Efficacy of Removal of Sucrose-Supplemented Interproximal Plaque by Electric Toothbrushes in an In Vitro Model

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Electric toothbrushes were evaluated using a model of plaque removal by fluid shear forces. Sucrose supplementation during plaque development did not affect the removal of bacteria from biofilm exposed to low-energy shear but did increase their resistance to high-energy shear. The toothbrush supplying high-energy shear forces removed significantly more viable bacteria.

Dental plaque is a microbial biofilm that develops on non-shedding oral surfaces (24), and its composition and structure are strongly influenced by the amount of fermentable carbohydrates available (2, 3, 14, 17). The breakdown of these carbohydrates to acids by the oral microbiota (16) is a major factor in the etiology of dental caries. Constant-depth fermentors (CDFFs) (23) can produce oral biofilms in vitro which closely model those found in vivo. This is true in terms of both the composition of the microbial population (10, 19, 20) and the plaque architecture (13). Using an established system which models the action of electric toothbrushes upon CDFF-grown biofilms (5, 6), we previously showed that a Sonicare Plus toothbrush (SP) removed a significantly (P = 0.017) greater median percentage (51.12%) of viable biofilm bacteria than did a Braun Oral B 3D (D17) toothbrush (BO) (17.49%) (7) when the CDFF was supplied with a mucin-containing artificial saliva (18).

These brushing experiments were repeated under identical conditions by using oral biofilms grown in the presence of sucrose to determine if supplementing dental plaques with excess fermentable carbohydrate during growth affected the efficacy of plaque removal by electric toothbrushes.

The protocol for growing oral biofilms in a CDFF on a hydroxyapatite (HA) substratum and the brushing methodologies have been described previously (7). However, in this study sucrose was added to the CDFF 24 h after inoculation as 330-ml pulses of a 10% (wt/vol) aqueous sucrose (Sigma, Poole, United Kingdom) solution which was pumped into the CDFF three times a day, each pulse lasting 30 min. This level of sucrose supplementation equates to the total mean daily intake of adults in the United Kingdom (1).

A pair of HA disks containing biofilms were inserted into recesses located in plastic teeth (Fig. 1), which were in turn placed into a typodont model (SM-PVR-660; Columbia Dentoform, Long Island City, N.Y.) itself located in an exposure chamber containing 7 ml of a brushing solution (5). After a brushing time of 5 s, the number of bacteria removed from the biofilms was ascertained by removing an aliquot of brushing solution and determining the number of CFU on blood agar after 48 h of anaerobic incubation at 37°C. The number of bacteria remaining upon the disks after brushing was similarly determined following their removal by vortex mixing with phosphate-buffered saline and glass beads and plating as described above. The percentages of bacteria (in terms of CFU) removed from the biofilm by brushing were calculated by assuming the total number of bacteria initially present upon the HA disks to be the sum of the number of bacteria removed and the number of bacteria remaining.

A Mann-Whitney U test (a nonparametric comparison of the medians of two independent groups) showed that the median percentage of sucrose-supplemented plaque bacteria removed by the SP (32.00%, n = 7) after 5 s of exposure was significantly (P = 0.003) greater than that removed by the BO (15.31%, n = 5). The interquartile range of the data was calculated from the weighted averages (Table 1). The actual number of bacteria removed and remaining upon the HA disks after brushing is shown in Fig. 2.

In this study, a series of sucrose pulses, followed by rinsing with artificial saliva, was used to model the situation which occurs in vivo during the consumption of sucrose-rich drinks (11, 12), an approach which has been previously used to expose dental plaque to a cariogenic challenge (8, 9). Sucrose availability affects the composition and structure of oral biofilms (17, 19). It has also been shown that different species of bacteria have different responses to shear stress (21). This suggests that, since oral biofilms grown in the presence of sucrose have a different composition from that of biofilms grown in the absence of sucrose, their resistance to shear stress should also be different. Biofilm structure is also dependent upon nutrient availability (17): broadly speaking, excess nutrients tend to yield flat biofilms while nutrient-limited conditions result in finger-like projections attached to the bulk of the biofilm by narrow isthmi.

Oral bacteria store excess carbohydrates as intracellular and extracellular polysaccharide (EPS). Insoluble EPS with branched glucan or fructan chains contributes to the structural integrity of the biofilm matrix by the formation of a network of cross-linked linear macromolecules (22). An increase in the
production of EPS will, in turn, increase the physical stability of the plaque as a whole (15).

A comparison between the percentage of bacteria removed from sucrose-supplemented oral biofilms and a similar study using biofilms grown without available sucrose (5) showed an interesting disparity in the percentages of biofilm bacteria removed. There was almost no difference in the median percentage of plaque bacteria removed by the BO in biofilms grown with and without available sucrose: with sucrose, 15.31%; without sucrose, 15.86%. However, supplementation of CDFF-grown plaques with sucrose did appear to affect the percentage of plaque removed by the SP (with sucrose, 32.00%; without sucrose, 48.45%).

The reason why sucrose supplementation of plaque biofilms did not affect the efficacy of the BO toothbrush may be due to the relatively low-level fluid shear that it produced through the interproximal space (i.e., between the teeth), shear which was capable of removing bacteria associated with surface protrusions whether or not sucrose was available to the developing biofilm but was unable to remove the underlying bulk of the plaque. In a model similar to the one used in this study, the mean interproximal fluid flow velocity produced by a BO was 18 cm s⁻¹, while an SP produced a flow velocity of 31 cm s⁻¹ along with entrained air bubbles (4). Bacteria associated with surface protrusions will be preferentially removed from biofilm by these external forces since their attachment to the bulk of the biofilm-substratum is over a smaller area. The SP toothbrush, however, removes both the surface protrusions and deeper layers of the plaque biofilm (7).

Changes in the robustness of oral biofilms in response to dietary carbohydrate in the form of sucrose may be responsible for the observed differences, but further work is needed to ascertain if this is the case.

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TABLE 1. Median percentage of sucrose-supplemented oral biofilm removed from HA disks by two different electric toothbrushes, enumerated as CFU

<table>
<thead>
<tr>
<th>Toothbrush</th>
<th>n</th>
<th>Median (%)</th>
<th>IR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP</td>
<td>7</td>
<td>32.00</td>
<td>22.11–43.21</td>
</tr>
<tr>
<td>BO</td>
<td>5</td>
<td>15.31</td>
<td>7.40–16.38</td>
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IR, interquartile range.

REFERENCES