Reduction of formation of denture plaque by a protease (Alcalase®)

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The efficacy of a protease preparation (Alcalase®, Novo, Denmark) in preventing accumulation of plaque on the fitting surface of complete upper dentures was examined in a double-blind study. Seventeen denture wearers with clinically healthy oral mucosae participated in the study. The protease was dispensed in dissolvent tablets containing 15, 30, 60, 100, 300, 500, or 1000 mg of the enzyme. Enzyme tablets or placebo tablets were dissolved in 150 ml water at 50°C, and the dentures were soaked once daily for 15 min. Denture soaking was performed with or without brushing. The study consisted of 14 1-week periods. At the start of each period the dentures were cleaned by brushing until they showed no visible plaque after staining. At the end of each period plaque was stained and the dentures were photographed. The photographs were projected, and the percentage area of the fitting surface showing plaque deposits was scored with 10% intervals. Statistical analysis showed that there was a significant reduction of plaque scores with increasing enzyme concentration. The most marked reduction in plaque formation was seen when mechanical cleansing was combined with immersion in a 500-mg enzyme solution. At a concentration of 300 mg (or more) the enzyme immersion was as efficient as mechanical cleansing in preventing denture plaque accumulation. □ *Plaque; dentures; plaque control; enzymes*

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Microbial plaque on the fitting surface of dentures may cause denture stomatitis, which is a frequent complication of the wearing of removable dentures (4). Electron microscopical studies have shown that denture plaque is structurally similar to dental plaque and is composed mainly of gram-positive cocci and rods (18). However, in denture stomatitis yeasts are usually prevalent and apparently play a significant role as pathogens (2, 4, 10). Quantitative cultural studies of 1-week-old denture plaque have shown that denture plaque is a rapidly accumulating microbial plaque (9).

Clinical studies have shown that the control of denture plaque is essential to obtain and maintain a healthy oral mucosa in denture wearers (1, 7). Recently, the efficacy of certain enzyme preparations, i.e. mutanase and protease (Neutrase®), was tested (5, 8). The enzyme preparations reduced accumulation of denture plaque to such an extent that the state of the palatal tissues in patients with denture stomatitis improved significantly. The present study was designed to test prevention of denture plaque accumulation by another protease, Alcalase®, which was less expensive and more stable than the mutanase/Neutrase preparation.

Materials and methods

Patients

Seventeen subjects wearing complete dentures participated in the study. Nine were women and eight men, with a mean age of 61.4 ± 7.6 years and a mean denture experience in the maxilla of 21.6 ± 8.8 years. The subjects were selected for the study by the following criteria: 1) wearing complete dentures for more than 10 years; 2) showing a clinically healthy palatal mucosa; and 3) showing plaque on the fitting surface of the upper denture, as visualized by a disclosing solution.

Enzyme

The enzyme used in the experiments was Alcalase® (Novo Industry A/S, Bagsvaerd, Denmark), which is a subtilopeptidase (EC 3.4.21.14) formerly known as subtilisin type Carlsberg. This is a peptide peptidohydrolase that was formed during submerged fermentation of a strain of *Bacillus licheniformis*.

The enzyme is a rather unspecific protease, hydrolysing a wide variety of proteins with optimum activity at pH 8–9 (50% activity at approximately pH 5 and 12) and at about 60°C (50% activity at approximately 50°C and 70°C). It is extremely stable in solution, the activity being virtually unchanged after 60 min at 50°C, and showing a 50% reduction after 60 min at about 65°C. The Alcalase used in the present experiments was a highly purified preparation but otherwise similar to Alcalase preparations used in food industry and added to self-acting detergents. The enzyme was dispensed as dissolvent tablets containing a citrate buffer, sodium bicarbonate, and magnesium stearate. A detergent was added to reduce surface tension of the solutions. The composition of the detergent was 15% non-ionic surfactant, 50% sodium tripolyphosphate, 2% sodium silicate, 23% natrium sulfate, and 10% water. Enzyme tablets were prepared as dissolvent tablets with 15, 30, 60, 100, 300, 500, or 1000 mg of the enzyme + 1000 mg of detergent. Control tablets without enzyme, but otherwise with a similar composition, were prepared with or without detergent. Tablets containing 60 or 500 mg of the enzyme were also prepared without detergent.

Design of the study

The subjects participated in a total of 14 treatment periods, to test the clinical effect of the different enzyme concentrations and the various denture cleansing procedures. Since mechanical cleansing without enzyme treatment was performed in two different treatment periods, a total of 13 different treatments were tested (Table 1). Eleven subjects participated in all 14 treatment periods, 1 completed 11 treatment periods, and 5 subjects participated in only 7 treatment periods. The number of subjects participating in each of the 14 treatment periods is shown in Table 1. The sequence of treatments for each subject was randomized, and the subjects were not aware of which treatment they were receiving in the individual treatment periods.

Each tablet was dissolved in 150 ml of water at 50°C, and the patients were instructed to soak the dentures once daily for 15 min in a freshly prepared solution. Brushing was not allowed during treatment periods B–K and M (Table 1). In periods A, L, and N the patients were told to brush

Table 1. Experimental design of the study, indicating the different denture cleansing procedures tested

Period	No. of subjects	Alcalase, mg/150 ml	Detergent, mg/150 ml	Denture brushing
A	15	—	—	+
B	14	0	0	—
C	14	0	1000	—
D	14	15	1000	—
E	14	30	1000	—
F	14	60	1000	—
G	14	60	0	—
H	15	100	1000	—
I	15	300	1000	—
J	15	500	1000	—
K	15	500	0	—
L	14	—	—	+
M	14	1000	1000	—
N	14	500	1000	+

their dentures 'in the usual manner' (Table 1). No particular brushing technique was introduced. At the start of each treatment period the fitting surface of the upper denture was brushed professionally until it did not show plaque after staining; at the end of each treatment period plaque was recorded.

Identification and quantitation of denture plaque

The upper denture was rinsed under tap water and the fitting surface was stained with a 0.3% aqueous solution of proflavine monosulfate (6); rinsing under tap water was repeated, and the fitting surface of the denture was photographed (Medical Nikkor®, Kodak Ektacrome® ER 135-36), using a fixed object/film distance with standardized exposure time and photographic technique. The photographs were projected at ×10 magnification, and the percentage area of the fitting surface showing plaque deposits was scored with 10% intervals from zero to 100%. In addition, coded photographs of the individual dentures were ranked according to increasing amounts of plaque, and a mean rank was calculated for each period. The photographs were studied independently by two persons, who were unaware of the treatment received.

Statistical analysis

Statistical analysis of the percentage area covered by plaque was performed, using both analysis of variance and non-parametric tests (Cochran's Q-test and Friedman's test). The ranking of dentures according to increasing amount of plaque was analyzed statistically, using the same non-parametric tests as mentioned above. Since both analysis of variance and non-parametric tests of percentage area covered by plaque gave identical results, only results from analysis of variance are presented. Furthermore, since only minor differences were found in the scoring of the two examiners, the results are based on pooled estimates from the two examiners.

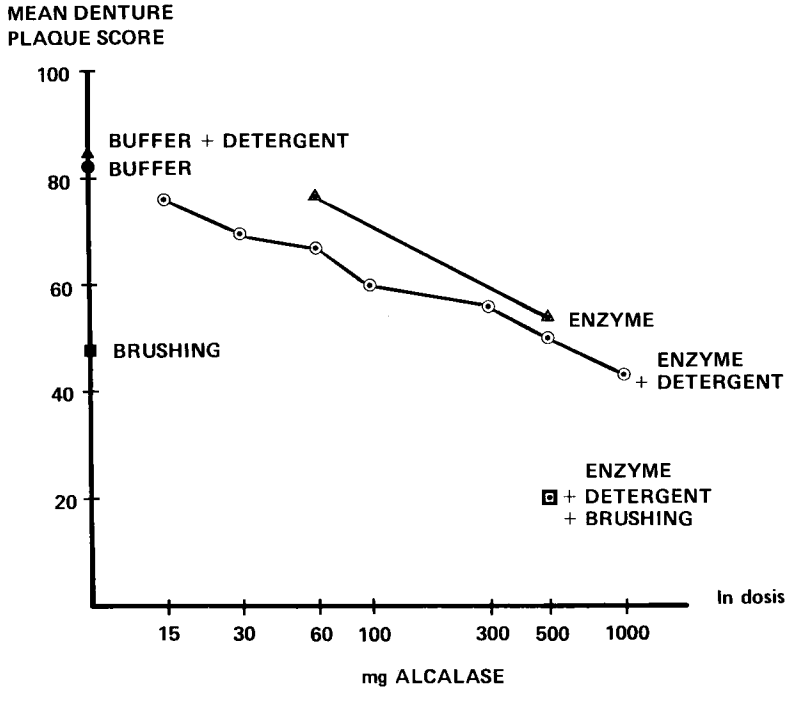

Fig. 1. Mean plaque scores after denture cleansing for 1 week, using the various cleansing schedules and enzyme concentrations. The results are based on pooled estimates from two examiners.

Results

Fig. 1 and Table 2 show the mean area of the surface which was covered by plaque. The mean values are adjusted after estimation of missing data for those patients who did not complete the entire study. There was a consistent and highly significant reduction of plaque scores with increasing enzyme concentration ($P<0.001$). Brushing and immersion in a 500-mg enzyme solution produced an equivalent reduction of the mean area covered by plaque as compared with immersion in placebo from about 80% to about 50%. Furthermore, immersion in a 500-mg enzyme solution + brushing produced an additional reduction of the percentage of the mean area covered by plaque from about 50% to about 20%; indeed, in five subjects the fitting surface of the denture showed no visible plaque after this cleansing procedure.

Table 3 shows that the effect of mechanical cleansing and the effect of enzyme concentration was statistically significant. Furthermore, Fig. 1 shows that the effect of adding base to the enzyme at two different enzyme concentrations (60 mg and 500 mg) increased the effect of the enzyme. Table 3, however, shows that no effect of the base *per se* and no statistically significant effect of addition of base to enzyme at these two concentrations could be found. The most marked effect on plaque formation was found when mechanical cleansing was combined with 500 mg enzyme. No statistically significant interaction was, however, observed between mechanical cleansing and enzyme.

When the effect of mechanical cleansing was compared with that of immersion with different enzyme concentrations, the enzyme concentration had to be increased to 300 mg (or more) for immersion to be as efficient as mechanical cleansing in preventing denture plaque accumulation (Table 4).

Discussion

The present study has shown that it is possible to control denture plaque development by immersing dentures in an Alcalase solution for a period of 15 min once daily. Thus, a solution containing 300 to 500 mg of the enzyme per 150 ml was as efficient as routine brushing, and the combination of immersion in the enzyme solution and brushing reduced plaque formation very markedly.

With the plaque score index a significant dose-response relationship was found. The reliability of this index for the present material has been studied by testing inter- and intra-examiner agreement (15). The results indicated that the reliability of this plaque scoring system was very high, although the assessment of the area covered

Table 2. Mean percentage area of the fitting surface of the upper dentures covered by plaque after completing the various treatment periods. Pooled estimates for two observers. Means corrected for missing values

Mechanical cleansing		−	−	+	+
Detergent		−	+	−	+
Enzyme, mg	0	83%	84%	48%	
	15		76%		
	30		69%		
	60	77%	68%		
	100		59%		
	300		57%		
	500	54%	51%		20%
	1000		43%		

Table 3. Analyses of variance showing the effect of the three main factors (mechanical cleansing, detergent, and enzyme) and their interactions. Pooled estimates of two examiners

Source of variation	Degrees of freedom	S^2
A. Mechanical cleansing	1	71048***
B. Detergent	1	1308
C. Enzyme concentration	7	12610***
A × (B,C)	1	382
B × C	2	662
A × (B,C) + B × C	3	753
Experimental variation	172	920

*** $P < 0.001$.

by plaque was made without any actual measurements.

Earlier studies in humans and animals have shown that dental plaque accumulation to some extent could be prevented by mutanase, an enzyme with the potential to split α-1,3 linkages of glucan (12, 14). Furthermore, it has been shown that denture plaque accumulation was reduced by immersing dentures in a mutanase/protease solution (5, 8). The Alcalase used in the present study is a stable, efficient, and relatively inexpensive proteolytic enzyme. It was assumed that this enzyme might have the potential to disintegrate glycoproteins of the acquired pellicle and the plaque matrix and thereby impede colonization of the denture surface by microorganisms. It is the general consensus that the tooth pellicle is composed mainly of glycoproteins that are of salivary origin and that specific glycoproteins are dominant in the film formation (3, 17). Furthermore, salivary glycoproteins are involved in the process of bacterial aggregation (11, 13). The chemical composition of the denture pellicle and plaque matrix is not completely known, but seems to resemble that formed on tooth enamel (16). Furthermore, the ultrastructure of the plaque matrix and the microflora of denture plaque resemble tooth plaque (9, 18). It is reasonable to assume that the clinical effect of Alcalase is, in fact, due to a degradation of the acquired pellicle and the plaque matrix. This is supported by the patients' observation that the polished denture surfaces became smooth after immersion in solutions containing higher enzyme concentrations (unpublished observations).

The slope of the dose-response curve indicates that the optimal concentration of the enzyme had not been reached. Furthermore, some of the patients displayed relatively extensive accumulations of denture plaque even when enzyme treatment and brushing were combined. It is likely that surface irregularities of the dentures and inefficient brushing technique are causes for the inter-individual variations in degree of plaque formation. From a clinical point of

Table 4. Analysis of variance showing the difference between the effect of mechanical cleansing and immersion in various enzyme concentrations. Pooled estimates of two examiners

Source of variation	Degrees of freedom	S^2
100 mg + detergent	1	5280*
300 mg + detergent	1	3244
500 mg + detergent	1	1096
1000 mg + detergent	1	1036
Experimental variation	172	920

* $0.01 < P < 0.05$.

view it is not realistic to increase the enzyme concentration above 300 to 500 mg/150 ml to obtain a further reduction of plaque formation. Another possibility is to increase the immersion time, e.g. 2×15 min or 1×30 min daily. Furthermore, immersion in the enzyme solution should be supplemented by brushing, either by professionals or by the patient after careful instruction. Finally, clinical and technical procedures in denture treatment should aim at producing dentures with smooth surfaces to facilitate denture cleansing.

In conclusion, the results of the present study indicate that a commercial denture-cleansing product based on Alcalase would be a significant adjunct to denture brushing. As such, an enzymatic cleanser could facilitate and make possible an acceptable denture hygiene in at-home or institutionalized subjects wearing complete dentures, overdentures, or removable partial dentures.

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