Suppression effects of dental glass-ceramics with polarization-induced highly dense surface charges against bacterial adhesion

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This study investigated the surface characteristics and antibacterial ability capacity of surface-improved dental glass-ceramics by an electrical polarization process. Commercially available dental glass-ceramic materials were electrically polarized to induce surface charges in a direct current field by heating. The surface morphology, chemical composition, crystal structure, and surface free energy (SFE) were evaluated using scanning electron microscopy, energy dispersive X-ray spectrometry, X-ray diffraction, and water droplet methods, respectively. The antibacterial capacity was assessed by a bacterial adhesion test using *Streptococcus mutans*. Although the surface morphology, chemical composition, and crystal structure were not affected by electrical polarization, the polar component and total SFE were enhanced. After 24 h incubation at 37°C, bacterial adhesion to the polarized samples was inhibited. The electrical polarization method may confer antibacterial properties on prosthetic devices, such as porcelain fused to metal crowns or all ceramic restorations, without any additional bactericidal agents.

Keywords: Antibacterial capacity, Glass-ceramics, Surface modification, Surface charge, Surface free energy

INTRODUCTION

The accumulation of dental biofilm on tooth and prosthetic device surfaces is an important factor in the development of caries and periodontitis¹. As dental caries is a major cause of failure of all-ceramic restorations and porcelain fused to metal crowns^{2,3}, there is increasing interest in using dental materials that might inhibit plaque formation^{4,5}. Antibacterial coatings can be divided into two categories depending on how the antibacterial agent is delivered: active coating and passive coating⁶.

Passive coatings impede bacterial adhesion and/or kill bacteria upon contact without releasing bactericidal agents into the surrounding tissue⁶. During the formation of a dental biofilm on a prosthetic device surface in an oral cavity, four well-defined stages are recognized; transport to the surface, initial adhesion, attachment, and colonization/plaque maturation⁷. The initial adhesion results in a weak and reversible adhesion of the bacteria *via* their interaction with the surface at a certain distance through long and short-range forces. Interactions between bacteria and the surfaces of the prosthetic device are thought to be based on a variety of forces, including the Lifshitz-van der Waals, electrostatic, and hydrophobic forces and various specific receptor-ligand interactions⁸. These findings

indicate that the surface characteristics of materials play critical roles in the initial adhesion of bacteria to prosthetic devices and in the subsequent formation of biofilms⁶.

Dental glass-ceramics, which are mainly composed of silicon dioxide, are one of the most popular materials used in prosthetic devices because of their excellent esthetic value, biocompatibility, and stability⁹. Although *in vivo* studies have shown that the adhesion of bacteria and bacterial biofilm to glass-ceramics is lower than to other dental materials, such as gold alloy, titanium, amalgam, and resin composite¹⁰, further research into strategies to treat bacterial infections is needed because of the risk of material-associated infections¹¹.

Previously, it was reported that some ceramic biomaterials, such as titanium dioxide and zirconia, could be electrically charged by the dipole moment through an electrical polarization process^{12–15}. However, few studies are available on the application of electrical polarization to dental glass-ceramics. Moreover, the effect of electrical polarization on surface characteristics and bacterial adhesion is not yet fully understood. In this study, to create an antibacterial dental glass-ceramic material, we modified dental glass-ceramics using the electrical polarization process and characterized the surface morphology, chemical composition, crystal structure, and surface free energy of the glass-ceramics. The antibacterial capacity of the modified glass-ceramics was assessed by testing the adhesion of the bacterium

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Streptococcus mutans, which is known to cause dental caries.

MATERIALS AND METHODS

Fabrication of testing materials

Commercially available dental glass-ceramics (Cerec Blocs S3-M 14, Sirona Dental Systems, Bensheim, Germany) were cut into sections approximately 1 mm thick with precision diamond saws (IsoMet Low speed Saw, Buehler, IL, USA). The ceramic disks were ground with wet abrasive paper (Wet abrasive paper, Refinotec, Kanagawa, Japan), polished with diamond suspensions to 3 μm tolerance (Hyprez Diamond Slurry, Engis, IL, USA). The arithmetic average surface roughness (Ra) of specimens was characterized by laser microscope (VK-8510, Keyence, Osaka, Japan).

Surface modification of glass-ceramic disks

In accordance with the electrical polarization process reported in previous studies^{13,16}, the specimens were electrically polarized in a DC field of 0.1 or 1.0 kV/cm in air at 250°C for 1 h. The ceramic surfaces placed in contact with the anode and the cathode were defined as the N-surface and the P-surface, respectively. The control group was prepared using the same equipment by heating without applying the DC voltage (herein, it is termed the H-surface) (Fig. 1).

Surface characterization of polarized glass-ceramic disks

The crystal structure, micro structure, and chemical composition of the specimens were evaluated by X-ray

diffraction (XRD, D8 Advance, Bruker, Germany) analysis, scanning electron microscope (SEM, S-3400NX, Hitachi, Japan), and energy dispersive X-ray spectroscopy (EDS, S-3400NX, Hitachi, Oxford Instrument), respectively. Confirmation of the polarization was determined by the thermally stimulated depolarization current (TSDC) as previously described¹⁷. The stimulated depolarization current was measured from room temperature to 560°C in air at a heating rate of 5.0°C/min. The stored charges (Q) were calculated from TSDC spectra using the equation

$$Q = 1/\beta \int J(T) dT$$

where $J(T)$ is the measured depolarization current density at temperature T and β is the heating rate.

Surface free energy (SFE)

The SFE of the specimens was evaluated by the Owens-Wendt method¹⁸. The static contact angles of the specimens ($n=30$) were measured according to the sessile drop technique using a contact angle meter (DM-301, Kyowa Interface Science, Saitama, Japan) with water, formamide (Wako Pure Chemical Industries, Osaka, Japan), and diiodomethane (Wako).

Bacterial adhesion test

Before testing bacterial adhesion, the specimens were immersed in artificial saliva^{19,20} (Table 1) with or without 1.4 wt% mucin suspension (Mucin from porcine stomach, Type II, Sigma-Aldrich, Mo, USA) at 37°C for 1 h to form a coating of salivary conditioning film.

The *Streptococcus mutans* MT 8148 (*S. mutans*) bacterium (kindly provided by Prof. Ichiro NAKAGAWA of Tokyo Medical and Dental University) were incubated in brain-heart infusion (BHI, Bacto Brain Heart Infusion, Becton, Dickinson and Company, Sparks, USA) broth at 37°C for 24 h to obtain 10^9 colony forming units (CFU)/mL bacterial suspension. The bacterial suspension was added to each well to cover all specimen surfaces and incubated at 37°C for 24 h. The specimens ($n=60$) were then gently washed with PBS, stained using acridine orange solution (-Bacstain- AO solution, Dojindo Laboratories, Kumamoto, Japan), and observed under a fluorescence microscope (Fluoview FV10i,

Fig. 1 Schematic illustration for fabrication of specimens.

Table 1 Chemical composition of artificial saliva

Material	Concentration (g/L)	Manufacturer
NaCl	0.4	Wako Pure Chemical Industries
KCl	0.4	Wako Pure Chemical Industries
CaCl ₂ (H ₂ O)	0.795	Wako Pure Chemical Industries
NaH ₂ PO ₄ (H ₂ O)	0.69	Kanto Chemical
Na ₂ S	0.005	Wako Pure Chemical Industries
Urea	1	Wako Pure Chemical Industries

Olympus, Tokyo, Japan). The amount of bacterial adhesion was estimated from the positive area ratio of acridine orange.

Statistical analysis

The chemical composition of each surface was analyzed *via* one-way analysis of variance (ANOVA) followed by a Tukey HSD test. A two-way ANOVA was used to assess significant differences between the means of the studied polar component, dispersive component, and total SFE (applied voltage and surface polar character). A three-way ANOVA was used to assess significant differences in the means of the positive area ratio among selected factors (applied voltage, surface polar character, and mucin coating). When no interaction was found, a one-way ANOVA followed by a *t*-test with Bonferroni correction was used to assess for significant differences between the different applied voltage and surface polar character conditions. $p < 0.05$ was considered statistically significant.

RESULTS

Surface characterization

The average surface roughness of polished dental glass-ceramics used in this study was $0.23 \pm 0.02 \mu\text{m}$. The SEM micrographs of dental glass-ceramic surfaces with or without a DC field are shown in Fig. 2. All sample surfaces were relatively smooth but presented some polishing flaws. There were no remarkable changes between before and after applying electrical fields. Any changes in the chemical composition of the surfaces were explored by EDS analysis (Table 2). The glass-ceramic block used in this study was composed of sodium, potassium, silicon, and calcium. There were no significant differences in the chemical composition of the polarized (1.0 kV/cm) and non-polarized specimens (0 kV/cm). The crystal structures of the polarized (1.0 kV/cm) and non-polarized glass-ceramic blocks were mainly nepheline ((Na, K) AlSiO_4) and anorthoclase ((Na, K) AlSi_3O_8) as measured by XRD analysis (Fig. 3).

Surface charges of polarized glass-ceramics

The behavior of a polarized material can be estimated

Fig. 2 SEM images of dental glass-ceramics modified through the electrical polarization process. (a) H-surface, (b, d) N-surface, (c, e) P-surface, (b, c) 0.1 kV/cm, (d, e) 1.0 kV/cm, Bar, 5 μm .

Table 2 Chemical composition of non-polarized and polarized dental glass-ceramics analyzed by energy dispersive X-ray spectrometry

Element	H-surface	N-surface	P-surface
Na	10.7 $\pm$ 0.8	11.5 $\pm$ 0.2	10.7 $\pm$ 0.9
Al	20.4 $\pm$ 0.6	21.5 $\pm$ 0.3	20.5 $\pm$ 1.5
Si	60.5 $\pm$ 0.7	58.8 $\pm$ 0.8	60.2 $\pm$ 2.1
K	8.0 $\pm$ 0.5	7.4 $\pm$ 0.2	8.2 $\pm$ 0.4
Ca	0.4 $\pm$ 0.2	0.7 $\pm$ 0.0	0.4 $\pm$ 0.2

Fig. 3 XRD results of non-polarized and polarized dental glass-ceramics.

Fig. 4 Representative thermally stimulated depolarization current (TSDC) spectra of non-polarized and polarized specimens.

Table 3 The polar and dispersive component and total surface free energy (SFE) of modified dental glass-ceramics

Surface character	H-surface	N-surface	P-surface
Applied voltage (kV/cm)	0	0.1	1.0
Polar component (mJ/m ²)	9.5±2.3	21.9*±5.3	18.9*±4.8
Dispersive component (mJ/m ²)	34.4±2.5	28.4±3.4	28.0±2.0
Total SFE (mJ/m ²)	43.9±1.0	49.1*±2.9	48.3*±1.7

*: $p < 0.05$.

by TSDC measurements. Representative TSDC spectra of the polarized and non-polarized dental glass-ceramics are shown in Fig. 4. The depolarization current of the polarized samples was observed at *ca.* 200°C and reached at maximum value at *ca.* 420°C (0.1 kV/cm) and 480°C (1.0 kV/cm). The stored charges of the 0, 0.1 and 1.0 kV/cm samples were 0, 37 and 103 mC/cm², respectively. The half-lives of the 0.1 and 1.0 kV/cm samples at 37°C were 1 and 65 years, respectively.

SFE of polarized glass-ceramics

The surface free energies were calculated using the Owens-Wendt method and are shown in Table 3. Because the interactions in the polar component, dispersive component, and the total SFE between the surface character and the applied voltage were not significant, the SFE was evaluated by one-way ANOVA followed by Bonferroni multiple comparison test. The polar component and the total SFE were enhanced by an electrical polarization applied at voltages of 0.1 and 1.0 kV/cm. However, the SFEs were not affected by the polar character (N- and P-surface). Furthermore, we evaluated the effect of the amount of applied voltage on

the SFEs. There were no significant differences between the polar components, dispersive components and total SFEs for both applied voltages (0.1 and 1.0 kV/cm).

Bacterial adhesion test

The degree of bacterial adhesion was evaluated by the positive area ratio of acridine orange solution (Figs. 5 and 6). The means and SDs of the positive area ratio are presented in Fig. 7. Because the interactions (surface character *vs.* applied voltage, surface character *vs.* mucin coatings, applied voltage *vs.* mucin coatings, and surface character *vs.* applied voltage *vs.* mucin coating) in the positive area ratio were not significant, we evaluated the positive area ratio by one-way ANOVA followed by a Bonferroni multiple comparison test. Regardless of the presence or absence of a mucin coating, bacterial adhesion was inhibited by the electrical polarization process. The polar character, the amount of applied voltage, and the presence of a mucin coating had little effect on bacterial adhesion.

Fig. 5 Fluorescence microscope images of adherent bacteria on the dental glass-ceramics coated without mucin suspension.
(a) H-surface, (b, c) 0.1 kV/cm, (d, e) 1.0 kV/cm, (b, d) N-surface, (c, e) P-surface. Bacteria were stained with acridine orange solution (green). Bar, 30 μ m.

Fig. 6 Fluorescence microscope images of adherent bacteria on the dental glass-ceramics coated with mucin suspension.
(a) H-surface, (b, c) 0.1 kV/cm, (d, e) 1.0 kV/cm, (b, d) N-surface, (c, e) P-surface. Bacteria were stained with acridine orange solution (green). Bar, 30 μ m.

Fig. 7 The quantity of bacterial adhesion to the dental glass-ceramics.
*: $p < 0.05$

DISCUSSION

The results of this study indicate that the electrical polarization process offers promise as a noninvasive surface modification method to impart antibacterial properties to dental glass-ceramics. Antibacterial biomaterial strategies for the prevention of infections are divided into two types based on the mechanisms of biofilm formation: bactericidal/bacteriostatic coatings and anti-adhesion coatings^{21,22}. Anti-adhesion coatings are achieved by surface modifications to directly alter surface properties, including chemical composition and reactivity, hydrophilicity and hydrophobicity, surface roughness or texture, and surface charge²². Although certain polymer coatings, such as hydrophilic polymethacrylic acid, polyethylene oxide or protein-resistant polyethylene glycol, provide significant inhibition of bacterial adhesion⁶, their stability in the oral environment is not well understood. In this study, to estimate the sustainability of the antibacterial effect, the half-lives of polarized samples at 37°C were analyzed from the TSDC measurements. Although many studies have reported on the survival rates of porcelain-fused metal crowns and all-ceramic restorations, no long term (*i.e.*, over 20-years) clinical trials have been conducted^{2,3}. Polarized samples prepared with 1.0 kV/cm voltage showed sustained, controlled surface charges for over 20 years, indicating that a 1.0 kV/cm application of electrical polarization can confer antibacterial properties.

The surface charges of dental glass-ceramics were controlled through the electrical polarization process. Electrical polarization using an external electric field can manipulate the electric dipoles; subsequently, stored surface charges are observed by TSDC¹³. The TSDC spectra of polarized dental glass-ceramics showed several peaks characteristic of charge carriers. In an earlier study, we successfully controlled alkali ions in 45S5-type bioactive glass through the electrical polarization process¹⁶. The glass phase of dental glass-

ceramics contains alkali oxides, such as sodium and potassium oxide, which can move through the network of the glass matrix²³. Alkali ion carriers have been reported to jump among sites around the non-bridging oxygen ions by an applied DC field during the electrical polarization process²⁴. Electrical polarization using an external electric field also controls the dipoles in the crystal²⁵. Furthermore, dental glass-ceramics also contain a crystal phase²⁶. Anorthoclase and nepheline constitute the crystal phase of dental glass-ceramics. They are part of the alkali feldspar group and contain sodium and potassium ions, which are dominant charge carriers in feldspar minerals²⁷. Therefore, the major charge carriers in dental glass-ceramics are sodium and potassium ions. Additional studies will be required to identify the factors that contribute to the temperature-dependent peaks in the TSDC spectra.

Electrical polarization can be used to alter the SFE of dental glass-ceramics without physically changing the surface structure, the chemical composition or the crystal structure. SFE is a thermodynamic approach that describes the equilibrium state of atoms in the surface layer of a material²⁸. The Owens-Wendt method determines the dispersion and polar components of SFE from the measurements of the contact angle in bipolar and polar liquids¹⁸. Some of the factors that have an impact on the measured value of the contact angle include surface roughness and physical and chemical homogeneity²⁸. In this experiment, surface roughness was relatively uniform for all samples and was also found to be equally smooth after SEM analyses. Furthermore, EDS and XRD analyses showed no obvious difference in the surface structure and crystal structure among the samples. These results suggest that electrical polarization alters the SFE by manipulating the dipoles moments in dental glass-ceramics.

Electrical polarization reduced bacterial adhesion to dental glass-ceramics even in the presence of mucin protein coating. Biofilm formation in the oral cavity is

initiated by the adsorption of salivary proteins and oral bacteria onto a tooth or prosthetic device surface²⁹). The mucins are a family of heavily glycosylated proteins, which are the major organic components of saliva. Because they contain substantial amounts of sialic acid and sulfate residues³⁰, mucin coatings confer a negative charge to all surfaces, and then electrostatic interaction will take place. Although electrostatic repulsive forces exist between negatively-charged bacteria and mucin-free N-surfaces or mucin-coated N and P-surfaces⁷), our data showed that the amount of bacterial adhesion to mucin-free N-surface and mucin-coated substrates was not significantly different from that adhered to mucin-free P-surface and mucin-free substrates, respectively. The amount of bacterial adhesion in this study may depend on not only an initial adhesion but also a detachment caused by wash protocol. The detachment strength of bacteria from a substrate is associated with a surface wettability³¹). Due to the hydrophilicity of polarized samples with no relation to their polar character, adhering bacteria were more readily detached from the polarized samples than from non-polarized glass-ceramics.

CONCLUSION

In conclusion, this study provides new and important methods for the surface modification dental glass-ceramics to inhibit bacterial adhesion, including mucin-aided adhesion. The electrical polarization process mediates dipole behavior in the dental glass-ceramics and increases the surface free energy without the need for additional agents. Furthermore, this process avoids the alteration of the surface chemical composition, crystal structure, or morphology. However, the antibacterial effect of this method on polarized dental glass-ceramics coated with several oral salivary proteins is unclear and warrants further examination *in vivo*.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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