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Biofilms in the Edentulous Oral Cavity

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Keywords
Complete dentures; biofilm; microbiota; edentulous; DNA–DNA hybridization; checkerboard.

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This research received the following support, grants, and awards: NIDCR grant DE14368; American College of Prosthodontists (ACP)/P&G Research Fellowship in Complete Denture Prosthodontics; Academy of Prosthodontics Foundation Research Grant; Greater New York Academy of Prosthodontics (GNYAP) Research Award; ACP/ESPE Research Fellowship in Geriatric Prosthodontics; Northeastern Gnathological Society's Granger-Pruden Award.

This study was also awarded first place in the table clinic competition at the 2005 ACP Annual Session in Los Angeles, CA.

Accepted December 12, 2007

doi: 10.1111/j.1532-849X.2008.00301.x

Abstract

Purpose: The oral cavity presents numerous surfaces for microbial colonization. These surfaces produce biofilms of differing complexities unique to each individual. Several studies have looked at biofilms in dentate patients. There has been limited research regarding biofilms on dentures or soft tissues of edentulous patients. The purpose of the present investigation was to provide meaningful data describing microbial ecological relationships in the oral cavity of edentulous patients and to evaluate the microbiota on hard and soft tissue surfaces and saliva in edentulous patients wearing complete dentures.
Materials and Methods: Sixty-one edentulous subjects with complete maxillary and mandibular dentures were recruited. "Supragingival" biofilm samples were taken from 28 denture teeth for each subject. Biofilm samples were also taken from the dorsal, lateral, and ventral surfaces of the tongue, floor of mouth, buccal mucosa, hard palate, vestibule/lip, "attached gingiva," and saliva. Samples were individually analyzed for their content of 41 bacterial species using checkerboard DNA–DNA hybridization. Levels and proportions of each species were determined for every sample location.
Results: Periodontal pathogens such as *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* were clearly present in the samples from the edentulous subjects. Microbial profiles in samples from the soft tissue surfaces differed among site locations. Samples from the dorsum of the tongue exhibited the highest bacterial counts followed by the "attached gingiva" and the lateral surfaces of the tongue, while the lowest mean counts were found in samples from the buccal mucosa and labial vestibules. Using cluster analysis of the proportions of the test species, three clusters were formed. The first cluster comprised saliva, supragingival plaque, and the lateral and dorsal surfaces of the tongue. The second cluster comprised the other six soft tissue surfaces. Species on the denture palate formed a third cluster.
Conclusions: One of the major findings in this study was the detection of periodontal pathogens, *A. actinomycetemcomitans* and *P. gingivalis,* in the edentulous subjects, as these species were thought to disappear after removal of all natural teeth. This finding has implications regarding future dental treatment and the general health of individuals. Distinct patterns of microbial colonization were seen on the different soft tissue surfaces. Thus, this investigation provided the first step in defining the organisms that are associated with edentulous patients on both soft (mucosa) and hard surfaces (denture). The study also provided meaningful data that described microbial ecological relationships in the oral cavity of edentulous subjects. The authors believe that this study is the first comprehensive assessment of the microbiota in the complete denture-wearing subject.

There has been some controversy over the number of current and future edentulous patients worldwide. Several studies have suggested that fluoridation and changing demographics are leading to the falling rate of edentulism.[1,2] Other authors have indicated that edentulism is on the rise.[3-5]

In 1994, Lang[3] reported that the number of edentulous persons over 65 years of age in need of complete dentures in the United States and Canada appeared to be decreasing as a percent of the total population; however, he also stated that the total number of patients needing these services by the year 2030 will

be almost the same as it is today. A survey by Cates in 1989[5] showed that the geriatric population in the United States was increasing and was expected to continue to rise through the 21st century. Despite changing demographics, treating complete denture geriatric patients is expected to be a large part of dental care well into the next century.[3-7]

Douglass et al[4,8] concluded that the adult population within the United States in need of complete dentures would increase from 33.6 million in 1991 to 37.9 million in 2020. They emphasized the need for complete denture prosthodontic training in dental education, as a sizable minority of the patient population will continue to need complete denture services, despite the previous assumption of declining rates of edentulism. Part of the process to optimize treatment of edentulous patients is the need to understand the microbiota present not only on mucosal surfaces, but also on the surfaces of prostheses.

The oral cavity presents numerous surfaces for microbial colonization. These surfaces are colonized by biofilms of differing microbial complexity unique to each individual.[9] Several studies have described biofilms in dentulous patients,[10-12] but there have been relatively few studies of the microbiota of the mucous membranes or saliva in edentulous subjects and even fewer looking at the microbiota on complete dentures. Studies of the edentulous oral cavity of infants prior to tooth eruption have suggested that *Prevotella melaninogenica* was the most frequently isolated anaerobic species found in 70% of infants.[13] Other common anaerobes detected in edentulous infants included *Fusobacterium nucleatum, Veillonella* species, and non-pigmented *Prevotella.* The source of the anaerobes appeared to be the mother, because there was a correlation between maternal salivary concentration and the infant's colonization by these species, particularly *P. melaninogenica.*[14]

At the other end of the age spectrum, the microbiota of 51 edentulous subjects (mean age 74 years) with complete dentures was studied using culture techniques.[15] Biofilm samples taken from the intaglio (tissue) surfaces of the dentures as well as the palate, buccal mucosa, dorsum of the tongue, and saliva were analyzed using nonselective and selective media techniques. "Black-pigmented *Bacteroides*" were found in 96% of subjects, while yeasts were found in 49% of subjects. *Streptococcus mutans* was found in 84% of saliva samples; 92% of the samples yielded lactobacilli.

Data in the literature have suggested that species such as *S. mutans* required hard surfaces for sustained colonization,[16-19] even though they might be detected in dentate subjects at low levels on the soft tissues.[20,21] It has also been shown that *S. mutans* essentially disappeared from the oral cavity when all the teeth were extracted and reappeared if hard surfaces were provided in the form of dentures.[16-19] Other investigators have stated that *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* disappeared from the oral cavity after extraction of all teeth and did not reappear even when hard surfaces such as dentures were provided.[22,23] These species have also been reported to have a strong association to various systemic diseases in the dentate population.[24-31]

These data are intriguing in that they suggest that teeth are essential for colonization of species such as *A. actinomycetemcomitans* and *P. gingivalis*. Further, hard surfaces appear essential for the colonization of *S. mutans*. Theilade and Budtz-Jorgensen[32] examined the predominant cultivable microbiota on removable dentures in subjects with denture-induced stomatitis. They suggested that the gingival crevice as well as the fluid passing through the gingival crevice might be essential for the colonization of most Gram negative rods, including common species such as *F. nucleatum, Prevotella intermedia,* and *Prevotella nigrescens.* The data from studies examining the oral microbiota in edentulous patients are fragmentary, often derived from small number of samples and/or patients, using techniques that are not able to detect low number of organisms and a wide spectrum of bacterial taxa.

The purpose of the present investigation was to examine the microbiota of biofilms that form on dentures and the oral soft tissues and in the saliva of edentulous, denture-wearing subjects using checkerboard DNA–DNA hybridization.[33] This technique is used to determine sequence similarity between DNAs of different origin and the amount of sequence repetition within one DNA. It is a useful tool for the enumeration of bacterial species in large samples of microbiologically complex systems.[33-35]

Materials and methods

Subject population

The subject population consisted of 61 edentulous subjects (54% male, 46% female) who used complete maxillary and mandibular dentures on a daily basis. The baseline characteristics of these subjects are presented in Table 1. Subjects of any racial/ethnic group were accepted for study as long as they were in good general health.

To be included in the study, subjects had to be over 20 years of age, have been edentulous for at least 1 year and worn complete maxillary and mandibular dentures on a daily basis.

Subjects who had received antibiotic therapy in the 3 months prior to the start of the study or who had any oral lesions (e.g., candidiasis, ulcerations, leukoplakia, oral cancer) or a systemic condition that required antibiotic coverage for routine dental procedures (e.g., heart conditions, joint replacements) were excluded from the study.

Soft tissue samples

Microbial samples were taken from eight separate oral soft tissue locations in each subject using MasterAmp™ buccal swab brushes (Epicentre Technologies, Madison, WI). Four hundred and eighty-eight samples were obtained by gently stroking each

Table 1 Demographic features of the subjects

Mean age in years (± SD)	59.6 (± 11.3)
% Males (N)	54 (33)
% Females (N)	46 (28)
% Current smokers (N)	43 (26)
% White* (N)	64 (39)
% African–American (N)	34 (21)
% Asian (N)	2 (1)

N denotes the actual number of subjects.

*1 subject in the White group reported being Hispanic.

site in an area large enough to yield sufficient number of microorganisms for DNA probe analysis. The samples were collected from three areas of the tongue: one from the dorsum of the tongue, one from the ventral surface, and one sample from both the lateral surfaces of the tongue. This was done by first taking a sample from the left lateral surface of the tongue and then using the same swab to take a sample from the right lateral surface. Similarly, there was one sample for both the left and right buccal mucosa and the maxillary and mandibular labial vestibules, respectively. Microbial samples were also taken from the floor of the mouth, hard palate, and the maxillary anterior "attached gingiva" (fixed keratinized tissue). The samples were placed in separate tubes containing 0.15 ml Tris EDTA (TE) buffer (10 mM Tris-HCL, 0.1 mM EDTA, pH 7.6), and 0.15 ml 0.5 M NaOH was added.

Denture samples

Twenty-eight separate microbial samples were taken from the dentures of each subject while the dentures were in the mouth. Each sample was obtained using separate sterile curettes from the mesio-buccal surface of every denture tooth. The samples were placed in separate tubes containing 0.15 ml of TE buffer and processed as described for the soft tissue samples. A total of 1708 samples from the denture teeth were collected. An additional sample from the midpoints of the exterior, polished surfaces of each denture hard palate was taken using a MasterAmp™ buccal swab brush. All the dentures had polished exterior surfaces without any rugae.

Saliva samples

Each subject provided a sample of whole unstimulated saliva by expectorating into a sterile tube. A total of 61 samples were collected. A 0.2-ml sample of whole saliva was mixed with 0.15-ml sterile, filtered TE buffer. A 0.2-ml sample of this mixture was transferred to a new tube, and 0.15 ml of 0.5-M NaOH was added. The samples were processed as described for the soft tissue and denture samples.

Microbial analysis of the samples

All samples were analyzed using checkerboard DNA–DNA hybridization to determine the levels of 41 bacterial species presented in Table 2.[33] In brief, the samples were placed in separate Eppendorf tubes containing 0.15-ml TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.6), and 0.15 ml of 0.5-M NaOH was added to each tube. The samples were lysed, and the DNA placed in lanes on nylon membranes using a Minislot device (Immunetics, Cambridge, MA). After fixation of the DNA to the membranes, the membranes were placed in a Miniblotter 45 (Immunetics) with the lanes of DNA at 90° to the lanes of the device. Digoxigenin-labeled whole genomic DNA probes to 41 bacterial species were hybridized in individual lanes of the Miniblotter. After hybridization, the membranes were washed and then incubated with anti-digoxigenin antibody conjugated with alkaline phosphatase. Signals were detected using AttoPhos substrate (Amersham Life Science, Arlington Heights, IL) and were read using a Storm Fluorimager (Molecular Dynamics, Sunnyvale, CA), a computer-linked instrument that read the intensity of the fluorescence signals resulting from the probe-target hybridization. Two lanes in each run contained standards at concentrations of 10^5 and 10^6 cells of each species. The sensitivity of the assay was adjusted to permit detection of 10^4 cells of a given species by adjusting the concentration of each DNA probe. Signals were evaluated using the Storm Fluorimager and converted to absolute counts by comparison

Table 2 Species for which DNA probes were prepared for the study

Aggregatibacter actinomycetemcomitans 43718 and 29523	*Actinomyces israelii 12102*
Actinomyces gerencseriae 23840	*Actinomyces odontolyticus 17929*
Actinomyces naeslundii genospecies 1 12104	*Campylobacter gracilis 33236*
Actinomyces naeslundi genospecies 2 43146	*Campylobacter showae 51146*
Campylobacter rectus 33238	*Capnocytophaga ochracea 33596*
Capnocytophaga gingivalis 33624	*Eikenella corrodens 23834*
Capnocytophaga sputigena 33612	*Eubacterium saburreum 33271*
Eubacterium nodatum 33099	*Fusobacterium nucleatum ss polymorphum 10953*
Fusobacterium nucleatum ss nucleatum 25586	*Fusobacterium periodonticum 33693*
Fusobacterium nucleatum ss vincentii 49256	*Leptotrichia buccalis 14201*
Gemella morbilliorum 27824	*Peptostreptococcus micros 33270*
Neisseria mucosa 19696	*Prevotella intermedia 25611*
Porphyromonas gingivalis 33277	*Prevotella nigrescens 33563*
Prevotella melaninogenica 25845	*Selenomonas noxia 43541*
Propionibacterium acnes 11827 and 11828	*Streptococcus constellatus 27823*
Streptococcus anginosus 33397	*Streptococcus intermedius 27335*
Streptococcus gordonii 10558	*Streptococcus mutans 25175*
Streptococcus mitis 49456	*Streptococcus sanguinis 10556*
Streptococcus oralis 35037	*Treponema denticola B1*
Tannerella forsythia 43037	*Veillonella parvula 10790*
Treponema socranskii S1	

All strains were obtained from the American Type Culture Collection (ATCC, Manassas, VA), except *Treponema denticola B1* and *Treponema socranskii S1,* which were obtained from The Forsyth Institute (Boston, MA).

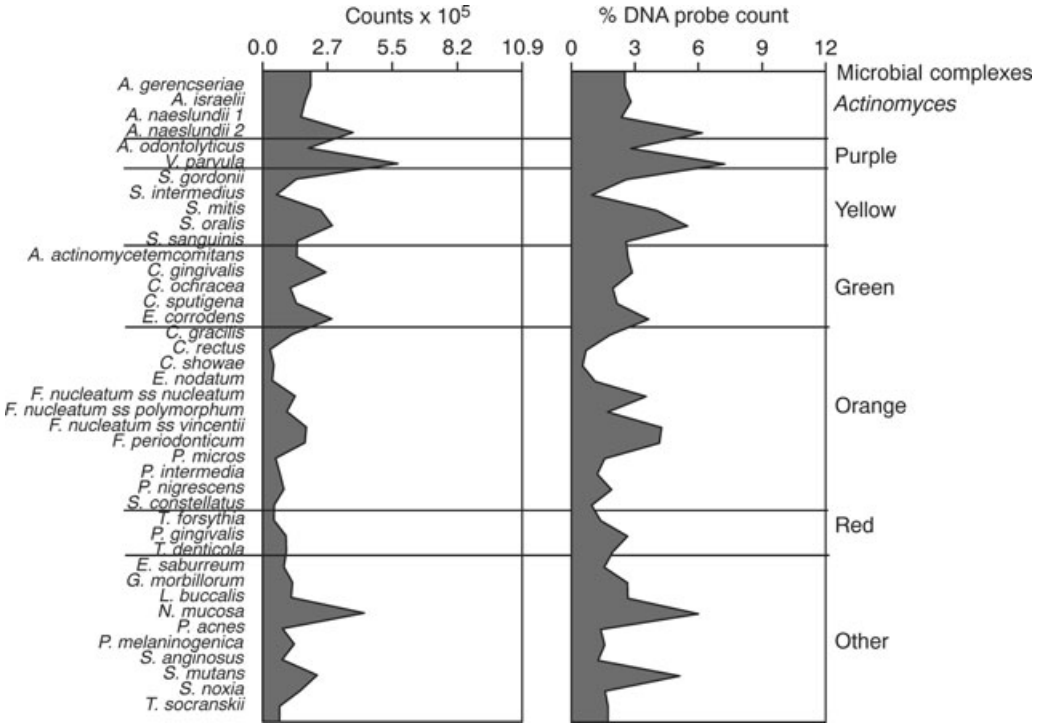

Figure 1 Microbial profiles of mean counts ($\times 10^5$) and mean% DNA probe counts of 41 bacterial species in 61 edentulous subjects. Counts for each species were averaged separately across up to 28 sites in each subject and then across subjects. Similarly, the proportion that each species comprised of the total DNA probe count was determined at each site, and then averaged within and then across subjects. The species were ordered according to the complexes described by Socransky et al.[34]

with the standards on the same membrane. Failure to detect a signal was recorded as zero.

Data evaluation

Microbiological data available for each of the 61 subjects were the counts of 41 test species in "supragingival" biofilm samples taken from the mesial aspect of each denture tooth in each subject. The counts for each species from 28 "supragingival" sites were averaged within each subject and then averaged across subjects. In a similar fashion, the percentage of the total DNA probe count was determined for each species at each site in each subject and averaged within and then across each subject. The mean values for each species were depicted graphically as "microbial profiles" ordered according to the microbial complexes.[34]

Counts and proportions of 41 test species were available for one sample per subject from each of the following oral surfaces: tongue dorsum, tongue lateral, tongue ventral, floor of mouth, buccal, hard palate, vestibule/lip, attached gingiva, and the exterior surface of the denture hard palate as well as the counts and proportions of each species in a sample of unstimulated saliva. The counts for each species were averaged across subjects for each intraoral location. Significance of difference in counts or proportions of each species among intraoral locations were determined using the Friedman test and adjusted for multiple comparisons.[36]

Cluster analysis was performed on the mean proportions of the 41 species in samples from eight soft tissue surfaces, saliva, denture teeth and the exterior surfaces of the hard palate of the dentures. Similarities were computed using the chord coefficient[37] and sorted using an average unweighted linkage sort.[38]

Results

Microbiota of denture biofilm samples

Figure 1 presents the mean counts ($\times 10^5$) and mean proportions of the 41 test species in the biofilm samples from the denture teeth of 61 edentulous subjects. Mean counts of the *Actinomyces* species, *V. parvula*, *Streptococcus* species, with the exception of *S. intermedius*, *C. gingivalis*, *E. corrodens, N. mucosa,* and *S. mutans* were quite high, while mean counts of many of the orange complex species and the entire red complex species were relatively low. The mean proportions followed a similar pattern. A striking feature was the presence of the periodontal pathogens, *A. actinomycetemcomitans* and *P. gingivalis.*

Microbiota of soft tissue and saliva samples in edentulous subjects

Figure 2 presents the mean total DNA probe counts for saliva samples, eight soft tissue surfaces and the polished, and exterior

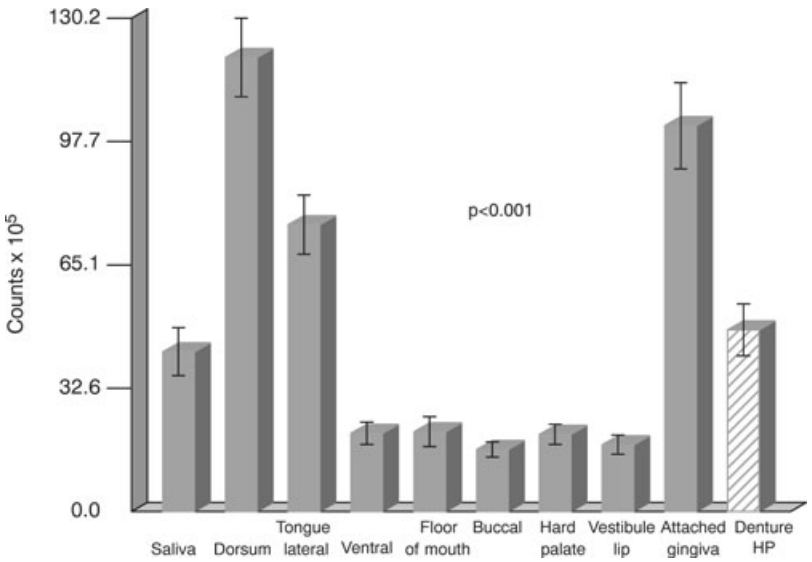

Figure 2 Mean total DNA probe counts ($\times 10^5$, $\pm$ SD) in samples of saliva, eight soft tissue surfaces, and the denture hard palate from 61 edentulous subjects. Total counts were averaged across subjects for each sample location separately. Significant differences among sample locations were sought using the Kruskal–Wallis test and adjusted for multiple comparisons.[36]

surface of the denture palates. Samples from the dorsal surfaces of the tongue exhibited the highest bacterial counts, followed by the "attached gingiva" (fixed keratinized tissue) and the lateral surfaces of the tongue. The lowest mean counts were found in samples from the buccal surfaces and the labial vestibules. Mean proportions of 31 of the test species differed significantly among sample locations, with the exception of *A. naeslundii genospecies 2, S. gordonii, S. sanguinis, C. ochracea, F. nucleatum ss polymorphum, F. periodonticum, S. constellatus, L. buccalis, N. mucosa,* and *S. mutans* (Fig 3). The pattern of colonization differed among species. For example, *S. mitis* and *S. oralis* were found in lower proportions in saliva, the dorsal and lateral tongue surfaces, and the denture hard palates when compared to their proportions on the other soft tissue surfaces. *P. melaninogenica* was found in the highest proportions on the dorsal surfaces of the tongue. *A. odontolyticus, C. sputigena,* and *G. morbillorum* were detected in the highest proportions on the polished surfaces of the denture palates.

Comparison of the microbiota of the tongue dorsum, hard palate, and polished (exterior) denture palate

Figure 4 presents the mean total DNA probe counts in samples from the dorsal surfaces of the tongue, hard palate, and the polished (exterior) surfaces of the denture palate for the 61 edentulous subjects. The total DNA probe count was highest on the dorsal surfaces of the tongue and lowest in samples from the hard palates when comparing the three groups. The total DNA probe count for the polished, exterior surfaces of the denture palates was higher than that seen on the subjects' hard palates. When comparing the three surfaces for the 41 bacterial species, 40 species showed a significant difference among the three groups with p-values <0.001 (Fig 5). The exception was *S. mitis,* which was found in high levels in all three locations. Significant differences in mean proportions were observed for 21 of the test species.

Cluster analysis was employed to group the mean microbial profiles of the sample locations. The technique employed the minimum similarity coefficient and an average unweighted linkage sort using the mean species proportions of samples from saliva, the eight oral soft tissue locations, and the denture palatal surfaces (Fig 6). Two clusters were formed with $>85\%$ similarity consisting of the dorsal and lateral surfaces of the tongue, supragingival plaque, and saliva (cluster 1); and the "attached gingiva" (fixed keratinized tissue), hard palates, labial vestibules, buccal vestibules, ventral surfaces of the tongue, and floor of the mouth (cluster 2). The polished (exterior) surfaces of the denture palates did not cluster with the other sample locations.

Discussion

This study presents cross-sectional data of the microbiota of dentures, oral soft tissues, and saliva of 61 edentulous subjects wearing both maxillary and mandibular complete dentures. All subjects in this investigation had been edentulous for at least 1 year. The "supragingival" plaque composition of the biofilms that formed on eight soft tissue surfaces, polished palatal surfaces (external) of the dentures, denture teeth, and the microbiota of saliva samples were examined between the 61 subjects and then for different intraoral locations within the same subject.

The 41 test species examined in the current investigation were those often found in studies of plaque and soft tissue biofilms in dentate subjects.[10,35] These species were also found in the "supragingival" plaque samples and soft tissue biofilm samples of the edentulous subjects. The results from the current investigation are significant in that much of the recent research has concentrated on the oral health of dentate patients, including examination of the association between oral disease and systemic health. Given the results of the current investigation, it may be important to provide the denture-wearing population care and follow-up similar to their non-denture-wearing counterparts.

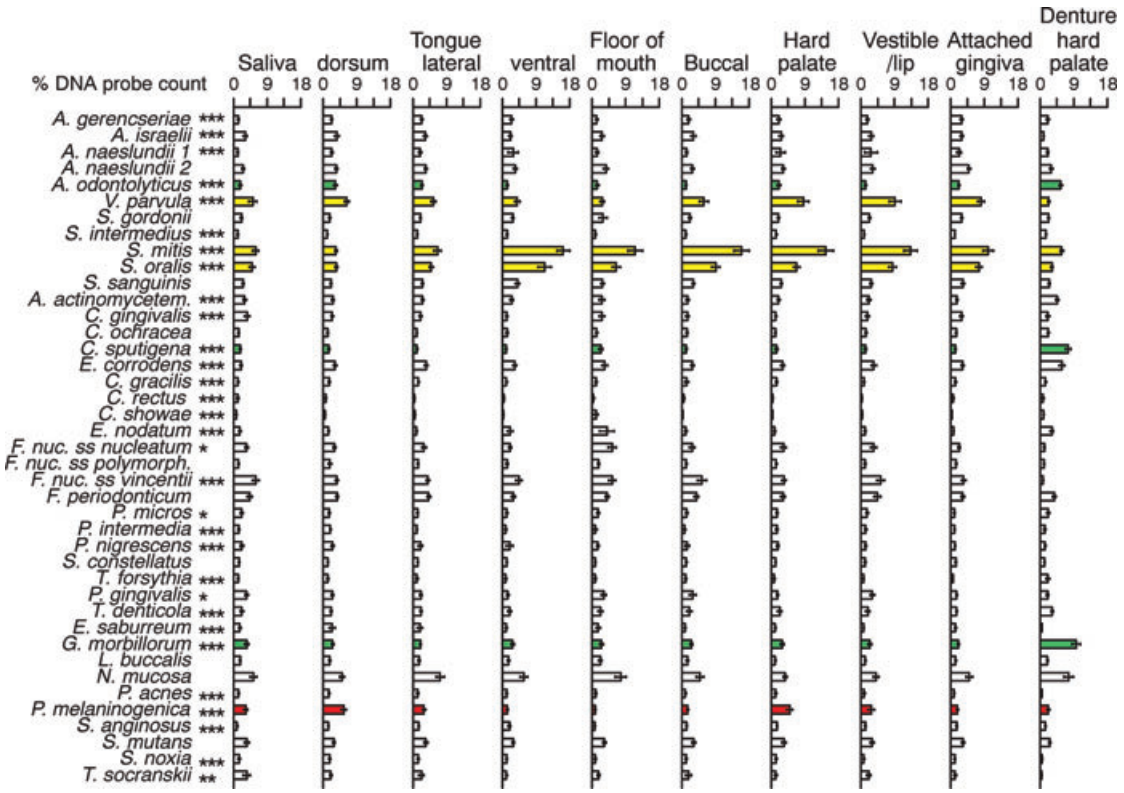

Figure 3 Mean% DNA probe counts (± SD) of 41 species in samples of saliva, eight soft tissue surfaces, and the denture hard palate from 61 edentulous subjects. The proportion that each species comprised of the total DNA probe count was computed and averaged across subjects for each sample location separately. Significant differences among sample locations was sought using the Kruskal–Wallis test and adjusted for multiple comparisons: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. The colored bars represent species that were markedly different among sample locations.

The composition of the microbiota of different soft tissue surfaces, saliva, and the polished (exterior) surfaces of the denture palates was examined for 41 test species. On average, the test species could be found in samples from all locations, although there were marked differences among surfaces in the microbiota with significant differences detected for 31 species. The highest mean total counts and counts of most species were detected on the dorsal surfaces of the tongue. The "attached gingiva" (fixed keratinized tissue), which represented the anterior ridges of edentulous maxillae, harbored the second highest mean counts, while the lowest counts were found on buccal mucosal surfaces and the labial vestibules.

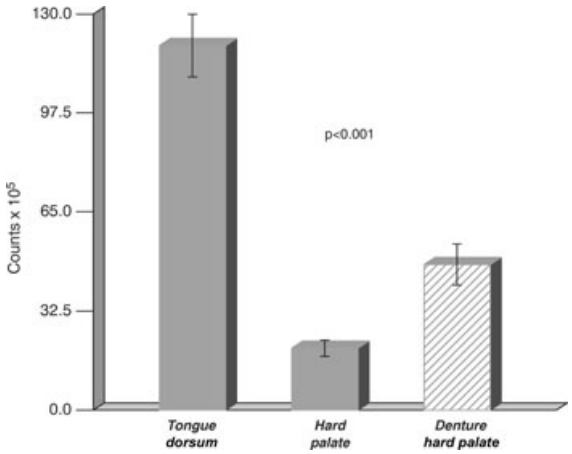

Figure 4 Mean total DNA probe counts ($\times 10^5$, ± SD) in samples from the tongue dorsum, hard palate, and the exterior surface of the denture hard palate from 61 edentulous subjects. Total counts were averaged across subjects for each sample location separately. Significant differences among sample locations were sought using the Kruskal–Wallis test and adjusted for multiple comparisons.

Cluster analysis of the mean microbial profiles of the samples demonstrated that the microbiota of the lateral and dorsal surfaces of the tongue and saliva were similar to one another; however, they were different from the microbial profiles of the remaining six surfaces. This is similar to the findings in dentate subjects of Mager et al,[10] who found the proportions of bacterial species differed markedly on different intraoral surfaces and that the microbiota of saliva was most similar to that of the dorsal and lateral surfaces of the tongue. The microbiotas of the soft tissues resembled each other more than the microbiotas that colonized the teeth both above and below the gingival margin.

An important finding of the current investigation was that the periodontal pathogens *A. actinomycetemcomitans* and *P. gingivalis* were found in both the supragingival and soft tissue samples of the edentulous subjects. This finding is in contrast

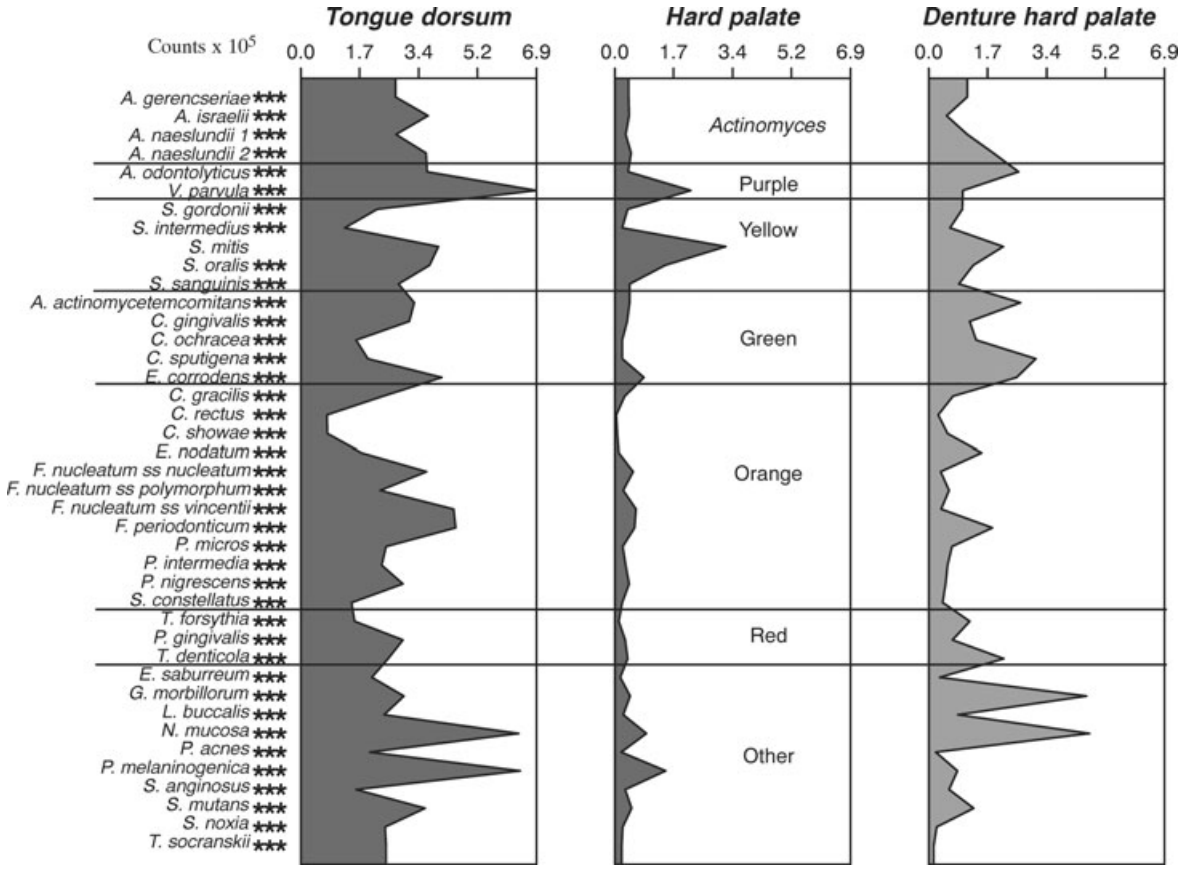

Figure 5 Mean counts ($\times 10^5$) of 41 species in samples from the tongue dorsum, hard palate, and the exterior surface of the denture hard palate from 61 edentulous subjects. Counts were averaged across subjects for each sample location separately. Significant differences among sample locations were sought using the Kruskal–Wallis test and adjusted for multiple comparisons: $^{***}p < 0.001$.

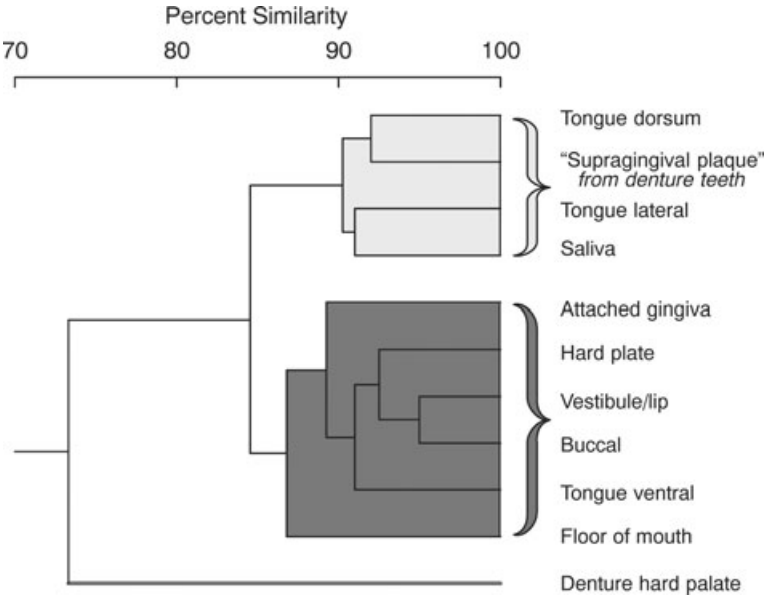

Figure 6 Dendrogram of a cluster analysis of the mean species proportions in samples from saliva, eight oral soft tissue surfaces, the denture teeth, and the exterior of the denture hard palate in 61 edentulous subjects. A minimum similarity coefficient and an average unweighted linkage sort were employed. Two clusters were formed at >85% similarity. The exterior surface of the denture hard palate did not cluster with the other sample locations.

to reports in the literature that suggested that these species disappeared from the oral cavity after extraction of all teeth and did not reappear even when hard surfaces, such as complete dentures, were provided.[22,23] Furthermore, several investigators have reported a strong association between these periodontal pathogens and various systemic diseases,[24-31] although only biofilms in dentulous subjects were examined. The results of the current study suggest that complete denture patients may also be at risk for systemic disease from these two periodontal pathogens if they gain access to the circulation via trauma or pathology of the oral mucosa.

An interesting comparison was that of the microbiota of the tongue dorsum and the outer polished surface of the denture palates. It might be expected that the levels and types of bacteria seen on the dorsum of the tongue would be similar to those found on the palatal surfaces (polished, external) of complete dentures; however, in the cluster analysis of the microbiota, the microbiota of the denture palate did not cluster with that of any of the other surfaces. The effect of the different types of surfaces for initial attachment is the probable cause for such a difference in the levels and types of species on the two surfaces. A larger number of bacteria will likely adhere to the tongue due to papillae providing an increased surface area and possibly a more consistent moist environment. The highly polished external surface of the denture palates appeared to minimize colonization, leading to fewer microbes.

There were certain limitations associated with this study. One limitation was that probes to only 41 microbial species were employed. While this is far greater than any previous study, it is recognized that a substantial portion of the microbiota may not be represented. It has been suggested that over 700 species, many of which are uncultivable, can colonize the oral cavity;[9] however, the 41 probes used in this study have been shown to account for about 50% to 55% of the biomass in biofilm samples from dentate subjects.[35] Additional probes that should be considered in future denture studies would be *Candida albicans* and *S. salivarius.*

One might also argue that the microbiota seen in the denture-wearing subjects in this study could be attributed to the presence of "hard-tissue," in the form of complete dentures. It would be interesting to examine the composition of biofilms on the soft tissues of subjects with no natural teeth remaining and no hard-tissue replacements present in the form of either complete dentures or dental implants.

With a rise in the size of the elderly population, one can now also expect an increase in complete-denture patients, and therefore, it is critical for oral healthcare providers to pay equal attention to the dental needs of edentulous patients. This investigation provided an extensive examination of the microbiota of a limited number of edentulous, denture-wearing subjects. It is hoped that these data could have an impact on oral healthcare in complete denture patients.

Conclusions

The results of this study demonstrated that the periodontal pathogens *A. actinomycetemcomitans* and *P. gingivalis*, which were thought to be eliminated with the extraction of all natural teeth, were seen in significant numbers in the edentulous subjects. Microbial profiles differed according to the specific surfaces for colonization. The microbiota of the denture teeth, lateral and dorsal surfaces of the tongue, and saliva were similar to one another, and differed from the microbiota of the other six soft tissue surfaces and denture hard palates, which formed distinct cluster groups.

When comparing the mean total DNA probe counts among the dorsal surfaces of the tongue, denture palates, and the subjects' palates, the highest mean counts were found on the tongue dorsum, followed by the polished (exterior) surface of the denture palate, and were lowest on the hard palate.

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