

Full Length Research Paper

# Tissue inflammatory response and salivary *Streptococcus mutans* count with three different denture cleansers

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This study was conducted to detect the clinical effect on mucosa and the bacteriological effect on saliva of three types of commercially available denture cleansers. Forty edentulous patients free from any oral or systemic diseases were selected in this study. Conventionally constructed dentures were delivered. At the beginning of the study the clinical condition of the mucosa was recorded, and salivary samples were taken for bacterial culture. The patients were divided into four groups each of ten patients. For every group a denture cleanser was prescribed and patients were instructed to use it according to manufacturer's instructions, any other method of cleansing was prohibited. The fourth group was a control group that did not use any denture cleanser. Patients were recalled every month for checkup of the oral mucosa and inspection of the dentures. The results indicated that chemical cleansing of dentures decrease the total number of colony forming units of microorganisms and the number of *Streptococcus mutans*. The condition of the mucosa also improved with the decrease of the oral microorganisms. Chemical cleansers containing peroxide were better than those containing sodium hypochlorite.

**Key words:** Denture cleansers, *Streptococcus mutans*, patients.

## INTRODUCTION

Provision of acrylic resin dentures is believed to alter the make up of oral microflora by encouraging the growth of certain microorganisms. This change is believed to occur as a result of the roughness of this material even if highly polished and finished and the ability of various microorganisms to adhere and colonize various surfaces of this material (Arai et al., 2009; da Silva et al., 2008; Millsap et al., 1999; Nair and Samaranayake, 1996).

Patients wearing removable dentures represent a good percentage of total dental patients. One of the most common difficulties that these patients may encounter is denture cleanliness.

Patients are usually concerned with the hygiene of the denture, and consequently they demand a clean denture that is free of food debris, calculus, stains and undesirable odour. Patient discomfort and mucosa irritation may result if denture cleanliness is not kept at an acceptable level. Clinicians on the other hand are primarily concerned with the detrimental effects of these

deposits on the underlying oral mucosa as well as the adjacent hard tooth structures.

Denture cleaning is primarily carried out mechanically using a tooth brush and a suitable detergent or a tooth paste or an ultrasonic cleaning machine (Abelson, 1985, 1981).

However, if the patient is elderly enough to have reduced manual dexterity, then a combination of mechanical and chemical denture cleansing is indicated (Budtz-Jorgensen, 1979). Chemical denture cleansing is carried out mainly by immersion of the denture in a suitable material that is capable of eliminating or reducing disease producing micro-organisms (Nikawa et al., 1999; de Freitas et al., 2011).

Available denture cleansers include: alkaline hypochlorite denture cleansers (Handa et al., 2008; Jagger et al., 2002; Oliveira et al., 2006), effervescent peroxide cleansers (Handa et al., 2008), acid cleansers (Nikawa et al., 1994), disinfectants and enzymes

(Nikawa et al., 1994).

Efficacy of various denture cleansers against a variety of micro-organisms has been demonstrated (Barnabe et al., 2004; Dills et al., 1988; Morgan and Wilson, 2000; Thorgeirsdottir et al., 2006)

*Streptococcus mutans* is believed to have a role in dental caries (Fitzgerald et al., 1983; Nikawa et al., 1998). The provision of such acrylic resin dentures if accompanied with colonization with this microorganism will have more change to produce deleterious effects on adjacent natural teeth (Nikawa et al., 1998).

This study is conducted to evaluate the effect of three types of commercially available denture cleansers which are: Fittydent "super cleansing tablets", Corega "denture cleansing tablets; bio formula", and Clorox "sodium Hypochlorite solution". Their effects are studied clinically on the supporting oral mucosa and bacteriologically on total colony forming units and on *S. mutans* in saliva.

## MATERIALS AND METHODS

Forty completely edentulous male patients were selected randomly. Patients included in the study were of male gender, medically fit, non smokers, non alcohol drinkers, new denture wearers, had healthy oral mucosa and extremely resorbed ridges. However, cases with: severe undercuts, or extremely thin covering mucosa, or recent extractions that are less than six months were excluded.

All patients were provided with complete upper and lower acrylic dentures constructed in the conventional method and were provided with the following instructions:

- i. Patients were instructed to take the denture out of their mouths during night and keep it in tap water only.
- ii. They were instructed to clean the dentures with tap water without brushing. Any other method of cleansing was prohibited.

Regular appointments for denture care were arranged to carry out any necessary adjustments for the denture.

Patients were asked to come again after four weeks of denture insertion to make base line recordings for this study, divide the patients into groups and start the study.

### Denture cleansing

Patients were divided into four groups that are equal in number according to the allocated type of denture cleanser:

**Group I:** In the first group, patients used "Fittydent, super cleansing tablet" (Fittydent International, GMBH, A-7423 Pinkafeld, Austria).

**Group II:** The second group used "Corega, cleansing tablets, bioformula" (Stafford-Miller Ltd., Dungarvan, Co., Waterford, Ireland).

For groups I and II patients were instructed to put the tablet in a glass of warm water containing sufficient water to cover the denture which was left in there overnight. Next morning and before wearing the denture it is washed under running water.

**Group III:** The third group used sodium hypochlorite solution prepared by diluting "Clorox" household bleach (Clorox International Trading Co.). The solution is prepared by adding 1 part sodium

hypochlorite to three parts tap water. The denture was immersed overnight. Next morning and before wearing the denture it was washed under running water.

**Group IV:** The fourth group was a control group; patients wore their dentures without using any cleanser. The dentures were, however, immersed in tap water.

### Clinical evaluation of inflammation in the tissues

The clinical condition of the denture supporting mucosa of every patient was evaluated at the same time of sample collection by evaluating signs of inflammation including redness, swelling, and soreness of mucosa. Only sharply outlined redness that was related to the fitting surface of the denture was considered for the grading of inflammation. Inflammation was described as: No inflammation, mild and sever inflammation.

### Bacteriological method

Samples were obtained at the following times: before denture insertion, one month after denture insertion immediately before the use of the allocated denture cleanser, two months after denture insertion (one month after denture cleanser use), four months after denture insertion (three month after denture cleanser use), and seven months after denture insertion (six month after denture cleanser use).

1 ml of saliva was taken in a sterile test tube. The following dilutions were prepared for bacterial count: 1:10, 1:100, 1:1000, 1:10000, 1:100000 and 1:1000000.

Diluted saliva samples were cultured on blood agar as well as on Mitis sucrose bacitracin. Blood agar is prepared from Nutrient agar to which 5 defibrinated horse blood is added (Emilson and Bratthall, 1976).

0.1 ml of each dilution was added to dry plates of both media and spread by a sterile L-shaped glass rod.

Plates were incubated in a candle jar in an incubator at 35°C for 72 h (Budtz-Jorgensen et al., 1978). Trials were made and the optimum dilution that gave separate countable colonies was 10 for MSB and 10 for blood agar.

On calculation of number of colony forming units (CFU) for blood agar, it is multiplied by 15, and number of *S. mutans* colonies on MSB is multiplied by 10<sup>3</sup>. Colonies were identified by film stained with gram stain.

### Statistical analysis

Paired t-test was used to identify significant differences between the groups. One way analysis of variance and the least significant difference test (Proc ANOVA) were run to test the significance between the change in bacterial count within each two intervals as well as the bacterial count in each interval between the three cleansers and the control group.

The change in bacterial count was expressed as: the change in number of total colony forming units, the change in number of *S. mutans*, the change in percent of *S. mutans* to total colony forming units.

## RESULTS

A total number of 40 male patients were recruited for this study with age range of 53 to 59 years. The patients

were divided equally into 4 groups.

## Clinical results

### ***Before denture insertion***

At the time of denture insertion the mucosa looked clinically healthy with no traumatic ulcers, hyper plastic tissues, or inflammation in all patients.

### ***One month after denture insertion (immediately before denture cleanser use)***

**Group I:** Severe inflammation was detected in four patients, mild inflammation was detected in four patients and there was no inflammation in two patients.

**Group II:** Severe inflammation was detected in three patients, mild inflammation was detected in five patients and there was no inflammation in two patients.

**Group III:** Severe inflammation was detected in six patients, slight inflammation was detected in three patients and there was no inflammation in one patient.

**Group IV (the control group):** Severe inflammation was detected in five patients, mild inflammation was detected in three patients and there was no inflammation in two patients.

### ***Two months after denture insertion (one month after denture cleanser use)***

**Group I:** Severe inflammation was detected in three patients out of the four detected in the previous visit, mild inflammation was detected in three patients out of the four detected in the previous period and there were no inflammation in four patients.

**Group II:** Severe inflammation was detected in three patients, mild inflammation continued to show in four patients of the five detected in the previous patients and there was no inflammation in three patients.

**Group III:** Severe inflammation continued to show in five patients out of the six detected in the previous period, mild inflammation was detected in three patients and there was no inflammation in two patients.

**Group IV:** Severe inflammation was detected in four patients, slight inflammation was detected in five patients and there was no inflammation in one patient.

### ***Four months after denture insertion (three months after denture cleanser use)***

**Group I:** Severe inflammation continued to show in two

patients out of three detected in the previous period, the third showed no inflammation, mild inflammation continued to show in two patients out of the three patients detected in previous period, and one was cured and there was no inflammation in six patients.

**Group II:** Severe inflammation continued to show in one patient out of two detected in the previous period, the other showed slight inflammation, mild inflammation was detected in four patients and there was no inflammation in five patients.

**Group III:** Severe inflammation; three patients out of five detected in the previous period continued to show severe inflammation, one patient showed mild inflammation, and one patient showed no inflammation, mild inflammation was detected in four patients and there was no inflammation in three patients.

**Group IV:** Severe inflammation; the three patients detected in the previous period continued to show severe inflammation, in addition to another patients, mild inflammation was detected in four patients and there was no inflammation in two patient out of the three detected in the previous period.

### ***Seven months after denture insertion (six months after denture cleanser use)***

**Group I:** Severe inflammation continued to show in the two patients detected in the previous period, mild inflammation continued to show in the two patients detected in the previous period and there was no inflammation in six patients.

**Group II:** Severe inflammation continued to show in the same patient detected in the previous period, mild inflammation continued to show in the four patients detected in the previous period and there was no inflammation was detected in five patients.

**Group III:** Severe inflammation; the three patients detected in the previous period continued to show severe inflammation, mild inflammation was detected in three patients and there was no inflammation was detected in four patients.

**Group IV:** Severe inflammation in four patients detected, three patients from the previous period continued to show severe inflammation in addition to another patient, mild inflammation was detected in three patients and there was no inflammation in two patients.

## Bacteriological results

### ***Total colony forming units count in saliva cultured on blood agar***

**Comparison between the four groups:** Before denture

**Table 1.** Statistics for total C.F.U. count in saliva of patient using different cleansers.

Variable	Mean	D	S.D.	S.E.
<b>Before insertion</b>				
Group I	0.138 x 10 <sup>8</sup>	a	0.008 x 10 <sup>8</sup>	0.002 x 10 <sup>8</sup>
Group II	0.138 x 10 <sup>8</sup>	a	0.008 x 10 <sup>8</sup>	0.002 x 10 <sup>8</sup>
Group III	0.138 x 10 <sup>8</sup>	a	0.008 x 10 <sup>8</sup>	0.002 x 10 <sup>8</sup>
Group IV	0.138 x 10 <sup>8</sup>	a	0.008 x 10 <sup>8</sup>	0.002 x 10 <sup>8</sup>
<b>After 1 month</b>				
Group I	4.000 x 10 <sup>8</sup>	a	1.471 x 10 <sup>8</sup>	0.465 x 10 <sup>8</sup>
Group II	3.700 x 10 <sup>8</sup>	a	1.619 x 10 <sup>8</sup>	0.512 x 10 <sup>8</sup>
Group III	2.765 x 10 <sup>8</sup>	a	1.659 x 10 <sup>8</sup>	0.524 x 10 <sup>8</sup>
Group IV	3.700 x 10 <sup>8</sup>	a	1.619 x 10 <sup>8</sup>	0.522 x 10 <sup>8</sup>
<b>After 2 months</b>				
Group I	2.525 x 10 <sup>8</sup>	a	0.584 x 10 <sup>8</sup>	0.312 x 10 <sup>8</sup>
Group II	2.805 x 10 <sup>8</sup>	a	1.083 x 10 <sup>8</sup>	0.342 x 10 <sup>8</sup>
Group III	2.415 x 10 <sup>8</sup>	a	1.545 x 10 <sup>8</sup>	0.488 x 10 <sup>8</sup>
Group IV	3.741 x 10 <sup>8</sup>	a	1.653 x 10 <sup>8</sup>	0.523 x 10 <sup>8</sup>
<b>After 4 months</b>				
Group I	0.940 x 10 <sup>8</sup>	a	0.342 x 10 <sup>8</sup>	0.107 x 10 <sup>8</sup>
Group II	1.695 x 10 <sup>8</sup>	ab	0.702 x 10 <sup>8</sup>	0.222 x 10 <sup>8</sup>
Group III	2.015 x 10 <sup>8</sup>	b	1.323 x 10 <sup>8</sup>	0.418 x 10 <sup>8</sup>
Group IV	3.742 x 10 <sup>8</sup>	c	1.669 x 10 <sup>8</sup>	0.528 x 10 <sup>8</sup>
<b>After 7 months</b>				
Group I	0.475 x 10 <sup>8</sup>	a	0.261 x 10 <sup>8</sup>	0.082 x 10 <sup>8</sup>
Group II	0.925 x 10 <sup>8</sup>	ab	0.409 x 10 <sup>8</sup>	0.129 x 10 <sup>8</sup>
Group III	1.565 x 10 <sup>8</sup>	b	1.152 x 10 <sup>8</sup>	0.364 x 10 <sup>8</sup>
Group IV	3.743 x 10 <sup>8</sup>	c	1.658 x 10 <sup>8</sup>	0.524 x 10 <sup>8</sup>

d= Least significant different between group; a & b & c = Means with no common letters differs significantly; S.D. = Standard deviation ; S.E. = Standard error.

insertion no significant difference was found between mean of total colony forming units in the four groups.

First postoperative visit, there was non-significant increase in the mean of total colony forming units in saliva of patients of the four groups.

Second postoperative visit: the mean of the total colony forming units in the saliva of the patients was decreased; however this decrease was not significantly different from the results of the previous visit Group IV showed non significant increase in total colony forming units.

Third postoperative visit the mean of the total colony forming units in the saliva of the patients continued to decrease. The decrease was significant in group I when compared to Group III, and it was non significant when compared to group II. There was also non significant difference between groups II and III. On the other hand, group IV showed an increase in the mean of total colony forming units.

Fourth postoperative visit, the total colony forming units

continued to decrease. This decrease was significant in Group I when compared to Group III, while no significant difference was noticed between Groups I and II, and between Groups II and III. Group IV showed increase in the mean of total colony forming units (Table 1).

**Comparison between each two successive samples in the same group:** Before to one month after denture insertion (before denture cleanser use): All groups showed significant increase.

One month to two months after denture insertion (one month after denture cleanser use) Group I, II, and III showed significant decrease. Group IV showed non significant increase.

Two months to four months after denture insertion (three months after denture cleanser use): Groups I, II and III showed significant decrease. Group IV showed non significant increase.

Four months to seven months after denture insertion

**Table 2.** Paired t-test for total CFU for the interval (between two successive appointments) in saliva of patients using different cleansers.

Appointments	Variable	Mean d.	S.E.D.	t-value	P
First appointment	Group I	$3.862 \times 10^8$	$0.464 \times 10^8$	8.312	**
	Group II	$4.012 \times 10^8$	$0.380 \times 10^8$	10.532	**
	Group III	$3.077 \times 10^8$	$0.502 \times 10^8$	6.125	**
	Group IV	$3.562 \times 10^8$	$0.511 \times 10^8$	6.961	**
Second appointment	Group I	$1.195 \times 10^8$	$0.229 \times 10^8$	5.216	**
	Group II	$1.175 \times 10^8$	$0.260 \times 10^8$	4.507	**
	Group III	$0.350 \times 10^8$	$0.089 \times 10^8$	3.913	**
	Group IV	$0.050 \times 10^8$	$0.037 \times 10^8$	1.340	NS
Third appointments	Group I	$1.110 \times 10^8$	$0.476 \times 10^8$	6.283	**
	Group II	$1.585 \times 10^8$	$0.292 \times 10^8$	5.415	**
	Group III	$0.400 \times 10^8$	$0.108 \times 10^8$	3.685	**
	Group IV	$0.001 \times 10^8$	$0.049 \times 10^8$	0.020	NS
Fourth appointments	Group I	$0.770 \times 10^8$	$0.129 \times 10^8$	5.952	**
	Group II	$0.645 \times 10^8$	$0.083 \times 10^8$	5.647	**
	Group III	$0.450 \times 10^8$	$0.079 \times 10^8$	5.654	**
	Group IV	$0.001 \times 10^8$	$0.025 \times 10^8$	0.387	NS

(six months after denture cleanser use)

Group I, II and III showed significant decrease. Group IV showed non significant increase (Table 2).

### ***S. mutans* count in saliva culture on *Mitis sucrose bacitracin***

**Comparison between the four groups:** Before denture insertion, *S. mutans* was not found one months after denture insertion (before denture cleanser use), there was an increase in the mean of *S. mutans* count in each group with no significant difference between group I, II, III and IV.

Two months after denture insertion (one month after denture cleanser use), the mean of *S. mutans* count in the saliva of the patients decreased.

This decrease was not significant compared to the results of the previous period. Group IV showed increase in the mean of *S. mutans* count.

Four months after denture insertion (three months after denture cleanser use), the decrease in the mean of *S. mutans* count in the saliva of the patients continued.

The decrease was significant in Group I compared to Group II and in Group I compared to Group III, Group IV showed increase in the mean of *S. mutans* count.

Seven month after denture insertion (six months after denture cleanser use), the decrease in the mean of *S. mutans* count in the saliva of the patients continued.

This decrease was significant in Group I compared to

group III that is, there is more decrease in bacterial count in Group I than in Group III. Also significant decrease was found in Group II compared to Group III that is, there is more decrease in bacterial count in Group II than in Group III. No significant difference in the decrease of bacterial count between Group I and Group III. Group IV showed non significant increase in the mean of *S. mutans* count (Table 3).

### **Comparison between each two successive samples in the same group:**

One month to two months after denture insertion (Table 4) Group I, II and III showed significant decrease, Group IV showed non significant increase.

Two months to four months after denture insertion (Table 4): Group I, II and III showed significant decrease Group IV showed significant increase.

Four months to seven months after denture insertion (Table 4): Group I, II and III showed significant decrease. Group IV showed non significant increase.

For months after denture insertion there was no significant difference in percent of *S. mutans* to total colony forming units in all groups (Table 5).

Seven months after denture insertion there was no significant difference in percent of *S. mutans* to total colony forming units in all groups (Table 6).

This means that as the total C.F.U. decrease the number of *S. mutans* count decreases and the denture cleansers does not decrease the *S. mutans* specifically.

**Table 3.** Statistics for *S. mutans* count in saliva of patients using different cleansers.

Variable	Mean	D	S.D.	S.E.
<b>After 1 month</b>				
Group I	1.500 x 10 <sup>6</sup>	a	0.745 x 10 <sup>6</sup>	0.235 x 10 <sup>6</sup>
Group II	1.315 x 10 <sup>6</sup>	a	0.812 x 10 <sup>6</sup>	0.257 x 10 <sup>6</sup>
Group III	1.430 x 10 <sup>6</sup>	a	0.863 x 10 <sup>6</sup>	0.273 x 10 <sup>6</sup>
Group IV	1.500 x 10 <sup>6</sup>	a	0.745 x 10 <sup>6</sup>	0.235 x 10 <sup>6</sup>
<b>After 2 months</b>				
Group I	0.525 x 10 <sup>6</sup>	a	0.484 x 10 <sup>6</sup>	0.155 x 10 <sup>6</sup>
Group II	1.805 x 10 <sup>6</sup>	a	0.680 x 10 <sup>6</sup>	0.215 x 10 <sup>6</sup>
Group III	1.415 x 10 <sup>6</sup>	a	0.632 x 10 <sup>6</sup>	0.200 x 10 <sup>6</sup>
Group IV	1.741 x 10 <sup>6</sup>	c	0.714 x 10 <sup>6</sup>	0.225 x 10 <sup>6</sup>
<b>After 4 months</b>				
Group I	0.655 x 10 <sup>6</sup>	ab	0.415 x 10 <sup>6</sup>	0.131 x 10 <sup>6</sup>
Group II	0.395 x 10 <sup>6</sup>	b	0.216 x 10 <sup>6</sup>	0.068 x 10 <sup>6</sup>
Group III	0.965 x 10 <sup>6</sup>	a	0.761 x 10 <sup>6</sup>	0.240 x 10 <sup>6</sup>
Group IV	1.592 x 10 <sup>6</sup>	c	0.726 x 10 <sup>6</sup>	0.229 x 10 <sup>6</sup>
<b>After 7 months</b>				
Group I	0.366 x 10 <sup>6</sup>	a	0.311 x 10 <sup>6</sup>	0.098 x 10 <sup>6</sup>
Group II	0.155 x 10 <sup>6</sup>	a	0.086 x 10 <sup>6</sup>	0.027 x 10 <sup>6</sup>
Group III	0.740 x 10 <sup>6</sup>	b	0.619 x 10 <sup>6</sup>	0.196 x 10 <sup>6</sup>
Group IV	1.600 x 10 <sup>6</sup>	c	0.764 x 10 <sup>6</sup>	0.024 x 10 <sup>6</sup>

d = Least significant different between group; a, b and c = Means with no common letters differs significantly; S.D. = Standard deviation; S.E. = Standard error.

**Table 4.** Paired t-test for *S. mutans* in saliva of patients using different cleansers between successive appointments.

Appointment	Variable	Mean d.	S.E.D.	t-value	P
First and second appointments	Group I	0.345 x 10 <sup>6</sup>	0.138 x 10 <sup>6</sup>	2.497	**
	Group II	0.425 x 10 <sup>6</sup>	0.175 x 10 <sup>6</sup>	2.426	**
	Group III	0.335 x 10 <sup>6</sup>	0.083 x 10 <sup>6</sup>	4.019	**
	Group IV	0.015 x 10 <sup>6</sup>	0.031 x 10 <sup>6</sup>	0.473	NS
Second and third appointments	Group I	0.500 x 10 <sup>6</sup>	0.100 x 10 <sup>6</sup>	4.972	**
	Group II	0.495 x 10 <sup>6</sup>	0.110 x 10 <sup>6</sup>	4.493	**
	Group III	0.130 x 10 <sup>6</sup>	0.076 x 10 <sup>6</sup>	1.692	NS
	Group IV	0.075 x 10 <sup>6</sup>	0.024 x 10 <sup>6</sup>	3.078	**
Third and fourth appointments	Group I	0.290 x 10 <sup>6</sup>	0.041 x 10 <sup>6</sup>	7.010	**
	Group II	0.240 x 10 <sup>6</sup>	0.068 x 10 <sup>6</sup>	3.496	**
	Group III	0.225 x 10 <sup>6</sup>	0.058 x 10 <sup>6</sup>	3.857	**
	Group IV	0.008 x 10 <sup>6</sup>	0.027 x 10 <sup>6</sup>	0.287	NS

**Comparison between each two successive samples in each group:** One month to two months after denture insertion (Table 5). There was no significant change in group I and II but significant change in group III, group IV no change.

## DISCUSSION

The forty patients selected in this study were medically fit and healthy. The medical history and physical examination showed that patients did not have any systemic

**Table 5.** Statistics for *S. mutans* as percent of total C.F.U. count in saliva of patient using different cleansers.

Variable	Mean	D	S.D.	S.E.
<b>After 1 month</b>				
Group I	0.425	a	0.253	0.080
Group II	0.387	a	0.170	0.053
Group III	0.541	a	0.109	0.034
Group IV	0.673	a	0.629	0.199
<b>After 2 months</b>				
Group I	0.437	a	0.211	0.067
Group II	0.403	a	0.220	0.069
Group III	0.499	a	0.130	0.041
Group IV	0.560	a	0.472	0.149
<b>After 4 months</b>				
Group I	0.405	a	0.177	0.056
Group II	0.452	a	0.255	0.080
Group III	0.488	a	0.143	0.045
Group IV	0.592	a	0.515	0.163
<b>After 7 months</b>				
Group I	0.388	a	0.261	0.082
Group II	0.367	a	0.148	0.046
Group III	0.497	a	0.162	0.051
Group IV	0.607	a	0.557	0.176

d = Least significant different between group; a, b and c = Means with no common letters differs significantly; S.D. = Standard deviation; S.E. = Standard error.

**Table 6.** Paired t-test for *S. mutans* to total CFU in saliva of patients using different cleansers between successive appointments.

Appointment	Variable	Mean d.	S.E.D.	t-value	P
First and second appointments	Group I	-0.012	0.035	-0.36	NS
	Group II	-0.016	0.042	-0.38	NS
	Group III	-0.042	0.014	2.97	+
	Group IV	-0.11	0.099	-1.13	NS
Second and third appointments	Group I	-0.033	0.038	0.88	NS
	Group II	-0.040	0.019	-2.50	*
	Group III	-0.010	0.038	0.297	NS
	Group IV	-0.032	0.014	2.20	*
Third and fourth appointments	Group I	0.015	0.058	0.264	NS
	Group II	0.084	0.057	1.470	NS
	Group III	-0.009	0.046	-0.196	NS
	Group IV	0.014	0.020	0.698	NS

diseases particularly those that could affect oral microflora including diabetes mellitus, malignancies and tuberculosis. Diabetic patients are prone to the growth of

microorganisms on denture surface (Budtz-Jorgensen et al., 1978). Furthermore, xerostomia, nutritional and vitamin deficiencies may all enhance mucosal

inflammation (Renner et al., 1979). Certain endocrine disturbances may affect the condition of the oral mucosa. To reduce the effect of the endocrine disturbances on the oral environment, female patients were not included in this study.

Only new denture wearers were included in this study. This was important since old prostheses may affect the oral environment. Also, all patients were given at least 6-month healing period after extractions to allow for alveolar bone resorption which mainly takes place during that period. Furthermore, oral microflora may be influenced by extraction.

Extremely thin or hyperplastic covering mucosa, may be a source of mucosal inflammation under dentures, altering the microbial flora of the oral cavity, accordingly, these cases were excluded. Severe bilateral bone undercuts may be a factor enhancing mucosal inflammation, so they were avoided. Extremely resorbed ridges may be a source of ill fitting denture inducing mucosal inflammation which is a factor altering the microbial flora of the oral cavity (Love et al., 1967).

Habits like tobacco smoking and drinking alcohol were also shown to change the microbial flora of the oral cavity (Myers and Krol, 1974). Hence, smokers and alcohol drinkers were excluded.

Drug therapy affects the microbial flora of the oral cavity; e.g. antibiotics, antihistaminics. So, at the time of taking the sample, if patients were under drug therapy taking the sample was postponed (Budtz-Jorgensen et al., 1978).

All dentures were constructed using heat-cured acrylic resin cured at 60 to 80°C in 8 h. The degree of curing affects the amount of residual monomer, degree of porosity, amount of stresses induced in the denture base and its dimensional stability. It was important to ensure dimensional stability of the denture base because it affects its fitness and consequently the thickness of saliva film and its flow under the denture that consequently affect the microbial condition.

Another important factor that was controlled in this study is oral and denture hygiene. Patients were motivated and instructed to maintain an optimum oral and denture hygiene. So patients had to clean the dentures after every meal, take the dentures out of the mouth during sleep and keep them soaked in denture cleansers during night for the study period of 6 months.

A sample of unstimulated saliva was taken before delivery of the denture to record the bacteriological count of the oral microbial flora. Patients were allowed a one month period after delivering the dentures prior to using denture cleansers. This period was thought to be sufficient for a change in the oral microbial environment into a stable condition. Also during that period any necessary adjustments were made to the dentures.

The control group did not use denture cleansers; they used the conventional methods of denture cleansing. This was important to study the effect of the denture

alone without the associated effect of denture cleansers.

Unstimulated saliva samples were investigated in this study, as it will give broader picture about the effect of the chemical cleansers on the whole oral environment including the denture and the oral mucosa. Changes in the microbial flora of saliva and its composition are reflection to what is happening in the oral mucosa and the denture. In this study wearing of complete dentures for one month altered the microbial flora of the oral cavity; there was significant increase in the total colony forming units together with the appearance of *S. mutans*. These results coincide with previous studies which found that samples of saliva from edentulous patients before wearing dentures contained no *S. mutans*, however, these started to appear after denture wearing (Carlsson et al., 1969). Also, it was shown that streptococci contribute to a wide range of cultivable micro-flora of plaque on removable dentures in patients with healthy oral mucosa (Theilade et al., 1983). Alteration of microbial flora and appearance of *S. mutans* after denture wearing is most probably due to the presence of micro-porosities in the acrylic resin surface to which salivary pellicle is attached then the pellicle get colonized with microorganisms and denture plaque is formed (van Reenen, 1973; Budtz-Jorgensen et al., 1981; Edgerton et al., 1987; Ratzow, 1964).

Unfortunately, even the properly cured denture has microporosities. The microporosities absorb oral fluids creating a favorable medium for the growth of microorganisms. Hygiene of the denture is inversely correlated with denture porosity (da Silva et al., 2008; Harrison et al., 2004; Hong et al., 2009). This is not the case in chrome Cobalt denture bases because of their better fitness, attainment of well polished surface and lack of microporosities (Elsaid et al., 1974).

Denture plaque is undoubtedly a major factor in oral mucous inflammation under dentures (Abelson, 1981; Sreenivasan et al., 2004). Areas of inflammation of the oral mucosa that appeared after wearing denture may be due to changes in the oral environment as a result of denture wearing altering the microbial oral flora. Also the presence of inflammation may alter the microbial oral flora.

In the present study the use of denture cleansers altered the oral microbial flora. Generally the degree of mucosal inflammation was decreased together with decrease of total colony forming units and *S. mutans* during the whole follow up period in the three cleansers studied.

The changes were not of the same degree in the three cleansers Fittydent and Corega showed the best results followed by sodium hypochlorite which was the least effective in improving the oral conditions.

After the period of the experiment, it was found that all denture cleansers decrease the total colony forming units count as well as *S. mutans* count. The least decrease was in the group using sodium hypochlorite "Clorox"

more decrease occurred in the two other groups, with no significant difference in decrease of bacterial count between these two groups.

It is worthy to mention that all cleansers reduced the count of *S. mutans* at the same rate of reduction of total bacterial count as there was no special effect on *S. mutans* alone.

During the follow up period and as the bacterial count decreased, the condition of the mucosa improved. The improvement of the oral mucosa condition after the use of denture cleansers is due to removal of denture plaque, debris and stains that dramatically reduces the incidence of inflammation of the supporting mucosa (Palenik and Miller, 1984).

The decrease of the total colony forming units and of the *S. mutans* noticed during the six months period of denture cleansing use can be explained by the mechanism of action of cleansers I and II effervescent tablets. Alkaline peroxides work basically through an oxygen releasing mechanism which loosens debris and removes light stains from the denture surfaces (Fitzgerald et al., 1983). Peroxide cleansers are a combination of an oxygen liberator such as perborate or per-carbonate and alkaline detergent (Denture cleansers, 1983). On adding these constituents to water they produce an alkaline solution of hydrogen peroxide. Oxidizing agents are incorporated for their effects on cleansing and stain removal. They also act as anti bacterial agents. The higher the pH of the solution the more effective it is. Also, releasing of bubbles of oxygen from the effervescent peroxide exerts a mechanical cleansing action. The alkaline detergent constituent acts as a protein dissolvent increasing the cleansing efficiency of the solution. The statistically non-significant difference reported in this study between the effects of cleansers I and II is most probably due to the fact that they are nearly chemically similar, with the same mode of action. The continuous decrease in the total colony forming units and *S. mutans* together with the improvement in mucosal condition suggests that their use provides better oral condition.

The recommended period for using the cleanser to provide better results, needs further study as prolonged use of these chemicals may have an adverse effect. The results of the present study coincide with previous studies which showed that peroxide containing cleansers are better than other cleansers (Ghalichebaf et al., 1982; Augsburg and Elahi, 1982; Moore et al., 1984).

However, other researchers believe that chlorine-containing cleansers are better than those containing peroxides in reducing the volume of plaque (Altman et al., 1979). The action of alkaline hypochlorite (cleanser III) is different; it removes light stains and food debris with a bleaching action. Hypochlorite acts directly on the organic matrix of plaque causing dissolution of polymer structure (Budtz-Jorgensen, 1979).

In conclusion, this study showed that the hypochlorite cleanser used for six months is less effective in removing stains and debris when compared to the peroxide

cleansers. Also, hypochlorite has a bleaching effect on acrylic resin changing its color (Hong et al., 2009). Further studies should be conducted to investigate the effect of these cleansers on the physical properties of the dentures.

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