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Simplified Technique for Sonication and Processing of Dental Plaque Samples

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A simplified method for processing dental plaque samples was devised and compared to previously used methods. Analysis of 36 nonstandardized subgingival plaque samples from various states of periodontal health and disease and 12 standardized supragingival plaque samples demonstrated that there was no significant difference between the recovery offered by the two techniques. Comparable recovery, increased convenience, and adaptation to the clinical setting suggests that implementation of this simplified technique may be of great value.

One of the major goals of periodontal research is the characterization of microorganisms resident in the gingival sulcus and periodontal lesion (5). Recent technical and conceptual developments have greatly improved the ability to isolate and cultivate bacteria colonizing the tooth surface and the gingival sulcus. For complete characterization, whole plaque samples must be dispersed to break up bacterial cell aggregates and diluted to reduce the number of bacteria which will be characterized.

Although the dispersive ability of sonic disruption was originally described by Williams and Eikenberg (7), very little information is available regarding its use in oral microbiology research. The goal of sonication as a method to disperse plaque samples is to effectively break up bacterial cell clumps while minimizing the destruction of viable cells. Robrish et al. (6) demonstrated that gram-negative bacteria are more sensitive to sonic disruption than gram-positive organisms, suggesting that sonic disruption of plaque is a technique for the enrichment of gram-positive organisms rather than a technique for gaining representative samples. Manganiello et al. (2) compared anaerobic and aerobic sonic dispersion of supragingival plaque. Up to 75% of the supragingival bacteria samples were recovered by using anaerobic sonication and prereduced anaerobically sterilized (PRAS) transport solutions. Continuous anaerobiosis was achieved by flushing all PRAS solutions with oxygen-free gas through sterilized cannulas introduced into the PRAS vials.

Even though these methods were improvements over more conventional techniques not employing anaerobic techniques of sampling, dispersion, dilution, plating, and cultivation, they are inconvenient. In general, these techniques are elaborate, expensive, and time consuming, requiring several technicians and complex equipment. A new, simplified technique was devised for plaque dispersion and processing which offered many advantages over the previously used technique. The purpose of the present investigation was to determine whether the simplified method of plaque dispersion and processing offered comparable recovery of microorganisms to the previously used technique.

MATERIALS AND METHODS

Plaque samples. A total of 36 plaque samples were taken from subgingival sites of 16 patients exhibiting different periodontal conditions, including patients with periodontal abscesses, aged but healthy patients, patients with pregnancy gingivitis, and those with idiopathic juvenile periodontitis (periodontosis) (Table 1). Eighteen samples were processed with the previously used techniques, whereas the remaining 18 samples were processed using the simplified methods.

Twelve standardized supragingival plaque samples were also taken from the first molar in one patient, so that a more accurate comparison of the sampling techniques could be made. Samples were standardized in the following manner. Three adjacent teeth, the maxillary second premolar and the first and second molars, were given a thorough prophylaxis by a dental hygienist. Subsequently, these teeth were not brushed for a period of 72 h, when a supragingival plaque sample was removed from 1 mm^2 of the buccal surface of the first molar with a modified gas-flushed syringe (3). The area was recleaned, and the plaque was again allowed to develop for 72 h when a second sample was taken from the same location. The two samples were processed using either the simplified or previously used techniques. This was repeated six times in one individual who exhibited periodontal health (gingival index = 0), so that the plaque samples would be as homogeneous as possible, facilitating a more accurate

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comparison of the sampling methods.

Sample processing. The method previously used for processing plaque samples involved the use of rubber-stoppered 15-ml vials as the transport container of the PRAS holding solution (Fig. 1). Plaque samples were placed into the dispersion solution which contained 1/4-strength Ringers solution supplemented with 0.0001% resazurin, 0.05% cysteine, and 0.1% sodium metaphosphate to prevent bacterial reaggregation after sonic dispersion (2). To maintain continuous anaerobiosis, sterilized gassing cannulas delivered oxygen-free gas into the PRAS solutions whenever the rubber stoppers were removed (2, 3). Plaque dispersal was accomplished by introducing the sterile end of a Branson sonic probe into the PRAS solution for a duration of 5 s at a frequency of 6 μ. Serial dilutions of the dispersed plaque were made by transferring 1-ml portions of dispersion solution into 9 ml of PRAS full-strength Ringers solution with serological pipettes. Anaerobiosis was maintained in the dilution tubes by using additional gassing cannulas.

The simplified method for plaque processing involves the use of septum-fitted serum vials (M. Wei-

TABLE 1. *Source and distribution of patients*

Patient type	No. of patients	
	New method	Old method
Aged, healthy	3	5
Periodontal abscess	2	3
IJP[a]	1	—
Pregnancy gingivitis	2	—

[a] IJP, Ideopathic juvenile periodontitis (peridontosis).

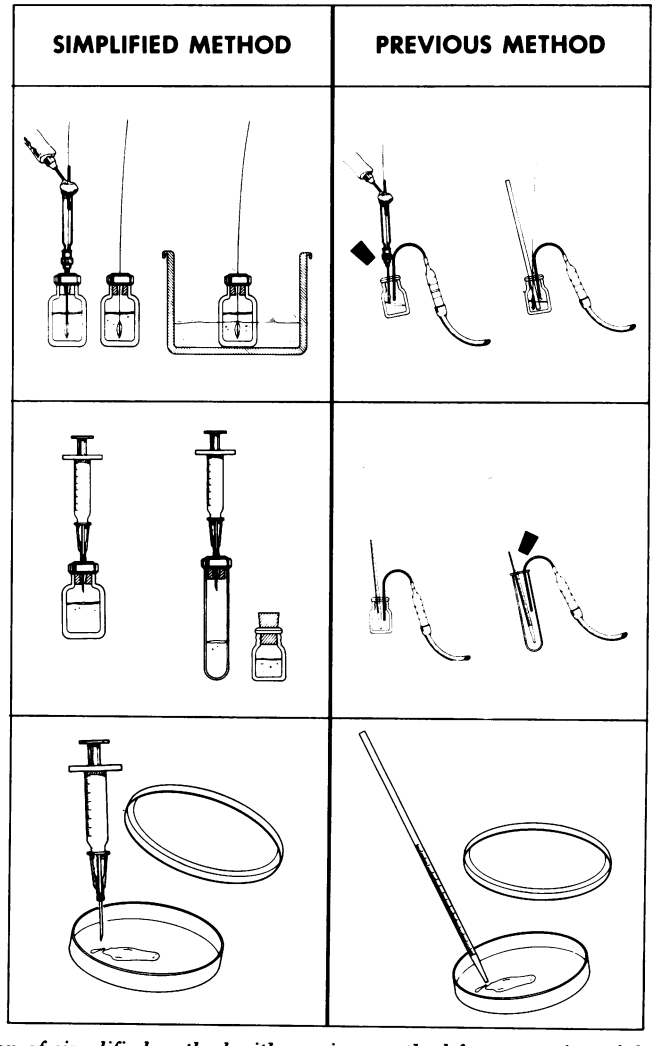

FIG. 1. *Comparison of simplified method with previous method for processing of dental plaque samples, showing dispersion (top), dilution (middle), and plating (bottom).*

ner, V. Grinenko, B. Kaplan, and M. G. Newman, J. Dent. Res. 57:676) (Fig. 1). After the sample is taken it is withdrawn into the syringe, when the blunt needle of the sampling device is replaced with a sterile sharp needle. The needle can puncture the septum, allowing the sample broach to enter the PRAS solution. The syringe and needle are retracted, leaving the broach in the PRAS solution with the septum forming a gas-tight seal around it. The broach is removed after sonic disruption (see below) or at the time the serum bottle is disassembled or discarded. These procedures achieve anaerobiosis as measured by resazurin indicator without the need for a complicated gassing apparatus. Plaque dispersal was then accomplished by placing the vial in a 37°C water bath contained in an ultrasonic dental instrument cleaner (Model T14, L&R) for 30 s. Subsequently, serial dilutions were made in 20-ml septum-fitted test tubes which also contained PRAS full-strength Ringers solution. Portions, 0.1 ml each, were then plated onto triplicate Trypticase soy agar plates with 5% sheep's blood (Baltimore Biological Laboratories) and 0.5% hemin and 0.05% menadione. For the previously used techniques, this was accomplished by using a pipette, whereas tuberculin syringes were used with the simplified methods. The plates were incubated at 37°C for 5 days in air, air plus 5% CO_2, and 10% CO_2–10% H_2–80% N_2 to quantitate organisms obligate to those atmospheres. Colony-forming units were counted for plates containing between 30 and 300 colonies. Viable counts were calculated by multiplying the colony-forming units by the appropriate dilution factors.

All colonies on one quadrant of the plate (described above) were picked as representative of each particular sample. These isolates were then subcultured and reincubated in the same atmosphere as orginally isolated. All bacteria isolated from the anaerobic atmosphere and those obligate to the air or air plus CO_2 environments were identified on the basis of colonial and cellular morphology, Gram stain, and atmospheric tolerances.

RESULTS

The mean viable count for the subgingival plaque samples obtained from all periodontal conditions for the simplified technique was 1.34×10^6 organisms, as compared to 1.54×10^6 organisms for the previous methods (Table 2). The Student's *t* test demonstrated that the difference between the mean recovery counts was not significant (statistical significance at the 5% level of confidence) (Table 3). The anaerobic and gram-negative nature of the subgingival plaque samples was analyzed to determine whether a full range of organisms, typically found with the previously used methods, could be isolated by using the newer techniques (Table 3). No significant differences were found between the mean recovery of anaerobes or gram-negative organisms for the two sampling techniques. Analysis of variance (1) also demonstrated no significant differences between the two methods for the anaerobic or gram-negative distribution.

TABLE 2. *Recovery of bacteria from subgingival plaque*

Method	Sample source	Bacteria		
		Viable counts	Anaerobic (%)	Gram negative (%)
Previous	Aged, healthy patient	1.36×10^6	12.5	17.5
		1.20×10^4	17.5	17.5
		2.36×10^4	80.6	71.0
		1.00×10^5	98.0	70.2
		2.12×10^4	20.0	45.0
		1.64×10^5	74.3	74.3
		2.03×10^6	65.8	63.6
		1.04×10^6	44.0	60.0
		1.04×10^6	52.5	42.5
		1.20×10^6	45.7	47.8
		2.00×10^3	5.0	0.0
		3.00×10^6	77.5	72.5
		2.56×10^6	39.5	55.8
	Periodontal abscess	3.20×10^5	90.5	48.1
		1.28×10^6	74.9	44.3
		1.92×10^5	62.5	82.5
		1.32×10^6	88.5	92.3
		1.20×10^7	34.4	53.1
	Avg	1.54×10^6	54.7	53.2
Simplified	Aged, healthy patient	1.12×10^6	81.3	96.9
		1.92×10^5	77.7	77.7
		1.08×10^6	12.0	12.0
		8.80×10^3	8.0	8.0
		1.04×10^5	20.0	20.0
		1.20×10^6	6.7	6.7
		2.00×10^5	10.0	10.0
		1.12×10^5	16.0	33.3
	Periodontal abscess	9.60×10^3	75.0	82.1
		4.00×10^5	42.3	47.4
		2.50×10^5	47.4	31.6
	IJP[a]	4.80×10^3	20.8	70.8
		1.00×10^7	96.3	61.5
	Pregnancy gingivitis	1.50×10^6	45.5	36.4
		2.00×10^6	42.3	50.0
		3.00×10^6	31.1	44.8
		1.00×10^6	51.2	42.1
		2.00×10^6	48.2	51.3
	Avg	1.34×10^6	40.7	43.5

[a] IJP, Ideopathic juvenile periodontitis (periodontosis).

To more precisely determine the ability of the two techniques to give comparable results, 1,080 bacterial isolates were characterized, and shown in Table 4. Gram-positive cocci, which form the most difficult to disperse cell aggregates, accounted for 30.5% of the samples processed by the previously used techniques, and 43.1% of the samples processed with the simplified methods. Student's *t* test and analysis of variance demonstrated no significant difference (at the 5% level of confidence) between the mean recoveries of gram-positive cocci or the distributions. Gram-negative rods, shown to be most sonic-sensitive to disruption, were recovered as 46.5% of the plaque samples for the previously used

TABLE 3. *Student's t test and analysis of variance between the recovery of microorganisms by two sampling techniques*[a]

Plaque sample	Recovery terms	Degrees of freedom	Observed Student's *t* score	Critical $t_{0.05}$	Observed *F* score	Critical $F_{0.05}$
Standardized, supragingival	Viability counts	10	0.117	1.81	1.03	5.05
	% anaerobic	10	0.1 34	1.81	1.72	5.05
	% gram negative	10	0.264	1.81	1.30	5.05
Nonstandardized, subgingival	Viability counts	34	0.235	1.69	1.41	3.24
	% anaerobic	34	1.47	1.69	1.07	3.24
	% gram negative	34	1.15	1.69	1.27	3.24

[a] Note that significance is determined by the observed *t* score or *F* score exceeding the $t_{0.05}$ or $F_{0.05}$ critical values (1). No data were significant at the 5% level of confidence.

TABLE 4. *Comparison of the differential recovery between two sampling techniques: nonstandardized plaque*

Sampling method	Gram-positive cocci	Gram-positive rods	Gram-negative cocci	Gram-negative rods
Previous	30.5%	16.5%	6.7%	46.4%
Simplified	43.1%	13.4%	4.2%	39.3%
t Score[a]	1.36	1.12	1.52	1.06
F Score[a]	1.15	1.62	1.23	1.47

[a] $t_{0.05}$ critical value = 1.69; $F_{0.05}$ critical value = 3.24. Note that significance is determined by the observed *t* score or *F* score exceeding the $t_{0.05}$ critical value or the $F_{0.05}$ critical value. No data were significant at the 5% level of confidence.

methods and as 30.3% for the simplified techniques. No significant differences were found for the recovery of gram-negative rods using the Student's *t* test and analysis of variance.

The recovery of organisms from the standardized plaque samples, using the simplified method, averaged 3.4×10^7 organisms, whereas the recovery for the previously used technique was 3.6×10^7 organisms (Table 5). Anaerobic recovery averaged 48.8% for the simplified technique and 47.5% for the previous methods. Recovery of gram-negative bacteria was 46.2% for the simplified methods and 48.9% for the previously used techniques. Student's *t* test and analysis of variance were calculated on the results of viable counts and anaerobic and gram-negative recovery, but no significant differences between sampling techniques could be found (at the 5% level of confidence).

DISCUSSION

The results indicate that the mean recovery rates and distribution of microorganisms as measured by viability counts and percentage of anaerobic and gram-negative organisms were comparable for the simplified technique and previously used techniques of plaque processing.

TABLE 5. *Recovery of bacteria from standardized supragingival plaque*

Method	Sample no.	Bacteria		
		Viable counts	Anaerobic (%)	Gram negative (%)
Previous	1	7.7×10^6	50.1	57.6
	2	9.1×10^6	62.1	59.1
	3	7.4×10^7	45.4	26.3
	4	6.4×10^7	64.1	71.6
	5	7.1×10^6	27.1	36.7
	6	5.4×10^7	36.4	41.1
	Avg	3.6×10^7	47.5	48.9
Simplified	1	9.1×10^6	64.4	51.7
	2	1.5×10^7	51.2	42.1
	3	5.1×10^7	30.1	28.1
	4	3.5×10^7	76.4	79.1
	5	9.7×10^6	28.2	31.2
	6	8.4×10^7	42.7	38.7
	Avg	3.4×10^7	48.8	46.2

This was true for standardized as well as nonstandardized plaque samples. Comparability was shown by the fact that the recovery of microorganisms by the simplified techniques was not significantly different from the recovery offered by the previously used methods. The additional characterization of bacterial isolates showed that even the most sonic-sensitive organisms, such as gram-negative rods, can be readily isolated by the simplified method of plaque processing.

In analyzing these results, it is important to point out the inaccuracies of comparing unstandardized plaque samples, as well as the problems associated with methods of plaque standardization. The unstandardized plaque samples were taken from patients with various periodontal conditions, including those with idiopathic juvenile periodontitis (periodontosis), those with periodontal abscess, aged but healthy patients, and those with pregnancy gingivitis. Since recent

studies have shown that qualitative and quantitative differences exist between the bacterial flora associated with these different periodontal conditions, direct comparison of sampling techniques cannot be accurately assessed by using samples from different clinical entities. However, the results from the two methods were similar to each other, indicating the ability to accurately recover the resident microorganisms associated with the particular state of periodontal health or disease.

Although exact quantification of the supragingival plaque samples was not achieved (4), the standardization procedure employed in this study gave homogeneous samples. Comparable recovery of microorganisms from these samples using both methods of plaque processing demonstrates the similarity of the two methods.

The results suggested that the simplified technique of plaque dispersion and processing affords comparable recovery to the previously used methods. The primary reason for employing this technique is the increased convenience of this method of plaque processing. Since the previous method required the removal of the rubber stoppers for all dispersion and dilution procedures, it may be more susceptible to contamination (M. G. Newman, unpublished data). With the vast expansion of knowledge in oral microbiology, a convenient and accurate technique for plaque sampling, readily adaptable to the clinical setting, will be of great value.

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