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Anticaries effect of compounds extracted from *Galla Chinensis* in a multispecies biofilm model

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Introduction: *Galla Chinensis* is a leaf gall known to have some antibacterial effects. Using an *in vitro* biofilm model of dental plaque, the present study aimed to evaluate the anticaries effects of *Galla Chinensis* and its chemical fractions.
Methods: A four-organism bacterial consortium (*Streptococcus sanguis*, *Streptococcus mutans*, *Actinomyces naeslundii*, *Lactobacillus rhamnosus*) was grown on hydroxyapatite (HA) discs, bovine enamel blocks, and glass surfaces in a continuous culture system and exposed to repeated solution pulses. *Galla Chinensis* extracts, sucrose solutions, and sodium fluoride solutions were pulsed into different flow cells. The pH value of the planktonic phase in each flow cell was recorded and the bacteria colonizing the biofilm on the HA discs were counted. Enamel blocks were observed using a polarized microscope and lesion depth was evaluated. The biofilm morphology was examined with a fluorescence microscope and the images captured were analyzed on an image analysis system.
Results: When *Galla Chinensis* extract, its chemical fraction, or fluoride was added to the sucrose solution, the planktonic phase pH remained higher than that in the sucrose alone. A lower level of colonization on the HA surface was also observed in the groups to which *Galla Chinensis* and fluoride were added compared with the control sucrose group, and this was reflected in both the total viable count and the biofilm imaging, which showed fewer cariogenic bacteria and a less compact biofilm, respectively. Enamel demineralization in both the fluoride group and the *Galla Chinensis* group was significantly less than that in the sucrose group.
Conclusions: *Galla Chinensis* and fluoride may inhibit the cariogenicity of the oral biofilm. *Galla Chinensis* appears to be a promising source of new agents that may prevent dental caries.

Key words: biofilm model; cariogenic bacteria; enamel demineralization; *Galla Chinensis;* oral biofilm

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Introduction

Dental plaque is defined as the diverse microbial community found on the tooth surface embedded in a matrix of polymers of bacterial and host origin (14, 16). Cariogenic bacteria can colonize and adhere to tooth surfaces and initiate plaque formation. Some cariogenic flora in dental plaque can metabolize carbohydrates to form tenacious biofilms, lower pH levels, and increase the possibility of enamel demineralization, leading to the formation of dental caries. Therefore the inhibition of the adherence of cariogenic bacteria on the tooth surface and the following decrease of pH is an important strategy in the prevention of dental caries.

Over the past two decades many studies on the anticariogenic effect of polyphenols extracted from different types of plants have been reported (19, 22, 25). In particular, the anticaries effects of tea polyphenols have been confirmed by many Japanese investigators (21). Plant polyphenols have been proven to have many physiological activities in humans, such as stimulation of phagocytic cells, host-mediated tumor activity, and a wide range of anti-infective actions (9). Direct inactivation of microorganisms by polyphenols has been reported: low concentrations of tannin modify the morphology of germ

tubes of *Crinipellis perniciosa* (1). Tannins in plants are found to inhibit insect growth and disrupt digestive events in ruminants (2). However, in the plant kingdom, the sources of polyphenols are numerous. Can the polyphenols present in other plants show the same anticariogenicity as the tea polyphenols?

Galla Chinensis, one such natural product, has been widely used in traditional Chinese medicine for thousands of years. *Galla Chinensis* is a leaf gall produced by parasitic aphids; it mainly comprises 60–70% gallotannin, 2–4% gallic acid, and some resin, fat, wax, starch, etc. According to the study by Li et al. (13), the ethyl acetate, ethanol, and water extracts of *Galla Chinensis* showed strong inhibitory activity against species of *Pseudomonas*, *Aeromonas*, *Alealigenes*, *Flavobacterium*, *Vibrio*, *Photobacterium*, *Moraxella*, and *Chromobacterium*. Previous studies proposed that *Galla Chinensis* had anticaries properties through the inhibition of the growth and acid production of certain cariogenic bacteria including *Streptococcus mutans* and *Lactobacillus rhamnosus* (11, 28). Furthermore, *Galla Chinensis* inhibited the adherence of oral bacteria to saliva-coated HA *in vitro* at values below the minimum inhibitory concentration (data not published). Therefore, we hypothesize that *Galla Chinensis* has anticaries properties that are important for dental application. The purpose of the present study was to examine the anticariogenic properties of extracts from *Galla Chinensis* in an oral biofilm model.

Materials and methods

Galla Chinensis extract preparation

Galla Chinensis (1000 g) was purchased from the Traditional Chinese Medicine Corporation of Chengdu (Sichuan, China). It was dried in an oven at 60°C for 3 days, then ground to a fine powder that was added to 600 ml distilled water. The mixture was stirred for 10 h at 65°C and then filtered. The extract was re-extracted with distilled water under the same conditions and then dissolved in 500 ml of ethanol (99.9%). After evaporation of the ethanol, 160 g of yellow porous solid (*Galla Chinensis* extract; GCE) was recovered. The GCE preparation (15 g) was further fractionated by adsorption chromatography on a Sephadex LH-20 column (Amersham Biosiences, Piscataway, NJ). Five grams GCE was dissolved in 10 ml distilled water and applied to the column. The column was eluted with deionized water (1500 ml), 30% ethanol (1500 ml), then 50% acetone (1500 ml), and finally with 100% acetone (1500 ml). Four fractions, GCE-A (deionized water fraction), GCE-B (30% ethanol fraction), GCE-C (50% acetone fraction), and GCE-D (100% acetone fraction) were obtained. According to our previous study, GCE and GCE-B have the strongest effects against bacterial growth and adherence, so they were selected in this study (27). A portion of GCE-B was further purified by successive column chromatography with a Diaion HP-20 column (8 cm × 20 cm) and a Sephadex LH-20 column (3 cm × 120 cm; Pharmacia-LKB Biotechnology, Uppsala, Sweden). The column was eluted with acetone–water (2 : 8, 3 : 7, 4 : 6, volume/volume) and two compounds were obtained, characterized as gallic acid and methyl gallate by spectroscopic methods including mass spectrometry and nuclear magnetic resonance.

Bacterial strains

The microorganisms grown in the continuous culture consortium were: *Streptococcus sanguis* ATCC10556; *S. mutans* ATCC25175; *Actinomyces naeslundii* WVU 627 (dental plaque isolate, WCUMS, Chengdu, China); and *L. rhamnosus* AC 413 (dental plaque isolate).

Primary continuous culture growth conditions

The system was set up as described previously with minor modifications (10). Briefly, overnight cultures of the four strains of bacteria grown in anaerobic brain–heart infusion broth (37°C, 20% CO_2, 80% N_2) were mixed and inoculated into a 250-ml vessel (Fig. 1: 1, the fermenter vessel) at 37°C under a gas phase of 5% (volume/volume) CO_2 in nitrogen, at a dilution rate of 0.1/h. The pH was maintained at 7.0 by the automated addition of 0.5 M NaOH. The biofilm growth medium contained: 2.5 g/l hog gastric mucin Type III (Sigma-Aldrich, St. Louis, MO), 2 g/l proteose peptone (Difco, Lawrence, KS), 1 g/l trypticase peptone (BBL, Shanghai, China), 1 g/l yeast extract (Oxoid, Cambridge, USA), 0.5 g/l glucose, 2.5 g/l KCl, and 0.1 g/l cysteine–HCl (Sigma-Aldrich). The medium was adjusted to pH 7.4 before being autoclaved. After inoculation, the consortium was allowed to establish for at least 3 days before the start of the biofilm experiments. Samples were taken from the vessel every day and cell counts were performed to monitor the growth of the bacteria.

Biofilm substrates

Enamel blocks for lesion formation studies were prepared from sound bovine incisors. The labial enamel surface was coarsely ground using a water-cooled, diamond-sintered abrading wheel (Malvern Instruments, Malvern, UK) and then polished on a glass plate using 1200-grade aluminum slurry (Electro Minerals Co., Manchester, UK). Enamel blocks were cut from this polished surface using a diamond-studded cutting wire (Well model 3242, Le Locle, Switzerland). Four sections, of approxi-

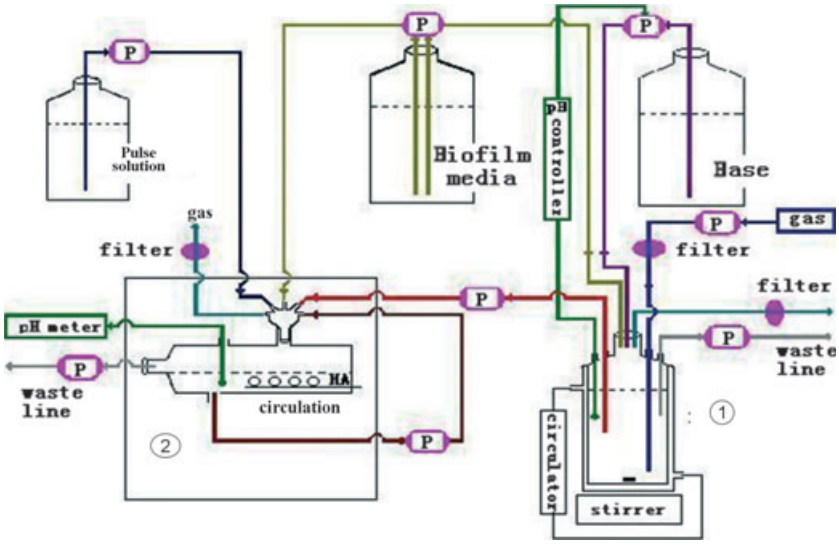

Fig. 1. Schematic diagram of the experiment biofilm system: 1, fermenter vessel; 2, flow cell.

mate area 6 mm × 1.7 mm, were cut from each tooth, the major axes running coronocervically.

The HA for use in biofilm bacteria counting and on glass discs for biofilm image analyses was obtained from the Laboratory for Oral Biomedical Engineering of the Ministry of Education of Sichuan University. Pairs of each type of substrate were mounted in each flow cell (Fig. 1: 2, flow cell) followed by sterilization.

Biofilm growth conditions

The biofilm model was established as reported by Hodgson et al. (10) (Fig. 1). This model is able to form stable and reproducible biofilm in different treatment groups. The model uses the amino acids, peptides, and mucin as the primary carbon source. The sucrose pulse is used to generate the cariogenic response. The production of reproducible biofilms in each group is achieved by using multiple second-stage vessels. After a stable population containing all four organisms had been established, four parallel connected flow cells were inoculated simultaneously with a mixture of planktonic phase from the continuous culture and with fresh medium (in a 1 : 9 ratio). A constant liquid level was maintained in each flow cell by means of a weir system (100 ml working volume). The growth medium was supplemented with 5 mmol/l phosphate buffer, pH 7.5. A total flow rate of 15 ml/h of combined inoculum/fresh medium was used to obtain a dilution rate of 0.75/h.

The contents of the flow cells were recirculated (60 ml/min) to achieve constant mixing. Liquid feeds to the flow cell, recirculation of contents, and removal of waste were controlled by peristaltic pumps. The pH of the planktonic phase was monitored over the course of the experiments using electrodes (E-331; Aurora Sci Co., Shanghai, China); these were sterilized in sodium hypochlorite and washed with sterile distilled water before use. Samples of the planktonic phases in each flow cell were collected every day. They were serially diluted in phosphate-buffered saline and spread on selective and non-selective media to monitor the bacteria in the flow cell as a quality control measure.

Biofilm treatments

To grow cariogenic biofilms on the test substrates, the flow cells were pulsed with 10 ml sucrose solution (25 mmol/l in water, which produced initial concentrations of 2.5 mmol/l in the bulk liquid phase) every 12 h. After 24 h, the initial biofilms were established and three types of treatment were then applied. The flow cell of the positive control group was pulsed with 10 ml 25 mmol/l aqueous sucrose solution containing 228 p.p.m. NaF; this produced an initial concentration in the flow cell of approximately 23 p.p.m. fluoride. The cells of the experimental groups were pulsed with 10 ml 4 mg/ml GCE or GCE-B (to give an initial concentration of 0.4 mg/ml) together with 25 mmol/l sucrose solution. The negative control group was pulsed with 10 ml 25 mmol/l sucrose solution only. For each flow cell, the response of the bacterial population to eight 1-min treatments, pulsed at 12-h intervals, was followed over a period of 5 days.

Enumeration of organisms

After 5 days of treatment, biofilm organisms were dislodged from the HA discs (only biofilm on HA discs were counted) by 20 s of sonication and 30 s of vortex mixing), serially diluted in phosphate-buffered saline, and spread on a range of selective and non-selective media. Total bacterial counts were performed on supplemented brain–heart infusion agar. *S. sanguis* and *S. mutans* were counted on mitis-salivarius and *A. naeslundii* was counted on brain–heart infusion agar containing 2.5 mg/l vancomycin after anaerobic growth for 4–7 days. *L. rhamnosus* was enumerated on Rogosa agar (*Lactobacillus* selective agar). Identifications were based on colony morphology and gram-staining reaction (10).

Enamel analysis

After treatment in the flow cells and swabbing for microbial population analysis, a thin section, approximately 250 μm, was removed from each block. The thin sections were polished to a final measured thickness of about 100 μm and analyzed for enamel demineralization using polarized light microscopy (Ellipse ME600L; Nikon, Tokyo, Japan) after imbibition in deionized water and quinoline. Digital images were taken using NIKON ACT-1 FOR L-1 software (Nikon) and the depths of the lesion in each group were measured with an image analysis system (IMAGE-PRO-PLUS, version 5.0; Media Cybernetics, Silver Spring, MD, USA).

Biofilm imaging

Fluorescence imaging of the biofilm structure on the glass discs was performed using a fluorescence microscope (Nikon, Tokyo, Japan) at 504–511 nm. Samples were stained with 10 mg/l fluorescein (Shanghai SSS Reagent Co. Ltd, Shanghai, China) at room temperature for 1 h, then rinsed with deionized water three times. Images were processed and analyzed (IMAGE-PRO-PLUS, version 5.0). Three images were captured from different areas of each glass disc. An appropriate manual threshold setting was applied to each captured image so that the surrounding background was eliminated from the calculations and, if necessary, incidental artifacts were removed; the areas of interest were then quantified. The area, mean density, and integrated optical density of the biofilm images were calculated where the area indicated the area covered by the biofilm and the optical density indicated the density of the biofilm. The lower the reading was the fewer bacteria there were or the lower the biofilm density was.

Statistical analyses

All experiments were independently repeated a minimum of three times, and the mean ± SD was calculated. Statistical comparisons between biofilm populations on different HA discs, the lesion depth determined by polarized microscopy, and the data collected by fluorescence microscopy were analyzed by one-way analysis of variance, followed by Tukey's test using SPSS 10.0 software (SPSS version 10.0 for Windows; SPSS Inc., Chicago, IL). A value of P less than 0.05 was taken as statistically significant.

Results

The pH response to pulses

The planktonic phases of the flow cells attained a steady state of pH 7.0 during the initial period of biofilm growth. Subsequently, pulsing with sucrose resulted in a pronounced decrease of pH (Fig. 2). The pH recovered over a 6-h period, with about 3 h at minimum pH. In the sucrose group, the lowest pH was about 5.0, while in the GCE, GCE-B, and NaF groups the lowest point was about pH 5.5–6.0, although they contained the same concentrations of sucrose.

Response of biofilm populations to pulses

Responses of the biofilm populations to different treatments are summarized in Table 1. The numbers of each bacterial type were significantly lower in the biofilm treated with NaF than in the biofilm

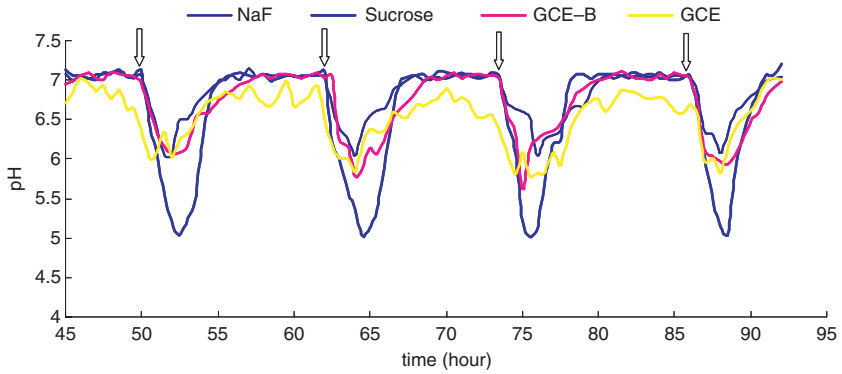

Fig. 2. The pH response in the flow cell against the pulsing. NaF = 228 p.p.m. fluoride as NaF + 25 mM sucrose; GCE = 4 mg/ml *Galla Chinensis* extract + 25 mM sucrose; GCE-B = 4 mg/ml *Galla Chinensis* extract-B + 25 mM sucrose; Sucrose = 25 mM sucrose. Arrows indicate one pulse to the flow cell.

exposed to sucrose alone. The total viable counts of bacteria on HA discs in the NaF, GCE, and GCE-B groups were lower than in those in the sucrose-only group. In the GCE and GCE-B groups the populations of *S. mutans* and *S. sanguis* were also significantly lower than in the sucrose group. The population of *A. naeslundii* in the GCE-B group was significantly lower than that in the sucrose group.

Effect of sucrose pulsing on enamel demineralization

When observed under the polarized light microscope, enamel sections subjected to any of the four treatments showed different levels of demineralization (Fig. 3). The depths of the lesion in the GCE (59.58 ± 6.69 μm) and NaF (40.68 ± 6.01 μm) treatment groups were significantly smaller than those in the sucrose group (93.67 ± 9.67 μm) and the GCE-B group (90.10 ± 8.71 μm). However, there was no significant difference between the GCE and NaF groups. Furthermore, a sound remineralization zone (which was negatively bi-refringent, while the lesion was positively birefringent) could be found in the NaF group as well as the GCE group. In the sucrose group and GCE-B group, in contrast, no remineralization zone could be observed.

Biofilm microstructure and image analysis

The images observed under the fluorescence microscope (magnification 20×) are shown in Fig. 4. In the sucrose group, with the pulses of 25 mM sucrose, the biofilm had an intensely fuzzy appearance (Fig. 4A). In the NaF group, the outline of each colony was fairly clear (Fig. 4B). The appearance of the biofilms from the GCE (Fig. 4C) and GCE-B (Fig. 4D) groups was between those of the NaF group and the sucrose group. With the software IMAGE-PRO-PLUS, we found that the area covered by biofilm in the NaF group was significantly lower than that in sucrose group. The integrated optical densities of the GCE, GCE-B, and NaF groups, were significantly lower than that of the sucrose group (Fig. 5).

Discussion

Dental plaque plays an essential role in the pathogenesis of dental caries. When supplied with carbohydrate there will be a shift from a benign biofilm to a cariogenic biofilm, which will contain a larger proportion of cariogenic bacteria and be more acidogenic and aciduric (16, 18). To control the oral biofilm, one approach is to remove or reduce the biofilm mass or its acidogenicity by using antibiofilm agents. Previous *in vitro* studies of antibiofilm agents using synthetic antimicrobial agents such as chlorhexidine gluconate and triclosan have proved the effectiveness of these agents (26, 27). However, excessive use can result in alteration of the oral cavity and also the development of bacterial tolerance. The search for natural antiplaque agents with safe efficacy and potent activity has been focused to reduce the use of synthetic antimicrobial agents in daily oral-care products. The study of natural products has been one of the most successful strategies for the discovery of new medicines (8): 78% of new antibiotics and 61% of new antitumor drugs approved by the Food and Drug Administration of the USA or by comparable organizations in other countries from 1983 to 1994 were natural products or derived from natural products (5).

Galla Chinensis has been used in traditional Chinese medicine and other oriental medicine systems for years. It inhibits the growth of intestinal bacteria and *Helicobacter pylori* (3, 7). It also inhibits tumor invasion by inhibiting the transcription of matrix metalloproteinase (20). Moreover, *Galla Chinensis* and gallic acid might be potent inhibitors for the treatment of heat-labile, enterotoxin-induced diarrhea. In our previous study (11), *Galla Chinensis* inhibited the adherence of planktonic oral bacteria to saliva-coated HA *in vitro* at a concentration below the minimum inhibitory concentration as well as inhibiting acid production by cariogenic bacteria (*S. mutans*, *A. naeslundii*, *L. rhamnosus*). It was therefore necessary to discover its anticaries effect in a multispecies biofilm system, which better resembles the true oral environment.

Our data showed that the extract of *Galla Chinensis* presents distinct inhibi-

Table 1. The effect of different treatments on the biofilm population on hydroxyapatite (HA) discs in flow cells

Treatment group		*S. mutans*	*S. sanguis*	*L. rhamnosus*	*A. naeslundii*	Total viable count
NaF (228 p.p.m.)	Log_{10} CFU ± SD	8.34 ± 2.12*	9.07 ± 2.09*	8.32 ± 1.22*	6.92 ± 0.13*	9.8 ± 0.19
	Proportion (%)	25.54	27.79	25.48	21.19	
Sucrose (25 mM)	Log_{10} CFU ± SD	15.7 ± 0.00	16.34 ± 0.17	16.63 ± 0.07	11.48 ± 0.22	11.21 ± 0.85
	Proportion (%)	26.1	27.17	27.65	19.08	
GCE-B (4 mg/ml)	Log_{10} CFU ± SD	11.62 ± 0.71*	12.32 ± 0.66*	8.92 ± 0.91*	7.22 ± 0.42*	11.3 ± 0.16
	Proportion (%)	28.99	30.73	22.26	18.02	
GCE (4 mg/ml)	Log_{10} CFU ± SD	13.49 ± 1.06*	14.18 ± 0.87*	15.99 ± 0.79	11.15 ± 0.47	10.41 ± 0.71
	Proportion (%)	24.62	25.86	29.17	20.35	

NaF = 228 p.p.m. fluoride as NaF + 25 mM sucrose; GCE = 4 mg/ml *Galla Chinensis* extract + 25 mM sucrose; GCE-B = 4 mg/ml *Galla Chinensis* extract-B + 25 mM sucrose; Sucrose = 25mM sucrose. *S. mutans*, *Streptococcus mutans*; *S. sanguis*, *Streptococcus sanguis*; *L. rhamnosus*, *Lactobacillus rhamnosus*; *A. naeslundii*, *Actinomyces naeslundii*. Data are presented as log_{10} colony-forming units (CFU)/disc and percentages.
*Significant difference from sucrose group, $P < 0.05$.

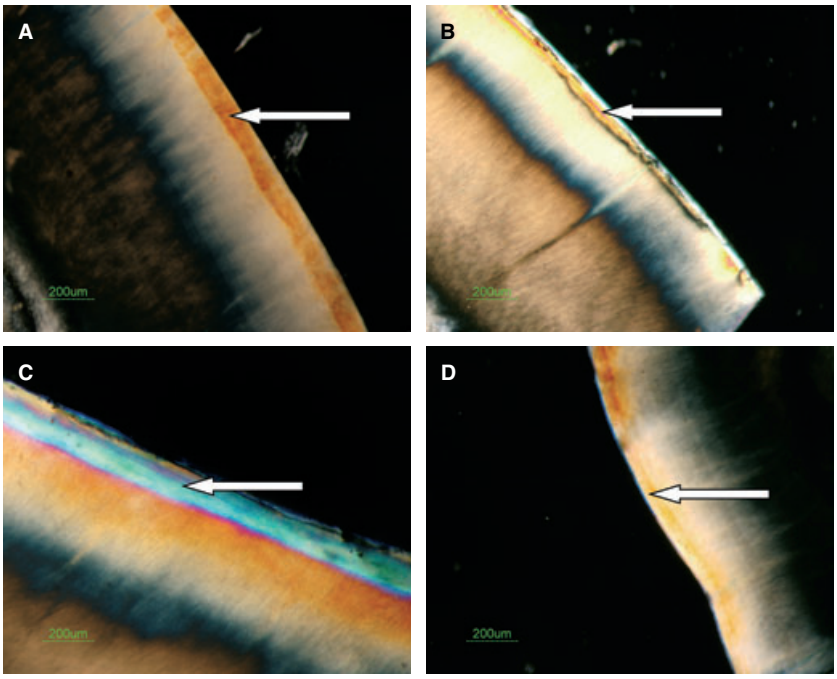

Fig. 3. Polarized light photomicrographs of longitudinal sections of bovine enamel showing caries-like lesions produced in the flow cells of each group: (A) sucrose group, (B) NaF group, (C) GCE group, and (D) GCE-B. Enamel sections are shown after imbibition in quinoline. Bar represents 200 μm and the white arrow indicates the remineralization zone. NaF = 228 p.p.m. fluoride as NaF + 25 mM sucrose; GCE = 4 mg/ml *Galla Chinensis* extract + 25 mM sucrose; GCE-B = 4 mg/ml *Galla Chinensis* extract-B + 25 mM sucrose; Sucrose = 25 mM sucrose.

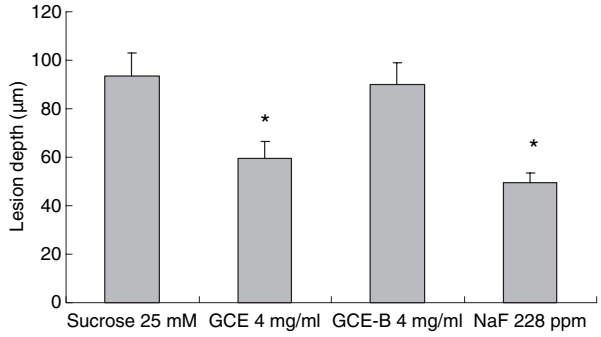

Fig. 4. The lesion depth as determined by polarizing microscopy. NaF = 228 p.p.m. fluoride as NaF + 25 mM sucrose; GCE = 4 mg/ml *Galla Chinensis* extract + 25 mM sucrose; GCE-B = 4 mg/ml *Galla Chinensis* extract-B + 25 mM sucrose; Sucrose = 25 mM sucrose. $^{*}P < 0.05$ compared with sucrose group.

tory effects against the multispecies oral biofilm. The pH measurements taken in the planktonic phase of the flow cells showed that both GCE and GCE-B could inhibit the acidogenicity of the experimental biofilm compared to addition of sucrose alone. Although the same concentration of sucrose was pulsed, the fall in pH in the planktonic phases for the GCE and GCE-B groups was restricted to 5.5 after each pulse, which is above the critical pH of enamel decalcification (12). The results here are consistent with our previous study of GCE use in planktonic oral bacteria. GCE and GCE-B contain several compounds classified as weak acids, of which tannic acid is a prime example. Weak acid is known to cause acidification of the cytoplasm of cells in an acid environment, which leads to inhibition of acid-sensitive enzymes, such as those of glycolysis (15). The polyphenoid compound from cranberry juice is found to have the same effect (6). Our data showed that the biofilm structure was also clearly affected by adding GCE and GCE-B in the sucrose solution: sucrose alone produced a more compact structure than the other treatment groups. In the GCE or GCE-B group, lower biofilm density and lower coverage area were observed. In our previous study on planktonic cells, we found that GCE and GCE-B could inhibit the adherence of *S. mutans* and *A. naeslundi* to saliva-coated HA *in vitro*. The rationale for the inhibitory effect of GCE and GCE-B on bacterial adherence is not clear, but polyphenols can form complexes with proteins and polysaccharides (9). It is probable that the inhibitory effects of GCE and GCE-B on the adherence of oral bacteria contribute to the affinity of GCE and GCE-B for the protein-binding site on the bacterial membranes and inhibit extracellular or cell-bound enzymes. Fewer *S. mutans* were found in the biofilm formed in the GCE-treated group, which may contribute to the less acidogenic and less cariogenic status of the biofilm. The ecological plaque hypothesis for dental caries (17) states that generation of a low pH environment from sugar fermentation results in an ecological shift to a more cariogenic microflora. The results presented here using a continuous culture system confirm that the ecological responses are consistent with this hypothesis. With carbohydrate supplementation in the flow cell, the population of cariogenic bacteria increased significantly, and there was a more compact biofilm structure. The results in this study indicate that the cariogenic shift of plaque was inhibited to some extent by the *Galla Chinensis* extract.

The bovine enamel section of the GCE group was less demineralized than that of the sucrose group. Previous studies in our laboratory (4) demonstrated that GCE and GCE-B could reharden the artificial carious lesions under dynamic, cyclic pH conditions. The results in this study support early findings that GCE could inhibit the demineralization of enamel in an *in vitro* biofilm model. This effect may contribute to the inhibition of cariogenic biofilm formation or to the reaction of GCE to the mineral content in the enamel; this requires further investigation. Combining the profile of enamel demineralization with the biofilm population, GCE-B presented a lower anticaries action than GCE in this study. Our preliminary chemical analyses of compounds extracted from *Galla Chinensis* (data not presented) revealed that GCE contains significant quantities of monomeric and polymeric polyphenols (e.g. gallotannin, gallic acid) and some other components (carbohydrates,

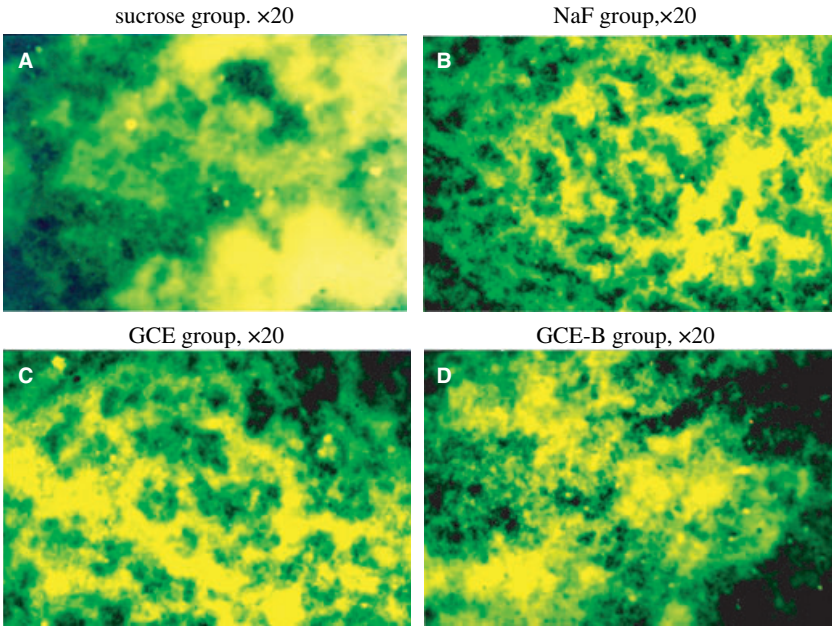

Fig. 5. The images of 120-h-old biofilm observed under the fluorescence microscope (magnification ×20). In the sucrose group (A), the biofilm has more extracellular polysaccharide, which gives it a fuzzy appearance. In the NaF group, the structure of the biofilm is more defined (B). The appearance of the biofilm from the GCE (C) and GCE-B (D) groups is between those of the sucrose and NaF groups. NaF = 228 p.p.m. fluoride as NaF + 25 mM sucrose; GCE = 4 mg/ml *Galla Chinensis* extract + 25 mM sucrose; GCE-B = 4 mg/ml *Galla Chinensis* extract-B + 25 mM sucrose; Sucrose = 25 mM sucrose.

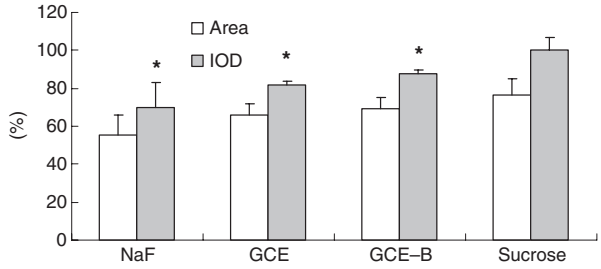

Fig. 6. Surface area (Area) coverage by biofilm and integrated optical density (IOD) of each group. Error bars show standard deviations from three independent replicates and illustrate the inherent heterogeneous nature of biofilm formation. *indicates that the IOD of that group was significantly different from that of the sucrose group ($P < 0.05$, analysis of variance, comparison for all pairs using Tukey test). NaF = 228 p.p.m. fluoride as NaF + 25 mM sucrose; GCE = 4 mg/ml *Galla Chinensis* extract + 25 mM sucrose; GCE-B = 4 mg/ml *Galla Chinensis* extract-B + 25 mM sucrose; Sucrose = 25 mM sucrose.

proteins, and other constituents). GCE-B, purified from GCE, contains only monomeric polyphenols, which were characterized as gallic acid and methyl gallate. Based on these findings, GCE may contain some additional components that inhibit the demineralization or enhance the remineralization of enamel.

In this study, fluoride, employed as a positive control, was effective in inhibiting the reduction of pH of the planktonic phase, the antibiofilm population (Table 1), and enamel demineralization (Fig. 3B). Fluoride can act to enhance remineralization in the cyclic demineralization–remineralization associated with acidification and alkalinization of dental plaque; and at the same time, it acts in multiple ways to affect the metabolism of cariogenic and other bacteria in the mouth (15). The result in this study was consistent with those of previous studies (23, 24).

In summary, the present study demonstrated the potential of the *Galla Chinensis* extracts GCE and GCE-B to limit acid accumulation from carbohydrate metabolism, reduce the proportion of cariogenic bacteria, and inhibit the demineralization of enamel in the model biofilm. *Galla Chinensis* may be a promising source of new agents to control dental caries.

Acknowledgments

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