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Relationship between fluoride release rate and anti-cariogenic biofilm activity of glass ionomer cements

Ngoc Phuong Thanh Chau[a,b], Santosh Pandit[a], Jian-Na Cai[a], Min-Ho Lee[c,**], Jae-Gyu Jeon[a,*]

[a] Department of Preventive Dentistry, School of Dentistry, Institute of Oral Bioscience and BK 21 Plus Program, Chonbuk National University, Jeonju 561-756, Republic of Korea

[b] Department of Odonto-Stomatology, Hue University of Medicine and Pharmacy, Hue University, 06 Ngo Quyen street, Hue City, Viet Nam

[c] Department of Dental Biomaterials, School of Dentistry, Chonbuk National University, Jeonju, Republic of Korea

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ABSTRACT

Objectives. The aim of this study was to evaluate acidogenicity and composition of *Streptococcus mutans* biofilms on glass ionomer cements (GICs) and then to determine the relationship between the anti-*S. mutans* biofilm activity and fluoride release rate of the GICs.

Methods. *S. mutans* biofilms were formed on discs prepared using five commercial GICs. Acid production and fluoride release rates of the biofilms on the GIC discs during biofilm formation (0–94 h) were determined. Next, 94-h-old *S. mutans* biofilms on GIC discs were analyzed to evaluate the biofilm composition (dry weight, bacterial cell number, and extra-cellular polysaccharide (EPS) amount) using microbiological, biochemical, and confocal laser scanning microscopy (CLSM) methods. Lastly, relationships between the fluoride release rate and changes in acidogenicity and composition of the biofilms were determined using a linear-fitting procedure.

Results. All of the tested GICs released fluoride ions. Of the GICs, the two that showed the highest fluoride release rates strongly affected acidogenicity, dry weight, and EPS formation of the biofilms. Furthermore, they reduced the bacterial and EPS bio-volumes and EPS thickness. However, the number of colony forming units (CFUs) of the biofilms was higher than that of the control. Generally, changes in the acidogenicity and composition (except for CFU count) of the biofilms on the GICs followed a negative linear-pattern of fluoride release rate-dependence ($R = -0.850$ to -0.995, $R^2 = 0.723$–0.990).

Significance. These results suggest that the anti-cariogenic biofilm activity of GICs is closely correlated with their fluoride release rate during biofilm formation.

* *Corresponding author*. Tel.: +82 63 270 4036.
** *Corresponding author*.
E-mail addresses: lmh@jbnu.ac.kr (M.-H. Lee), dentjjk@jbnu.ac.kr (J.-G. Jeon).

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1. Introduction

It has been well documented that biofilm formation occurs on the surfaces of different restorative materials within a short time after placement in the oral cavity [1,2]. The biofilms on the microgaps between restorative materials and tooth tissues can lead to secondary caries, which is responsible for half of all restorations fail within 10 years [3]. Similar to the dental caries process, the biofilms on these microgaps cause dissolution of the adjacent tooth surfaces and reduce the longevity of the restorative materials [4].

Although several studies have shown that the level of mutans streptococci is not necessarily related to the development of dental caries [5,6], many other studies have revealed *Streptococcus mutans* as one of the main causative pathogens for dental caries [7,8]. *S. mutans* can adhere to and accumulate on the tooth surface using extra-cellular polysaccharides (EPSs) produced by glucosyltransferases (GTFs) from sucrose [9]. Furthermore, this bacterium can metabolize dietary sugars to organic acids and withstand rapid and substantial fluctuations in the environmental pH [10]. The combination of these virulence properties allows *S. mutans* to effectively colonize the tooth surface and be sustained in the oral cavity.

To prevent secondary caries, fluoride-releasing restorative materials such as glass ionomer cements (GICs) and resin composites have been developed. Recently, several researchers have shown that nanocomposites containing calcium phosphate nanoparticles may reduce secondary caries [11]. Of the fluoride-releasing restorative materials, GICs have been used in various areas of restorative dentistry for several decades because of their biocompatibility and cariostatic properties [12,13]. Furthermore, GICs can reduce demineralization in adjacent hard tooth tissues [14,15]. Several studies have shown that the effectiveness of GICs may be related to their ability to release fluoride [13,16,17].

Biofilms on GICs can degrade material properties and roughen their surfaces, which in turn promote further biofilm formation, as well as material surface deterioration [18,19]. Recently, several studies have investigated bacterial adherence to and biofilm formation on GICs and have shown that these materials can affect bacterial adherence, acidigenicity, and biofilm formation [13,20]. However, although it is important to obtain information about whether GICs can affect composition of biofilms on GICs, little has been reported. Furthermore, there has been no study on the precise relationship between fluoride release level from GICs and changes in virulence and composition of biofilms on GICs.

Considering the advantages and widespread use of GICs in restorative dentistry, it would be worthwhile to test the hypotheses that fluoride level released from GICs can change virulence and composition of cariogenic biofilms. Therefore, the aim of this study was to investigate the changes in virulence, especially acidogenicity, and composition of cariogenic biofilms formed on GICs and then to determine the relationships between fluoride release level from GICs and changes in acidogenicity and composition of cariogenic biofilms using an *S. mutans* biofilm model.

2. Materials and methods

2.1. GIC disk preparation

Table 1 lists the materials used in this study. Five GICs were selected: Glaslonomer FX-II (Gla), Ketac Fil Plus Aplicap (Keta), Riva self-cure HV (Riva), GC Fuji Filling LC (GC), and GC Fuji II LC (GC2). The GICs used in this study were all commercially available, and the shade of all materials was A2 or A3. These GICs were used to prepare disk-shaped specimens according to the manufacturer's recommendations.

Disk-shaped specimens (12 mm in diameter and 1.2 mm in thickness) were prepared using polytetrafluoroethylene (Teflon) molds with a metal holder and glass slides to cover each face. Gla, Keta, and Riva specimens were self-cured. GC and GC2 specimens were light-cured for 20 s on each face using a light curing unit (G-Light, GC Corp., Japan). After curing, all specimens were polished sequentially with # 800 to # 1200 sand papers. Then the specimens were placed in a desiccator at room temperature. Hydroxyapatite discs (12 mm in diameter and 1.2 mm in thickness; Clarkson Chromatography Products, Inc., South Williamsport, PA, USA) were included as a control in this study.

2.2. Biofilm formation on GIC discs

The microorganism used for this study was *S. mutans* UA159 (serotype c). *S. mutans* biofilms were formed on saliva-coated GIC or hydroxyapatite (HA) discs placed in a vertical position in 24-well plates, as detailed elsewhere [21]. Briefly, the saliva-coated discs were generated by incubation with filter-sterilized (0.22 μm low protein-binding filter) human whole saliva for 1 h at 37 °C. For biofilm formation, the saliva-coated discs were transferred to a 24-well plate containing 1% sucrose (v/v) ultrafiltered (10 kDa molecular-weight cut-off) tryptone yeast-extract (UTE) broth with *S. mutans* UA159 ($2–5 \times 10^6$ colony forming units (CFUs)/ml). The biofilms were grown undisturbed for 22 h to allow initial biofilm growth. From this time point (22 h), the culture medium was changed twice daily (9 AM, 6 PM) until it was 94 h old. The culture medium was changed a total of six times. The pH value and fluoride concentration in the old culture medium were determined during the experimental period (until biofilms reached 94 h of age). The 94-h-old biofilms were used to analyze the biofilm composition. Each assay was performed in duplicate in at least six different experiments ($n = 12$).

2.3. Determination of fluoride concentration and pH value during biofilm formation

To evaluate fluoride release from GICs, the concentration of fluoride in the old culture medium was determined during the experimental period (22, 31, 46, 55, 70, 79, and 94 h). For the determination of fluoride concentration, a total of 2.8 ml of each medium was mixed with 280 μl of total ionic strength adjustment buffer (TISAB III). The fluorometer (Thermo Fisher Scientific, Orion, MA, USA) was calibrated using four standard solutions (0.1, 1, 10, and 100 ppm F^-). To evaluate the acidogenicity of *S. mutans* biofilms on GICs, the pH values of the old