Adherent bacteria cells in five dental materials: sonication effect

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Adherent bacterial cells on the surfaces of two dental porcelain ceramics, three composite resins and human enamel were examined using four types of bacteria strains. Their adherent cells were counted on saliva-coated and uncoated material surfaces after sonication, and contact angle and ζ potential were measured for each adherent cell tested. A correlation between contact angle and bacterial cells on an uncoated surface was found to be higher in two *Streptococcus sanguis* cells than in *S. mutans* Ingbritt and *S. sobrinus* OMZ 176, whereas there appeared to be a higher correlation between *S. mutans* Ingbritt or *S. sobrinus* OMZ 176 and ζ potential on the uncoated surface. On the saliva-coated surface, a significantly high correlation was found between the adherent cells, with the exception of *S. sanguis* ATCC 10 557, and the ζ potential. Contact angle and ζ potential values were small when the surfaces of the materials were coated with saliva, as compared with those on the uncoated surface. The sonication condition (120 s) of adherent cells on the surface of the material significantly depended on the types of bacteria cells, showing that *S. mutans* Ingbritt (> 50–60%) had a greater removal percentage than the others ($< 50\%$).

1. Introduction

Dental porcelain ceramics have been used in a manner similar to composite resins that promote bacterial growth [1, 2]: dental light-activated glass-ionomer cements have better adhesion and an antibacterial effect [3–9]. The light-activated glass-ionomer cements have hybrid systems, combining conventional glass-ionomer and visible light-cured resin chemistries [10–12]. ζ potential as a measure of electrostatic interaction on the surfaces of materials shows that composite resin has large values (minus) [5]. There is, however, little information about the sonication removal effect of dental ceramics and composite resins, which might affect the antibacterial effect of bacterial cells. Adherent cells on dental material surfaces during sonication are counted in this study, because it is considered that glazed porcelain ceramics and polished composite resins have different surface characteristics. This study thus examines the sonication effect of adherent bacterial cells in two dental porcelain ceramics, three composite resins and human enamel, which are exposed to four types of bacterial cells on saliva-coated and uncoated surfaces.

2. Materials and methods

The five dental materials examined in this study are listed in Table I, with details of brand name, manufacturer, batch number and sample code. Human enamel was used as a control material (code AE). The dental porcelains (A1, A2) were obtained by a glazing treatment, and the surfaces of resin composites (A3, A4, A5) were pressed against a glass surface during curing for 30 s by "quick light" (Morita, Kyoto, Japan). The enamel surface was polished finally with a No. 2000 emery paper. Sample A5 comprised 63.4 wt % bisphenol-A glycidyl dimethacrylate (bis-GMA), 34.1% triethylene glycol dimethacrylate (TEG-DMA), 0.5% camphorquinone (CQ), 1.95% dimethylaminoethyl methacrylate (DMAEMA) and 0.5% buthylated hydroxy toluene (BHT, Tokyo Kaser Co.). The sample was 1 mm thick with an area of 5×10 mm.

The surfaces of the samples were coated with saliva, which was collected unstimulated into chilled tubes and centrifuged for 20 min at 4 °C at $20\,000 \times g$, where g was the acceleration due to gravity, to remove debris. The cell adherent test was carried out for 2 h at 37 °C using four types of bacteria strains: *Streptococcus mutans* Ingbritt, *S. mutans* OMZ 175, *S. sobrinus* OMZ 176, *S. sanguis* ATCC 10 556, *S. sanguis* ATCC 10 557. The bacteria strains were routinely cultivated in a brain–heart infusion (BHI) matrix (Difco Laboratories, Tokyo) for 48 h [10, 11]. Five samples were coated with saliva, whereas the other five samples were not coated. The initial concentration of adherent cells was controlled with O.D. (optical density) = 0.3.

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TABLE I Dental materials used at an adherent cell test in this study: two porcelains, three resins and human enamel (a control sample)

Material	Manufacturer	Lot. No.	Code
Clapearl porcelain	Kuraray	00215	A1
VITA VMK68	GmbH	530	A2
Photo clearfil bright	Kuraray	11139	A3
Silux plus	3M	1EB2	A4
Base-resin[a]	–	–	A5
Human enamel	–	–	AE

[a]Comprises 63.4% Bis-GMA, 34.1% TEGDMA, 0.5% CQ, 1.95% DMAEMA, 0.05% BHT (all wt %).

After adhesion, the samples were sonicated at 20 kHz for 0–300 s in a glass tube (500 ml). They were fixed with 2.5% glutaraldehyde for 30 min at 4 °C, and stained with 1% acrydine orange. The adherent cells on the surfaces of the samples were counted arbitrarily at 20 sites under a fluorescence microscope (model BHS, Olympus).

The contact angle and ζ potential were measured as follows. The contact angle of ten samples (20 °C) was measured at three separate points by the horizontal projection technique using a contact angle meter (CA-A model, Kyowa). The ζ potentials of the surfaces of the crushed samples and the bacterial cells were measured with a particle micro-electrophoresis apparatus in a tris-HCl buffer (0.05 mol l^{-1}, pH = 7.2) at 25 °C, using a face ζ potential meter (type ZP-OM, Kyowa Kaimen). The values were calculated by the Helmholtz–Smoluchowsky formula [12].

3. Results

Table II indicates the mean values ± standard deviations of the contact angle (deg) and ζ potential (mV) in five materials and human enamel of uncoated and saliva-coated samples. Under the saliva-coated condition both values were comparatively lower than for the uncoated samples.

Table III indicates a correlation between contact angle or ζ potential and the number of adherent cells where four types of bacterial strains were used. High regression numbers were found for contact angles on uncoated surfaces with respect to two *S. sanguis* cells, whereas there was high correlation with adherent *S. mutans* Ingbritt and *S. sobrinus* OMZ 176 for ζ potential. The higher relation was found, except for *S. sanguis* ATCC 10557 when coated with saliva.

Figs 1–3 show the number of adherent bacterial cells in the different samples used. Adherent cell numbers had increased trends for *S. mutans* Ingbritt and *S. sanguis* ATCC 10556 on uncoated material surfaces (Fig. 1). A sonication time of >120 s gave less adherent cells (*S. mutans* Ingbritt) in both uncoated and saliva-coated composite resins (A3) (Fig. 2). The number of adherent cells on the surfaces of the specimen samples was less after 120 s of sonication as shown by four types of bacterial cells (Fig. 3).

4. Discussion

Adherent bacteria cells on the surface of five dental materials and human enamel were examined in this study. The effect of sonication in distilled water was

TABLE II Contact angle and ζ potential values of uncoated and saliva-coated surfaces of dental materials. The values show mean values ± standard deviations

	Contact angle (deg)		ζ potential (mV)	
Code	Uncoated	Saliva-coated	Uncoated	Saliva-coated
A1	36.5 ± 2.4	29.4 ± 3.4	− 57.0 ± 3.0	− 25.7 ± 1.1
A2	39.2 ± 3.3	28.4 ± 2.3	− 53.5 ± 3.7	− 23.7 ± 3.3
A3	68.5 ± 1.5	28.8 ± 4.9	− 31.7 ± 1.5	− 21.4 ± 1.2
A4	65.8 ± 2.2	34.1 ± 4.3	− 29.0 ± 3.2	− 20.6 ± 3.9
A5	60.2 ± 1.4	33.9 ± 1.9	− 25.9 ± 3.3	− 20.3 ± 3.2
AE	41.9 ± 5.3	26.8 ± 5.8	− 34.3 ± 2.7	− 25.3 ± 3.4

TABLE III Regression values of contact angle and ζ potential of uncoated and saliva-coated surfaces. The cells used in this study were *S. mutans* Ingbritt, *S. sobrinus* OMZ 176, *S. sanguis* ATCC 10556, and *S. sanguis* ATCC 10557. The statistical analysis was done by the student's *t*-test (p = probability)

	Contact angle		ζ potential	
Cell	Uncoated	Saliva-coated	Uncoated	Saliva-coated
S. mutans Ingbritt	0.798	0.729	0.883[b]	0.975[c]
S. sobrinus OMZ 176	0.769	0.766	0.837[a]	0.881[a]
S. sanguis ATCC 10556	0.831[a]	0.651	0.806	0.896[a]
S. sanguis ATCC 10557	0.824[a]	0.713	0.797	0.732

[a]$p < 0.05$.
[b]$p < 0.02$.
[c]$p < 0.01$.

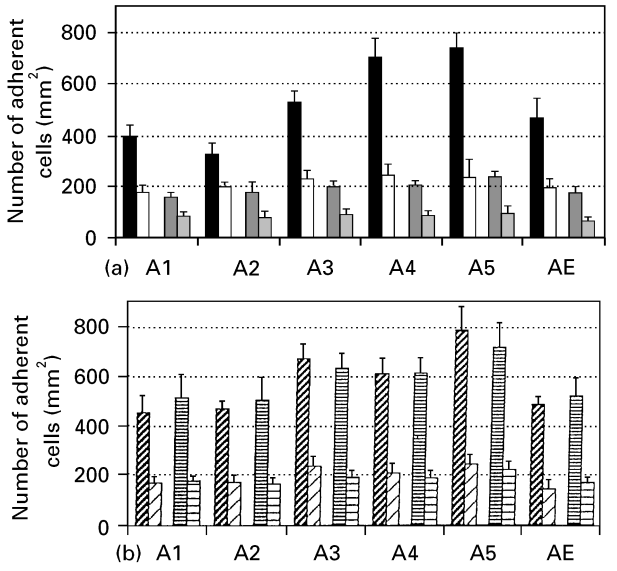

Figure 1 The number of adherent cells on uncoated and saliva-coated surfaces using *S. mutans* Ingbritt and *S. sobrinus* OMZ 176 (a) and *S. sanguis* ATCC 10 556 and *S. sanguis* ATCC 10 557 (b) Uncoated material: (■) *S. mutans* Ingbritt, (▩) *S. sobrinus* OMZ 176, (▨) *S. sanguis* 10 556, (▤) *S. sanguis* 10 557. Saliva-coated material: (□) *S. mutans* Ingbritt, (▩) *S. sobrinus* OMZ 176, (▨) *S. sanguis* 10 556, (▤) *S. sanguis* 10 557.

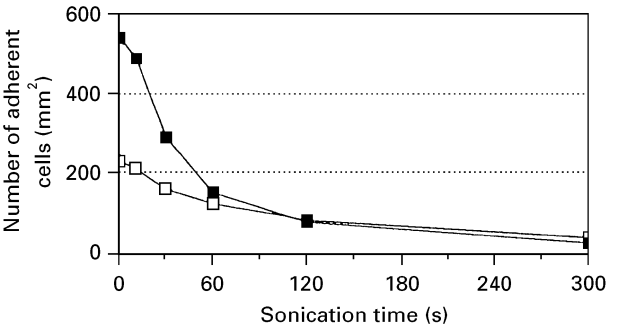

Figure 2 The change in the number of adherent cells (*S. mutans* Ingbritt) on uncoated (—■—) and saliva-coated (—□—) composite resin surfaces (A3) with increasing sonication time (0–300 s).

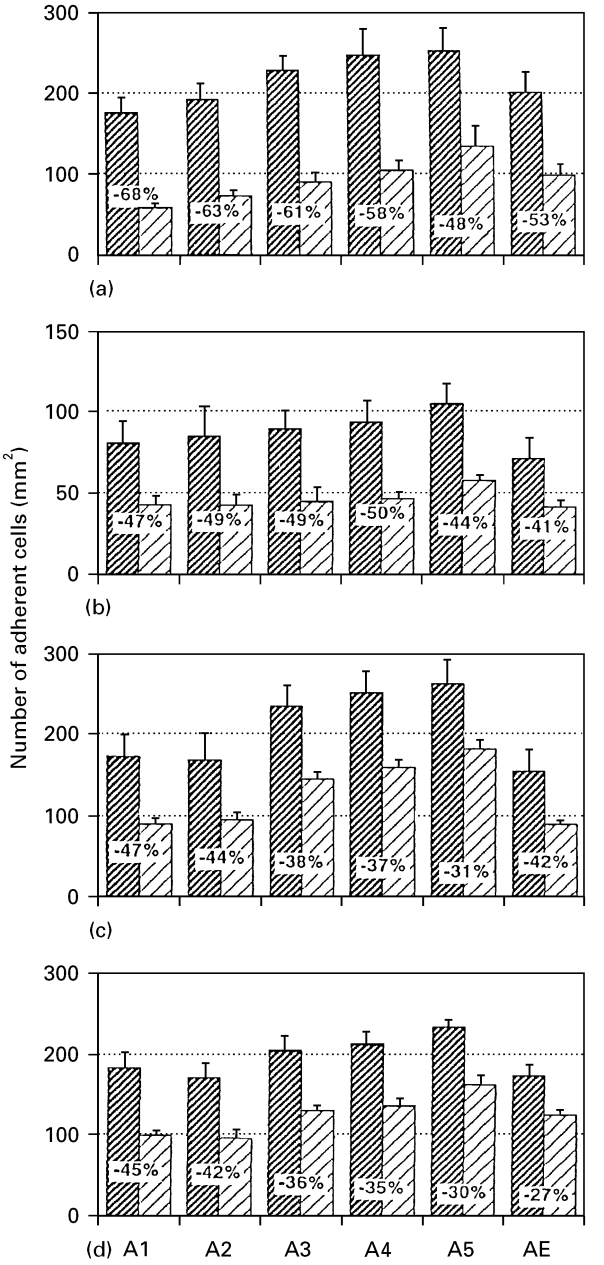

Figure 3 The number of adherent cells counted after sonication for (▨) 0 and (▨) 120 s. The method is described in detail in the text. The percentage values are the removal values during sonication testing. The adherent cells tested were (a) *S. mutans* Ingbritt, (b) *S. sobrinus* OMZ 176, (c) *S. sanguis* ATCC 10 556, and (d) *S. sanguis* ATCC 10 557.

effective for four bacteria cells that were reported for the glass-ionomers [7]. As measured by the cell adherent test, a sonication effect was clearly observed (Figs 1–3).

As reported by Satou *et al.* about the magnitudes of contact angle and ζ potential on composite resin surfaces for four types of bacterial cells [5], the contact angle (deg) was greater in two types of *S. sanguis* (37.9 ± 1.8; 45.3 ± 3.9) than in *S. mutans* Ingbritt (16.9 ± 1.5) and *S. sobrinus* OMZ 176 (30.7 ± 2.3), and the ζ potential value (mV) was lower in two *S. sanguis* (-27.6 ± 3.5; -22.5 ± 1.7) than in the other two bacteria (-37.5 ± 4.2; -36.3 ± 2.8). From these results, it is considered that there appears to be a clear correlation between the magnitude of the contact angle or ζ potential and the number of adherent cells. To examine electrostatic interaction during cell adhesion by means of ζ potential measurement, a saliva-coating surface treatment was needed for glazed, or polished, or as-cured material surfaces (Table II; Figs 1–3). Two types of bacterial cells (*S. sanguis*) had higher hydrophobicity associated with greater values of ζ potential (minus), showing that dental composite resins had a greater ζ potential. These values on dental material surfaces treated with saliva-coating were smaller than those on uncoated surfaces (Table II). This result suggests that there is a correlation between the contact angle, or ζ potential and the number of adherent cells; and a high correlation was found between the contact angle and hydrophobic *S. sanguis* and between the ζ potential and *S. mutans* or *S. sobrinus* on the uncoated surfaces. When saliva was coated to the surfaces of the materials, a clear correlation (ζ potential) was found, except for *S. sanguis* ATCC 10 557. These results suggest that electrostatic interaction is related to adhesion of bacterial cells. During sonication, adherent cells were removed by the

ultrasonicator between the surface and the saliva, and also at the cell–saliva interface (Fig. 2). Dental materials (dental ceramics, composite resins and human enamel) had different surface characteristics because of different surface treatment procedures, and a sonication effect of adherent cells on the surfaces of the materials was distinguished clearly in relation to contact angle and ζ potential.

This study confirmed that the adhesion of cells was controlled by hydrophobic surfaces in the sonication of adherent bacterial cells on material surfaces of two porcelain ceramics, three composite resins and human enamel. The saliva bound on the surface depended on the type of dental material, showing that there appeared to be a high correlation between saliva and the composition of the materials.

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