

An effect of mouth spray on denture microorganisms; An in vitro and an in vivo studies

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Summary

In this study a mouth spray (Janina™ Ultra White, Oral fresh spray, Janina International Ltd., Kent, England) is tested for invitro and invivo, its effect on denture microorganisms which are Candida albicans, Staphylococcus aureus and Streptococcus mutans. These microorganisms were cultured and after 48 hours incubation, suspension was prepared according to MacFarland technique. Then 5 ml a

mouth spray was added in the prepared suspension. Invitro results of this study showed that 3, 5, 10, 15, 30 and 60 min. later sample contains *Candida albicans* and *Streptococcus mutans* were found to be completely sterile in all tests. However *Staphylococcus aureus* was similarly found to be sterile in all test except 60 min. later only 9 colonies were found in the sample. For in vivo study, 30 patients are selected (x=68) whom are wearing their present dentures on average 3 years. The patients' dentures were firstly cleaned in an ultrasonic cleaner for 5 min. and then polished for 3 min. using abrasive agents. Patients were instructed not to clean their dentures for 3 days. When the patients came back for recall firstly smears were taken from the palatal surface of the upper denture which was used as a control. Than the mouth spray is sprayed on the palatal surface of the upper dentures. In the 1st group 10 min. after in the 2nd group 15 min. after and in the 3rd group 30 min. after smears were taken. Invivo results showed that 30 min. after using the spray no microorganisms were found on the samples ($p < 0.0002$). 60% and 30% reduction were seen in microorganisms after 15 and 10 min. respectively. From these invitro and invivo studies it can be concluded that the newly introduced the mouth spray is efficient for disinfecting the complete dentures.

Keywords: Denture hygiene, Complete dentures

Introduction

The complete denture can be cleaned in two possible ways; chemically or mechanically. Soap and brush are suggested the patients for mechanical cleaning. Although it has been widely accepted as an effective method, but mechanical methods for satisfactory cleaning denture is needed careful attention and effort of the patients. Therefore elderly people living in shelters and especially for the handicapped denture wearers cleaning a denture is a common problem. Also it has been pointed out that improper brushing causes the wear of the denture acrylic resin and damages the soft-lining material¹⁻⁴. The general impression is that available chemical cleansers are mostly effective on denture microorganisms⁵⁻⁷, however another report showed that some of the denture cleansers are not effective especially on *Candida albicans*⁸. The main disadvantage of these effervescent tablets is that dentures need to be kept in the glass of water a certain period of time which causing a pleasant view. Therefore it appears from the literature that there is no chemical denture cleaning material which is practical and effective in a short period of time. The aim of this study is to investigate in vitro and in vivo an effect of a mouth spray* on denture microorganisms.

*Janina™ Ultra White, Oral fresh spray, Janina International Ltd., Kent, England.

Material and Methods

In vitro Study:

For the invitro part of this study *Candida albicans* ATCC10231, *Staphylococcus aureus* ATCC 25923 and *Streptococcus mutans* CNCTC 8/77 Kuen 1458 were selected.

C. albicans in Sabouraud agar; *S. mutans* in Colombia agar and Brucella broth which 5% sheep blood is added; *S. aureus* in Muller Hinton agar were grown. Grown organism was

prepared with MacFarland standard no:1 to approximate a cell density of 3×10^8 CFU/ml and this suspension is diluted 1:5 to approximate a cell density of 6×10^7 CFU/ml.

These suspension was diluted 1/100 with sterile saline and 5 ml samples transferred into tubes, then 5 ml Janina suspension was also added in each tube. After 3, 5, 10, 30 and 60 min. 0.1 ml samples from each tube spreaded on selective media. The plates were incubated for 48 hours at 37^0 C. then the number of colony forming units (CFU) of microorganisms were calculated.

In addition, sterile distilled water which added suspensions of microorganisms were use as a positive control and sterile distilled water, which Janina suspension was added, used as a negative control.

In vivo study:

For the invivo part of this study 30 complete denture wearers were selected (age $x=68$) whom are wearing their present dentures on average 3 years. Patients' dentures were firstly cleaned in an ultrasonic cleaner for 5 min. and then polished for 3 min. using abrasives. Patients were instructed not to clean their dentures for 3 days. When the patients came back for recall, first smear was taken from the palatal surface of an upper denture, which was used as a control. Then the mouth spray is sprayed on the palatal surface of the upper denture (10 times and 5 cm away from the denture; 1 shut= 150 micl). First group of 5 patients' dentures for 30 min., 2nd group of 5 patients' dentures for 15 min. and 3rd group of 5 patients' dentures for 10 min. were kept on the bench after sprayed. At the end of the time 2nd smears were taken. In a similar period of time a matching number of patients' dentures are kept in saline.

Results

In vitro study;

After incubation colonies were counted and the results showed that number of microorganisms were increased in positive controls, however the negative controls were found sterile. Samples contains *C. albicans* and *S. mutans* were found to be completely sterile after 3, 5, 10, 15, 30 and 60 min. later. *S. aureus* similarly found sterile in the same period of time, however 60 min. later 9 colonies found in the sample.

In vivo study;

Several different microorganisms such as *Fusebakterium*, *Veillonella*, *Difteroides*, Black pigmented *Bacteroides* etc. are found analysis of smears which were taken from the patients dentures. Also *C. albicans* and *S. mutans* are seen most of the smears.

The results showed that there were no growth of microorganisms after 30 min. Although there is a considerable reduction between 80 to 99 % after 15 and 10 min. but there are still some microorganisms left on the denture surface.

The test of saline is never killed totally the microorganisms on the denture surface but it achieved some small reduction between 9 to 27 % after 30 min. The tests of saline even showed that some growth of microorganisms between 38 to 90 % after 15 and 10 min (Table 1, 2 and 3).

The results were evaluated using One way analysis of variance. The statistically significant result was only found after 30 min. treatment by the mouth spray ($P < 0.0002$).

Discussion

A study showed that only 60 % of elderly patients complete dentures were not found clean whom were living in shelters⁹. They showed that these elderly patients do not have a habit of cleaning dentures and the reason of not to clean was that they had to spent an

effort. Although they have been tough and stimulate to clean their dentures but investigators were not succeeded to increase percentage of clean dentures. Also mechanical cleaning of dentures is found insufficient for reducing the number of microorganisms on dentures and cleaning¹⁰⁻¹¹. It has even shown that mechanical cleaning may damage the denture acrylic and softlining material.

It well accepted that chemical disinfectants have some advantages over the mechanical cleaning such as effective disinfectant and ease of using. But some studies showed that not all the disinfectants effective on the most important microorganism "Candida albicans" for the dentures⁸. Therefore it has been suggested that all disinfectants should be investigated under same conditions in vitro and in vivo, in order to eliminate conflict between the studies¹². There is also much discussion on methodology of the studies which are investigating the efficacy of the chemical cleansers. In vitro part of the studies may showed different results from the in vivo study, because of the variation in soaking temperature, time and variation of the operators¹². After even 3 min. later effect of the spray was seen in vitro study, but in vivo study same effect was found 30 min. later. Therefore this differences may have been explained by the variations occurred in the in vivo and in vitro part of the study.

In vitro part of this study showed that 60 min. later 9 colonies of *S. aureus* were found. This result indicates that bacteriostatic effect of the spray appears to be reduced after 60 min. Therefore further investigation should be carried out bacteriostatic and bacteriocidal effect of the spray on microorganisms. However *S. aureus* has not considerable effect on edentate subjects. In vitro study is also showed that the mouth spray is highly effective on *S. mutans* and it is well known that *S. mutans* play a major role for the caries. In this aspect the spray may help the dentate patients who has a caries risk.

It appears from the literature that *C. albicans* is the most important microorganisms for dentures and it should be eliminated¹³. It well established from the our in vitro and in vivo studies that the spray is highly effective on *C. albicans*.

Conclusion

It does appear from this study that the mount spray is easy to use and effective on the *Candida albicans* therefore it can be used as a denture disinfectant.

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Treatment with mouth spray ; 10 min.			Treatment with saline ; 10 min.		
	Before	After		Before	Before
	Total viable count			Total viable count	
	Cfu/ml	Cfu/ml		Cfu/ml	Cfu/ml
Pt 1	8 x 10 ⁷	12 x 10 ³	Pt 6	10 x 10 ⁷	11 x 10 ⁷
Pt 2	3 x 10 ⁵	6 x 10 ⁴	Pt 7	12 x 10 ⁸	14 x 10 ⁸
Pt 3	17 x 10 ⁶	2 x 10 ⁵	Pt 8	13 x 10 ⁶	15 x 10 ⁶

Pt 4	8×10^7	12×10^3		Pt 9	12×10^7	13×10^7
Pt 5	3×10^6	8×10^4		Pt 10	9×10^6	15×10^6

Table 1

Treatment with mouth spray ; 15 min.			Treatment with <i>saline</i> ; 15 min.		
	Before	After		Before	After
	Total viable count			Total viable count	
	Cfu/ml	Cfu/ml		Cfu/ml	Cfu/ml
Pt 11	12×10^7	2×10^3	Pt 16	2×10^7	9×10^6
Pt 12	2×10^8	15×10^2	Pt 17	3×10^8	14×10^7
Pt 13	14×10^8	6×10^4	Pt 18	3×10^7	11×10^7
Pt 14	11×10^8	15×10^3	Pt 19	2×10^6	17×10^6
Pt 15	9×10^7	11×10^3	Pt 20	18×10^7	17×10^8

Table 2

Treatment with mouth <i>spray</i> ; 30 min.			Treatment with <i>saline</i> ; 30 min.		
	Before	After		Before	After
	Total viable count			Total viable count	
	Cfu/ml	Cfu/ml		Cfu/ml	Cfu/ml
Pt 21	12 x 10 ⁸	n.g.	Pt 26	11x 10 ⁷	10 x 10 ⁷
Pt 22	11 x 10 ⁸	n.g.	Pt 27	11 x 10 ⁷	8 x 10 ⁷
Pt 23	13 x 10 ⁸	n.g.	Pt 28	13 x 10 ⁸	8 x 10 ⁸
Pt 24	6 x 10 ⁸	n.g.	Pt 29	13 x 10 ⁷	8 x 10 ⁷
Pt 25	6 x 10 ⁸	n.g.	Pt 30	12 x 10 ⁸	9 x 10 ⁸

Table 3 (n.g.= no growth)

Captions

Table 1-) 10 min. treatment of the dentures with the mouth spray and saline showed no effect on the microorganisms.

Table 2-) 15 min. treatment of the dentures with the mouth spray and saline showed small reduction of the microorganisms.

Table 3-) 30 min. treatment of the dentures with the mouth spray and saline. Result showed no growth of microorganisms when the mouth spray is used.