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The effect of a microbead dentifrice on microbial load in oral microenvironments

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Abstract: *Objectives:* The human oral cavity contains several microenvironments or ecologic niches. While mechanical plaque control is well known to reduce the number of supragingival dental plaque bacteria, there is little data on antimicrobial effects in other oral ecologic niches. The present study examined the effects of mechanical plaque control using a microbead dentifrice on bacteria colonizing oral ecologic niches. *Methods:* Twenty-two adults (aged 18–70 years) including nine generalized moderate chronic periodontitis subjects and 13 periodontally healthy subjects having average gingival indices ≥1 and plaque indices ≥1.5 completed a 1 week washout phase and refrained from oral hygiene the morning of baseline sample collection. Microbial samples from supragingival dental plaque, buccal mucosa, dorsal surface of the tongue and whole mixed saliva were obtained. Subjects brushed with a microbead dentifrice and, after 10 min, sampling was repeated. The number of anaerobic bacteria was determined by culture on non-selective media and transformed to $\log_{10}$ for statistical analyses. *Results:* Mechanical plaque control using the microbead dentifrice resulted in statistically significant reductions in bacterial numbers in each ecologic niche ($P < 0.001$). The greatest reduction in the number of viable bacteria occurred in samples taken from the buccal mucosa (97.22%) followed by a 95.22% reduction in supragingival plaque bacteria, a 94.51% reduction in the number of bacteria on the dorsal surface of the tongue and a 91.57% reduction in the number of bacteria in whole mixed saliva. *Conclusions:* Mechanical plaque control using a microbead dentifrice reduces microbial load in microenvironments throughout the human oral cavity.

Key words: dental biofilm; formation control; microbiology; micro-organism; plaque; saliva; tongue

Introduction

The human oral cavity is not a homogeneous environment. Rather, the oral cavity is comprised of a number of microenvironments each harbouring a distinct microbial ecology comprised of diverse microbial species. The reduced oxygen levels in subgingival dental plaque, for example, favour colonization by higher numbers of bacterial anaerobes compared to supragingival dental plaque. Other microbial ecologic niches in the oral cavity include the teeth, buccal, palatal and floor of the mouth mucosa, dorsal and ventral tongue, tonsils and whole mixed saliva (1). Besides oxygen, the microbial composition of oral ecologic niches is influenced by factors such as the presence of shedding or

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non-shedding surfaces, salivary and gingival crevicular fluid (2, 3) and diet (4).

The bacteria that colonize the oral hard and soft tissues do not exist in a free-living (planktonic) state but as part of a microbial biofilm – an attached, multilayered, highly structured, microbial community (1). Bacteria in biofilms behave differently than free-living bacteria. They produce different virulence factors (5) and higher concentrations of antimicrobial agents are needed to kill bacteria in biofilms compared to free-living bacteria.

Numerous clinical studies, sometimes with microbiological analysis (6, 7), have demonstrated the cause-and-effect relationship between the accumulation of oral biofilms and the development of plaque-associated gingivitis (8). These studies also show that only a small number of the 1000 or more microorganisms infecting the oral cavity (9, 10) cause dental caries (11) and periodontal diseases (12). Routine mechanical plaque control – twice daily brushing and flossing with a fluoride dentifrice (13) – disrupts oral biofilms and reduces bacterial numbers thereby preventing most oral disease (14). However, most people do not perform meticulous oral hygiene as shown by the significant proportion of the population exhibiting dental caries and periodontal disease (11, 12, 15). Consequently, a number of agents are added to dentifrices and mouthrinses to control oral biofilms such as alcohols, cetylpyridinium chloride, chlorhexidine, detergents, fluorides, povidone iodine, triclosan and zinc citrate (16). Clinicians also administer local antimicrobials and prescribe systemic antibiotics to both control infections in the oral cavity and to inhibit biofilm formation (17). Further, there is continuing exploration for new agents that will target the pathogenic component of the oral flora such as novel plant extracts or phototherapy directed toward bacterial pigments (16).

Numerous clinical studies have established the efficacy of mechanical plaque control in removing supragingival dental plaques and, by extension, controlling subgingival dental plaques (18–20). There is little data, however, on the efficacy of oral hygiene in reducing bacterial load (the number of bacteria) in other oral ecologic niches. That is, there is little data on the effect of oral hygiene on bacterial numbers on, e.g. the buccal mucosa or dorsal tongue. The goal of reducing bacterial load in the oral cavity is of particular importance in view of the recently described relationships between oral infection and systemic diseases and conditions such as diabetes mellitus, heart disease and preterm low birth weight (21–23). Accordingly, the present study examined the hypothesis that mechanical plaque control with a microbead dentifrice can reduce microbial numbers throughout the oral cavity.

Study population and methodology

Subjects

The study protocol was approved by the Health Sciences Institutional Review Board at the University at Buffalo. Prospective subjects 18–70 years of age from Buffalo, NY were recruited from among the students, faculty, staff and patients of the dental clinics. Subjects were eligible for the study if they were in general good medical and dental health, did not have a serious medical condition such as diabetes mellitus, heart disease, liver or renal disease, or transmissible disease, e.g. AIDS, were not taking prescription medications and had not taken antibiotics, anti-inflammatory or anticoagulant drugs during the month preceding the study. Subjects were required to have at least 20 teeth and could not have fixed or removable dental prostheses. Subjects were required to have an average Gingival Index [Loe-Silness (24)] ≥1 and an average plaque scores ≥1.5 by the Turesky modification of the Quigley-Hein Plaque Index [T/Q-H (25)].

Table 1. **Subject demographics**

Patient number	Gender	Age (years)	Periodontal status
1	M	61	Generalized chronic periodontitis
2	F	32	Generalized chronic periodontitis
3	F	51	Generalized chronic periodontitis
4	M	59	Generalized chronic periodontitis
5	F	41	Periodontally healthy
6	F	42	Periodontally healthy
7	F	51	Generalized chronic periodontitis
8	F	62	Generalized chronic periodontitis
9	M	32	Periodontally healthy
10	F	41	Periodontally healthy
11	F	43	Periodontally healthy
12	F	53	Periodontally healthy
13	F	53	Periodontally healthy
14	M	25	Periodontally healthy
15	F	21	Periodontally healthy
16	F	60	Generalized chronic periodontitis
17	F	58	Generalized chronic periodontitis
18	F	25	Periodontally healthy
19	F	25	Periodontally healthy
20	F	52	Generalized chronic periodontitis
21	M	19	Periodontally healthy
22	M	24	Periodontally healthy

Twenty-two adults were enrolled in the study and all completed the study. Subjects participating in this study (Table 1) included six males and 16 females with a mean age of 42.3 years (range: 19–62 years). Subjects included 13 periodontally healthy subjects (four males, nine females, mean age: 34.1 years, range: 19–53 years) and nine subjects with chronic periodontitis (two male, seven female, mean age: 54 years, range: 51–62 years).

Subjects enrolled into the study were provided with a commercially available fluoride dentifrice and a soft-bristled toothbrush that they were instructed to use during a 1-week washout period. Subjects were instructed to discontinue all other oral hygiene for the remainder of the study.

Methods

Sample collection

Following the 1-week washout, subjects were scheduled for a baseline examination and sample collection. Subjects were

instructed to refrain from oral hygiene the morning of the examination and from eating, drinking or smoking for 2 h prior to the visit. A calibrated dentist performed an oral examination and collected samples before and 10 min after the subjects brushed their teeth (under supervision) for 1 min with 1.5 g of a microbead dentifrice using a manual toothbrush. Subjects were instructed to brush only their teeth and to avoid other areas in the mouth. The subjects rinsed their mouths with water after brushing. The dentifrice contains beads of uniform diameter containing flavour oils that break open during brushing. Samples collected before and after brushing with the microbead dentifrice included (i) unstimulated whole mixed saliva (≈1 ml) collected into a sterile tube, (ii) tongue samples collected from a randomly selected half of the dorsal surface of the tongue using a sterile tongue depressor (five passes per site) prior to brushing and from the opposite half 10 min after brushing. Samples were placed in 3 ml of phosphate buffered saline (PBS), (iii) buccal mucosa samples collected from a randomly selected left or right buccal mucosa using a sterile tongue depressor (five passes per site) prior to brushing and from the opposite half 10 min after brushing. Samples were placed in 3 ml of PBS and (iv) supragingival plaque samples collected from the buccal tooth surfaces in a randomly selected maxillary quadrant using a sterile 13/14 Columbia scaler and placed into 1 ml of PBS. Randomization in the selection of tongue, buccal mucosa and buccal tooth surface samples was by flip of the coin.

Microbiological procedures

Samples were dispersed by vortexing at maximal setting for 60 s followed by pulsed sonication (Branson Sonicator, output, 1, duty cycle, 50%; Branson Ultrasonics Corp., Danbury, CT, USA) with a cup horn for 30 s. Serial 10-fold dilutions were prepared in PBS. Dispersed samples were distributed onto trypticase soy agar with 5% sheep blood using a spiral plater (Spiral Systems Autoplater 4000; Spiral Biotech Inc., Norwood, MA, USA) and incubated in an anaerobic chamber for 1 week at 37°C. Following incubation, the number of colony forming units (CFU) was enumerated from plates having 20–200 colonies. The number of colony forming units is directly correlated to the number of viable bacteria in the samples.

Statistical analysis

The number of colony forming units ml^{-1} from each sample at each phase of the study was transformed to $\log_{10}$. Paired *t*-tests compared the log transformed viable counts from baseline and post-treatment for each sample. Mean differences in viable counts between baseline and post-treatment for each oral site were calculated and analysed by JMP software (Cary, NC, USA).

Results

The effect of brushing with the microbead dentifrice is shown in Table 2 and Fig. 1. Ten minutes after brushing, there were significantly lower average numbers of bacteria (colony forming units ml^{-1}) in each of the oral microenvironments sampled ($P < 0.01$). The largest percent reduction was found in samples taken from the buccal mucosa where the average number of bacteria went from 6.64 log colony forming units ml^{-1} to 4.93 log colony forming units ml^{-1} representing a 97.2% reduction (Table 3). There was an average 1.39 log (95.2%) reduction in bacterial numbers in supragingival dental plaque, an average 1.42 log (94.5%) reduction in bacterial numbers on dorsal tongue surface and an average 1.12 log (91.6%) reduction in bacterial numbers in whole mixed saliva. While each site demonstrated statistically significant reductions in the number of viable bacteria after brushing with the microbead dentifrice, there were no statistically significant differences between sample sites.

As might be expected, oral microbial load defined in this study as the number of colony forming units ml^{-1} in the sample sites, was generally higher in the nine subjects with chronic periodontitis compared to the 13 periodontally healthy subjects (Table 4). For baseline samples, there were higher numbers of bacteria recovered from chronic periodontitis patients in samples of dental plaque, buccal mucosa and whole mixed saliva as compared to periodontally healthy subjects. After treatment, the number of bacteria recovered from chronic periodontitis patient samples was generally still greater than that recovered from the periodontally healthy subjects. Only the baseline samples from the dorsal surface of the tongue showed higher bacterial numbers in the periodontally healthy subjects compared to the chronic periodontitis subjects.

Comparing periodontally healthy subjects with chronic periodontitis subjects, the largest post-treatment reduction in bacterial numbers occurred in samples of the buccal mucosa in periodontally healthy subjects where there was a 1.82 log reduction after treatment compared to baseline. The smallest post-treatment reduction in bacterial numbers occurred in samples of whole mixed saliva in periodontally healthy subjects where there was a 1.11 log reduction after treatment compared to baseline. None of the differences between periodontally healthy and chronic periodontitis groups achieved statistical significance.

Discussion

Patient directed approaches for routine oral hygiene are important in maintaining oral health. Common adjuncts for oral hygiene include a fluoride toothpaste, a soft-bristled toothbrush and less often, dental floss. Routine oral hygiene minimises accumulations of dental plaque and provides additional benefits such as maintaining fresh breath and a pleasing smile (19, 20).

A central feature of routine oral hygiene is elimination of supragingival plaque – a complex microbial biofilm (1, 5). While many studies have examined the efficacy of routine oral hygiene, most have employed clinical indices to ascertain effects on supragingival plaque and plaque-associated gingivitis (18–20). Even though oral micro-organisms are responsible for the initiation and progression of most oral infectious diseases – dental caries and periodontal disease (11, 12) – few studies

Table 2. **Effect of mechanical supragingival plaque control with a microbead dentifrice on bacterial numbers in oral ecologic niches***

	Supragingival plaque			Dorsal tongue			Saliva			Cheek		
Patient number	Baseline	Post-treatment	Change	Baseline	Post-treatment	Change	Baseline	Post-treatment	Change	Baseline	Post-treatment	Change
1	8.68	7.53	1.15	8.17	6.59	1.58	7.53	6.25	1.28	6.89	5.57	1.32
2	6.61	5.08	1.53	7.75	6.23	1.52	7.00	5.91	1.09	6.88	4.85	2.03
3	7.86	6.40	1.45	7.34	5.91	1.43	7.53	6.21	1.32	7.04	5.19	1.85
4	8.80	7.37	1.43	8.50	7.66	0.84	8.52	7.31	1.20	6.63	5.05	1.58
5	7.56	5.72	1.85	7.99	6.67	1.33	7.47	6.54	0.93	7.02	5.47	1.55
6	8.27	7.33	0.94	8.17	7.10	1.07	8.12	7.25	0.87	6.36	5.19	1.17
7	7.97	6.87	1.10	8.71	7.31	1.41	8.41	7.07	1.34	6.76	5.49	1.27
8	8.13	6.99	1.14	8.35	7.18	1.16	7.74	6.75	1.00	6.52	5.13	1.39
9	7.56	6.21	1.35	7.84	6.37	1.47	7.26	6.39	0.87	6.86	5.21	1.65
10	7.70	6.21	1.49	7.89	6.90	1.00	7.37	6.18	1.19	6.59	4.86	1.73
11	7.71	6.66	1.05	8.41	7.17	1.25	7.66	6.36	1.30	6.52	4.69	1.83
12	6.51	5.36	1.15	8.41	7.47	0.94	7.65	6.23	1.42	6.88	4.30	2.58
13	8.00	6.48	1.51	8.41	6.91	1.50	7.39	6.26	1.12	6.79	3.79	3.00
14	7.63	5.91	1.72	8.59	5.79	2.80	7.67	6.51	1.16	6.93	5.21	1.72
15	7.56	5.91	1.65	7.62	6.55	1.06	7.56	6.31	1.25	5.71	3.30	2.41
16	7.40	6.26	1.14	7.71	6.21	1.50	7.70	6.76	0.95	6.58	5.09	1.49
17	6.51	5.31	1.20	8.77	6.91	1.86	8.13	6.99	1.14	6.76	5.37	1.38
18	8.22	6.77	1.45	8.32	6.80	1.53	8.33	6.95	1.38	5.85	4.30	1.55
19	7.91	6.26	1.65	8.05	7.06	0.99	6.95	5.79	1.17	6.55	4.99	1.56
20	8.55	6.72	1.82	7.80	6.80	0.99	7.40	6.63	0.77	6.68	5.06	1.63
21	7.58	6.37	1.22	8.17	6.41	1.76	8.11	7.26	0.85	6.61	5.45	1.16
22	7.69	6.01	1.68	8.31	6.01	2.30	7.31	6.39	0.92	6.74	4.99	1.74

*Log CFU ml^{-1}.

have examined the effect of routine oral hygiene on the numbers and kinds of oral micro-organisms in dental plaque. Even fewer studies have examined the effects of routine oral hygiene on oral bacteria found in oral ecologic niches other than dental plaque such as the buccal mucosa, whole mixed saliva and dorsal surface of the tongue.

In the present study, we performed a concurrent assessment of the effects of mechanical plaque control with a microbead dentifrice on oral bacteria inhabiting different ecologic niches in the oral cavity i.e. dental plaque, buccal mucosa, tongue and whole mixed saliva. Microbiological analyses were conducted on the entire complement of micro-organisms recovered on enriched media (26). These techniques are widely used in clinical oral microbiology and recover both Gram-positive and Gram-negative organisms (7). In contrast to molecular techniques which enumerate both viable and non-viable bacteria (9, 10), anaerobic culture methods utilized in the present study focus solely on viable bacteria. This is particularly pertinent in surveys of different oral micoecologies since only viable bacteria can be transmitted between sites in the oral cavity (27) or between people (28, 29).

In this investigation, microbial samples were obtained from four different oral microenvironments in subjects 18–70 years of age. Since children and adolescents were not included, the findings are relevant only to adults. Supragingival dental plaque and whole mixed saliva were assayed based on previous reports relating the micro-organisms in these sites to clinical conditions (1). The dorsal surface of the tongue and the buccal mucosa were assayed based on more recent research (30–32). The dorsal tongue surface has been found to harbour a number of previously unknown bacterial species (30, 31) and buccal mucosa epithelial cells have been found to be infected with periodontal pathogens (32). The intracellular infection of buccal mucosa epithelial cells may serve as a bacterial reservoir for re-infection of the oral cavity and may provide pathogenic advantages to the survival of these micro-organisms (32).

Microbiological analysis of oral microenvironments is important in determining oral microbial load i.e. the total number of micro-organisms present in a site as well as the qualitative and quantitative relationships between oral microenvironments. Besides supragingival dental plaque, the oral microenvironments examined in the present study are not typically the focus of mechanical debridement/oral hygiene (13, 14). Accordingly, a standardized protocol was used in this study in which subjects were instructed to brush only their teeth and to refrain from hygiene in other areas of the oral cavity.

Statistical analyses of viable bacteria from the two phases of this study demonstrate significant reductions in oral bacteria after brushing. Inhibitory effects were observed on bacteria recovered from the supragingival dental plaque, whole mixed saliva, buccal mucosa and the dorsal surface of the tongue. Microbial reductions were observed amongst samples from each subject and the average bacterial reductions were 1 log or higher. It is likely that the reductions in bacterial numbers are due to enhanced dispersion of adherent plaques due to mechanical abrasions from the microbeads and to the formulation of the dentifrice. Several *in vitro* studies demonstrate

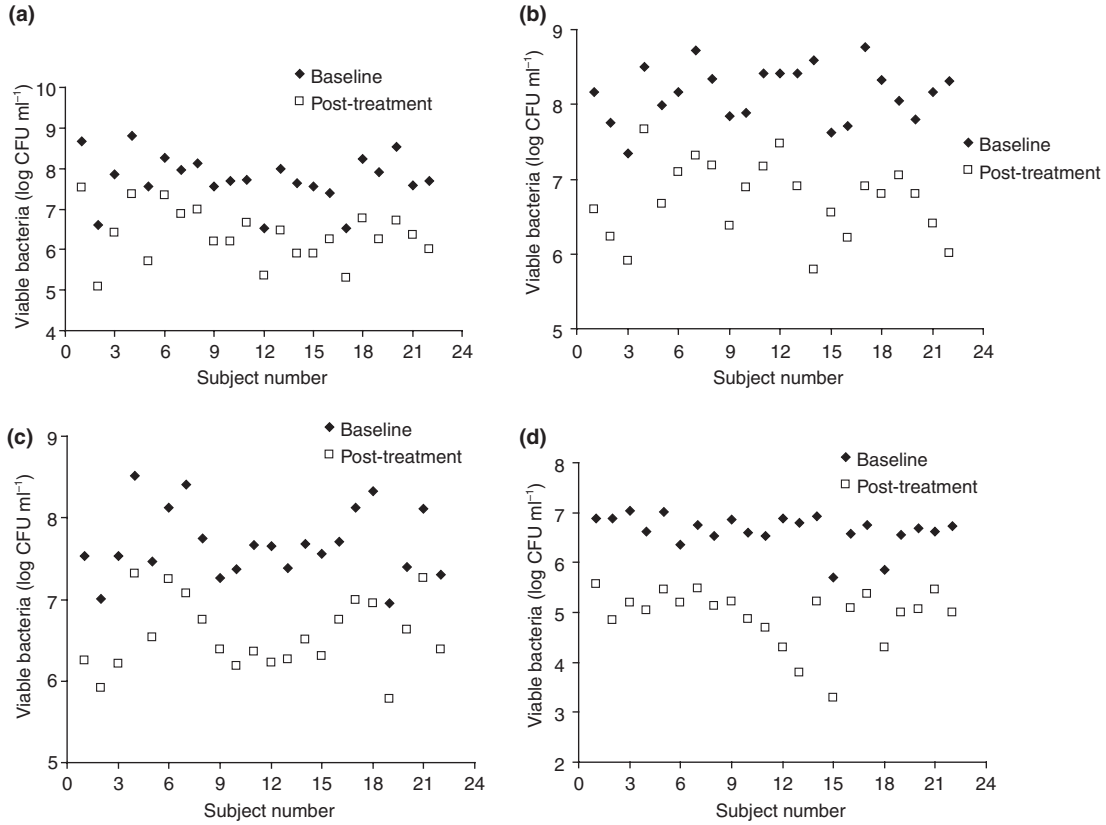

Fig. 1. Changes in the number of oral bacteria (log CFU ml^{-1}) following use of a microbead dentifrice in (a) supragingival dental plaque, (b) dorsal tongue surface, (c) whole mixed saliva and (d) buccal mucosa.

Table 3. **Summary of the effect of mechanical supragingival plaque control with a microbead dentifrice on bacterial numbers in different oral ecologic niches***

	Baseline	Post-treatment	Change	% reduction	*P* value
Dental plaque	7.75	6.35	−1.39 ± 0.27	95.22 ± 2.78	0.005477
Dorsal tongue	8.15	6.73	−1.42 ± 0.46	94.51 ± 0.46	2.48×10^{-12}
Buccal mucosa	6.64	4.93	−1.71 ± 0.46	97.22 ± 0.46	0.0000112
Saliva	7.67	6.56	−1.12 ± 0.19	91.57 ± 3.81	0.00022967

*Average log CFU ml^{-1} ± SD.

Table 4. **Microbial load in periodontally healthy and chronic periodontitis subjects***

	Baseline		Post-treatment		Change post-treatment		
Sample Site	Periodontally normal	Chronic periodontitis	Periodontally normal	Chronic periodontitis	Periodontally normal	Chronic periodontitis	*P* value
Dental Plaque	7.69	7.83	6.25	6.50	−1.44	−1.33	0.38
Dorsal tongue	8.17	8.12	6.71	6.76	−1.46	−1.37	0.83
Buccal mucosa	6.57	6.75	4.75	5.20	−1.82	−1.55	0.07
Saliva	7.60	7.77	6.49	6.65	−1.11	−1.12	0.41

*Average log CFU ml^{-1}.

that common surfactants can penetrate biofilms, bind to microbial cells, inhibit cell function and facilitate biofilm detachment (33–35). While these studies examined only a few oral bacterial strains, it is clear that the conditions in the mouth are vastly different from those established in the laboratory (36).

Several factors influence microbiological studies of the human oral cavity. There is considerable microbial variation

between subjects (37) and between oral microenvironments within subjects (1). There is even variation within the same bacterial species due to clonal heterogeneity and periodic population fluctuations (38, 39).

Subjects without predisposing health factors were used in the present study to reduce possible confounders. Twenty-two adults were recruited for the study and the resulting 176 samples represent a sizable number for analyses. Adults with gingival indices ≥1 and plaque scores ≥1.5 were recruited as generally representative of adult oral status. The subjects underwent a washout period with a fluoride dentifrice and a standardized oral hygiene regimen to reduce differences due to dentifrices and oral hygiene regimens. Procedures for sample collection were identical at each phase of the study and collection sites were randomized to minimize variations introduced during sampling.

As demonstrated in the present study, mechanical plaque control using a microbead dentifrice reduces microbial load in microenvironments throughout the human oral cavity. A recent study of 150 individuals demonstrated continuing oral health improvement following introduction of dentifrice and toothbrushes to a population relying on traditional oral hygiene procedures (40). In older adults, microbial load in the oral cavity appears to be related to aspiration pneumonia, the most common cause of acute care in this population (41–43). Weekly professional oral hygiene in conjunction with routine toothbrushing reduces the number of oropharyngeal bacteria (44) and the incidence of influenza (41, 43). Thus, results from this study provide a microbiological rationale for the role of routine oral hygiene in preventive programmes initiated by health care professionals (41, 43–45).

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