed in Zurich was used to product artificial caries lesions(Guggenheim et al., 2004).

Zurich biofilm model is a reliable tool that has been applied to various studies such as prediction of the in vivo efficacy of antimicrobials, and de- and

remineralizaton of enamel exposed to biofilms in vitro(Guggenheim et al., 2001; Guggenheim et al., 2004). White spot lesions on enamel adjacent to restorations were successfully developed in all the specimens in as few as 5 days. The experiments were repeated by two stages for each twelve specimens and the reproducible results were obtained.

For decades, as the method evaluating the demineralization of teeth on which artificial caries were formed, transverse microradiography (TMR) and polarized light microscopy (PLM) have been used as standardized methods. However, these methods require destructive preparation of specimens such as sectioning as thin as 100㎛ and the measurement gets difficult and time consuming(Ten Bosch and Angmar-Månsson, 1991). Quantitative, noninvasive assessments of demineralization in various caries studies were successfully performed using quantitative light-induced fluorescence (QLF)(Al-Khateeb, Ten Cate, Angmar-Månsson, et al., 1997; Ando et al., 1997; de Josselin de Jong et al., 1995; Hafstrom-Bjorkman et al., 1992).

QLF is a diagnostic technique that uses the natural fluorescence of teeth to discriminate between caries and sound enamel and to give a measure of lesion severity(Van Der Veen and de Jong, 2000). The fluorescence radiance of a carious lesion is lower than that of sound enamel. The dark appearance of a white spot lesion viewed with the fluorescence technique can be explained by the changes in scattering properties occurring in a lesion(Van Der Veen and de Jong, 2000). The fluorescence image of enamel with white spot lesions is digitized and the amount of fluorescence radiance loss is related to the mineral loss in the lesion(Hafstrom-Bjorkman et al., 1992). The fluorescence loss in the

lesion is quantified in comparison to the fluorescence radiance level of sound enamel in same specimen. Thus, biological variations between different teeth were compensated for contrary to conventional methods. Using QLF, carious lesions with a lesion depth as small as 5-10 $\mu$m can be detected and measured(de Josselin de Jong et al., 1995). Moreover, the laser fluorescence method has a lower discrimination threshold as compared with traditional methods(Hafstrom-Bjorkman et al., 1992; Ten Bosch and Angmar-Månsson, 1991).

Depending on the QLF analysis in this study, the mineral density of enamel adjacent to restorations was decreased after formation of artificial caries induced by *S. mutans* biofilm. The demineralization of specimen is quantified as ΔF and ΔQ value presenting the loss of fluorescence. Loss of mineral density should be evaluated and quantified by not only mean fluorescence loss but also the lesion area. Hence, the best representation of lesion is given by ΔQ(Van Der Veen and de Jong, 2000) and is comparable to the amount of mineral loss measured with longitudinal microradiography(Hafstrom-Bjorkman et al., 1992). ΔQ was significantly less in 0.5% ursolic acid group compared with control group and 0.1% ursolic acid group. The results showed that, when compared with conventional composite resin, composite resin containing 0.5% ursolic acid can have an inhibiting effect of the development of artificial caries lesions in vitro. The mechanism how ursolic acid inhibits the bacterial growth of *S. mutans* is not fully understood yet. It has been proved that ursolic acid can suppress dental plaque formation by inhibiting the glucosyltransferase activity of *S. mutans*(Kozai et al., 1987).

The result of the previous study of Kim showed that the CFU value was significantly decreased at 0.5% ursolic acid group compared with control group when sucrose was offered as the carbohydrate source(S. Kim, 2011). In this study, there was a statistically significant difference in secondary caries formation between 0.5% ursolic acid group and control group. 0.5% concentration of ursolic acid may be adequate to be incorporated into composite resin for presenting anticariogenic efficacy clinically.

The development of secondary caries around a restoration is likely to be influenced by various factors including not only antibacterial ability of restorative materials but also the physical properties of the restorative and adhesive materials, the quality of the hybrid layer and so on(Arnold et al., 2007). When the organic molecules are added to composite, the physical properties are expected to be lowered. Further study is required about the detrimental effects of ursolic acid in physical property of composite resin. The flexural strength of ursolic acid-containing composite resin was previously investigated and there were no significant differences in the values compared with the control(Kim, 2011). Another physical properties such as bonding properties, wear resistance, etc. are required to be investigated.

There are several limitations in present study. Artificial caries were induced for 5 days in this study. However, long term ability of ursolic acid-containing composite resin needs to be evaluated in further study. Although this model used bacterial biofilm to produce carious lesions in well plates, it cannot be an optimal simulation of the oral environment. For instance, this experiment was carried only with a single bacterium *S. mutans* not considering the microbial

interactions between oral bacteria. Attempts are required to mimic the diverse conditions present in the oral cavity which may affect dental caries development. This study evaluated the degree of outer lesion formation in the enamel around the restorations of composite resin containing ursolic acid. However, the secondary caries lesion consists of outer lesion as well as wall lesion, which is a narrower defect in the enamel or dentin along the cavity wall–restoration interface. Further investigation about the wall lesion around the restoration will allow the direct effect of ursolic acid–containing composite on inhibiting secondary caries to be understood.

# V. Conclusion

Within the limitation of this experimental study, it can be concluded that composite resin containing 0.5% ursolic acid might inhibit the caries induced by *S. mutans* biofilm around the restoration.

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## 국문 요약

# ursolic acid가 포함된 복합레진 수복물 주변의 우식 억제 효과

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원유경

1. 서론

복합레진 수복 실패의 주된 원인은 수복물 주변의 이차 우식 형성이다. 수복물 주변 이차 우식 형성을 예방하기 위한 하나의 방법으로 세균 피막 형성을 억제하는 항균성 수복물질을 사용할 수 있다.

이 연구의 목적은 천연항균물질인 ursolic acid가 첨가된 복합레진이 수복물 주변의 인공 우식 형성을 억제하는 효과가 있는지 알아보는 것이다.

2. 본론

4가지 농도의 ursolic acid (0, 0.1, 0.2, 0.5 wt%)를 Filtek Z350 (3M ESPE, St Paul, MN, U.S.A.)에 첨가하였다. 12개의 인간 발거 대구치의 협설면에 표준와동

(2mm x 3mm x 2mm)을 형성하고 항균 물질을 함유한 각 군의 실험레진으로 수복하였다. *S. mutans*에 의한 인공우식 모델을 이용하여 실험시편상에 이차우식 병소를 유발하였다. 각 실험 시편을 인공타액과 1% 농도의 당을 함유한 배지의 혼합물에서 5일간 배양한 결과 모든 시편에서 백색의 초기우식 병소가 형성되었다. quantitative light-induced fluorescence(QLF)를 이용하여 수복물 변연 주변의 법랑질 탈회를 정량적으로 분석하였다. 탈회 시편을 QLF-D Biluminator™ 2 (Inspektor Research Systems bv, Amsterdam, Netherlands)로 촬영하고, image analysis software QA2 (Inspektor Research Systems bv, Amsterdam, Netherlands)를 이용하여 촬영된 상의 형광 소실 정도를 비교하였다.

QLF 분석 결과, 0.5% 농도의 ursolic acid를 함유한 복합레진군에서 수복물 주변 법랑질의 형광 소실 정도가 0.1% 농도의 ursolic acid를 함유한 복합레진 군 및 대조군에 비하여 유의차 있게 적었다 ($p<0.05$).

3. 결론

본 실험연구의 결과에 의하면, 0.5% ursolic acid 가 첨가된 복합레진 수복물 주위로 *S. mutans* 에 의해 유발되는 우식 형성의 억제 효과를 기대할 수 있다.

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Key words : ursolic acid, composite resin, artificial caries