BALKAN JOURNAL OF STOMATOLOGY

BaSS

ISSN 1107 - 1141

Efficacy of a Mouth Spray on Denture Microorganisms: An *In Vitro* Pilot Study

SUMMARY

Complete dentures are contaminated by microorganisms, which can be a source of infection, such as denture stomatitis. The aim of this study was to test the efficacy of mouth spray against specific test bacteria and fungi, and to consider its potential.

3 bacteria and 1 fungus representing a broad microbial spectrum with a relevance of oral bacteria were used in 3 laboratory tests, including European suspension test, AOAC germicidal spray products test and in vitro denture disinfection test. The results of 3 laboratory tests showed that CloSYSII proved to be almost 100% effective for all microorganisms tested in this study. It can be concluded that the use of mouth sprays is efficient and easy way for disinfecting complete dentures.

Keywords: Complete Dentures; Microorganisms; Candida albicans

Altay Uludamar[1],Yasemin Kulak Ozkan[2], Nurver Üner[3]

[1]Dental Medical Clinic, Prosthodontic Department, Ankara, Turkey
[2] Marmara University, Faculty of Dentistry Prosthodontic Department, Istanbul, Turkey
[3]Marmara University, Faculty of Medicine Microbiology Department, Istanbul, Turkey

ORIGINAL PAPER (OP)
Balk J Stom, 2013; 17:70-74

Introduction

The presence of microbial film on the tissue surface of maxillary dentures is an important etiologic factor in denture stomatitis[1-4]. Denture base acrylic resin is easily colonized by oral endogenous bacteria and *Candida spp*, and possibly by extra-oral species, such as *Staphylococcus spp* or members of enterobacteriaceae[5-7]. This microbial reservoir can be responsible for denture-related stomatitis, especially in geriatric patients. Oral and denture hygiene of elderly individuals is extremely poor and denture cleaning is a common problem[8,9]. Proper routine cleaning of dentures is necessary to prevent denture stomatitis and maintain healthy supporting tissues. Effective plaque removal requires a degree of manual dexterity that is often lacking; especially among elderly patients[8]. The use of chemical denture cleaning agents produces more effective results, especially in geriatric patients and in people who have problems with wearing dentures[8,10]. A variety of experimental approaches have been tested in attempt to examine the efficacy of denture cleaning agents[6,11-13]. The general impression is that available chemical cleansers are effective on denture microorganisms[6,11,14]. However, some studies showed that not all of the disinfectants are effective on the most important microorganism for dentures - *Candida albicans*[15].

Effervescent tablets (alkaline peroxide types, enzyme types) are the most used denture cleaning agents[11,13,16-18]. 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. CloSYSII oral spray is the first pocket/purse size chlorine dioxide based oral spray on the market. The advantage of spray application is that it is quick and easy to apply and delivers a clean fresh portion of the solution each time it is used, whereas with a soaking regime the solution rapidly becomes contaminated, needs frequent replacement and can easily be knocked over. Therefore the aim of this study was to investigate *in vitro* and *in vivo* an effect of CloSYSII on denture microorganisms.

Material and Methods

The solution, CloSYSII (Portola Plaza Dental Group, CA, USA) consists of chlorine dioxide (6%). Its disinfectant properties were evaluated against a range of pathogenic bacteria, such as *Candida spp*, *S. aureus*,

S. mutans, *Neisseria* and *S. β-hemolyticus*. Previous studies[19] showed that analysis of swab samples taken from healthy complete denture wearers showed they contained considerable amounts of *α-hemolytic streptococcus*, *Neisseria*, *β-hemolytic streptococcus* and *C. albicans*. The same microorganisms were used in this study because they represent a broad spectrum of antimicrobial activity and they can colonize oral mucosa and be of potential risk for oral infections[19,20]. These microorganisms were purchased as a stock culture (KUKENS Study group, Department of Microbiology, University of Istanbul, Istanbul, Turkey).

European Suspension Test

For each test organism 10 ml of CloSYSII solution, supplemented with 0.5% bovine serum albumin to simulate "dirty conditions", was inoculated with 0.1 ml inoculums suspension to give approximately 1-5×10^7 cfu/ml[20]. Immediately after inoculation (Time 0) and then at intervals of 3, 5, 15, 30 and 60 min, 1 ml sample was transferred into 9 ml European standard test inactivator fluid to neutralize the antimicrobial action of the solution. Serial decimal dilutions were made with buffered peptone water and duplicate 1 ml portions used to prepare TSA (Tryptic Soy Agar) or SDA (Saubauraud Dextrose Agar) pour plates as appropriate. An inoculum control count was carried out by inoculating 10 ml sterile distilled water and sampling at Time 0. All plates were incubated at 37+/-2°C for 18-24 h and the numbers of bacterial and fungal colonies counted; by reference to the Time 0 count, the log reduction and percentage mortality of each test organism was calculated at each time point. The initial inoculum concentration for each test organism was 10^6 viable cells/ml, and the detection limit for recovery of viable cells was 10 ml.

AOAC Germicidal Spray Products Test

This test was to determine the efficacy of CloSYSII as a spray disinfectant. 7 replicate glass slides were inoculated for each organism. The initial inoculum concentration for each organism was 10^6 viable cells/ml. Glass slides were inoculated with 0.01 ml suspension of each organism by spreading over an area of 1 square inch. The slides were allowed to dry, sprayed with CloSYSII (10 times and 5 cm away; 1 spray=150 micl), and then allowed to stand at room temperature until the CloSYSII had evaporated (about 45min). They were transferred, using flame sterilized forceps, to screw cap glass containers each containing 20 ml subculture broth (nutrient broth for the bacteria and glucose/peptone broth for the fungi) and the containers shaken on a flat bed shaker for 2 min; since the primary subculture broths remained clear after 30 min of shaking, subculture into secondary broths was not required. The containers were then incubated at 37+/-2°C for 48 h for all strains except the trichophyton, which was incubated at 25-30°C for 7 days. After incubation the containers were examined for the presence of growth as judged by turbidity in the medium and scored as positive (+) for growth and negative (-) for no growth. Killing of the test organisms in 10 out of 10 of the treated slides was considered as giving presumptive evidence of disinfecting action[20].

In Vitro Denture Disinfection Test

6 acrylic resin samples (1cm^2) were obtained using polymetyl-methacrilate acrylic resin (Meliodent, Bayer Dental ltd, Germany) and immersed in isopropyl alcohol for 1h. They were placed in sterile laminar airflow cabined to allow the alcohol to evaporate and then immersed in sterile distilled water and rinsed by shaking for 1 min. Each denture piece was placed in empty sterile Petri dish and inoculated with 0.1 ml of a mixed suspension of 3 bacteria, each at a concentration of 1.5x10^6 cfu/0.1 ml, together with 0.5% bovine serum albumin. The inoculated denture pieces were then pre-incubated at 20°C for 4h. 3 dentures were treated with the solutions (9 sprays to wet the denture thoroughly) and 3 dentures acted as untreated control. The treated dentures were incubated app. 20°C for 2h. They were then all placed in separate sterile glass bottles containing glass beads and 100 ml of sterile recovery medium (Tryptone Soya Broth containing European Standard Test inactivator constituents), shaken vigorously for 1 min and then sample withdrawn to determine the numbers of surviving bacteria. Spread plates counts were preformed in duplicate TSA using 0.1 aliquots from serial decimal dilutions and after incubation at 37+/-2°C for 18-24 h[20].

Results

The results of European suspension test, AOAC germicidal spray products test and *in vitro* denture disinfection test are shown in tables 1-3.

European suspension test - the solution achieved 100% mortality of all 4 bacteria and *C. albicans* within 3 min of exposure. Since the initial inoculum concentration for each test organism was greater than 10^6 viable cells/ml and the detection limit for recovery of viable cells was 10 per ml, this represents a 5 log kill against each organism (Table 1).

AOAC Germicidal Spray Products test - the solution achieved 100% mortality of all 5 microorganisms tested. Since the initial inoculum concentration for each test organism was greater than 10^6 viable cells/ml, this represents a 6 log kill in each case (Table 2).

In vitro denture disinfection test - the solution achieved 100% mortality of the mixed bacterial changes inoculum in 2 of 3 replicated denture pieces tested, and 99% mortality in the third after exposure of 2h. These results represent a 5 log kill for the first 2 replicates and a 4 log kill for the third (Table 3).

*Table 1. The efficacy of CloSYSII in the European Suspension Test**

Time (min)	α-hem. Str.		β-hem-Str.		Neisseria		Candida albicans	
	Count (cfu/ml)	mortal (%)	Count (cfu/ml)	mortal (%)	Count (cfu/ml)	mortal (%)	Count (cfu/ml)	mortal (%)
SDW at 0	1.75		1.25		1.05		1.71	
0	15	99.99	<10	100	<10	100	<10	100
3	<10	100	<10	100	<10	100	<10	100
5	<10	100	<10	100	<10	100	<10	100
10	<10	100	<10	100	<10	100	<10	100
15	<10	100	<10	100	<10	100	<10	100
30	<10	100	<10	100	<10	100	<10	100
60	<10	100	<10	100	<10	100	<10	100

* All counts are expressed as $\times 10^6$

Table 2. The efficacy of CloSYSII in the AOAC Germicidal Spray Products test.

Replicate slide	A-hem. strep		β-hem strep		Neisseria		C.albicans	
	Cont	Test	Cont	Test	Cont	Test	Cont	Test
1	+		+		+		+	
2	+		+		+		+	
3		-		-		-		-
4		-		-		-		-
5		-		-		-		-
6		-		-		-		-
7		-		-		-		-
8		-		-		-		-
9		-		-		-		-
10		-		-		-		-
11		-		-		-		-
12		-		-		-		-

Table 3. The efficacy CloSYSII in the in vitro denture disinfection tests

Replicate	Bacterial count (cfu/ml)		Mortality (%)
	Control	Denture	
1	6.8 x 105	77	99.9
2	7.00 x 105	0	100
3	6.25 x 105	0	100

Discussion

Schou et al[9] showed that only 60% of elderly patients who were living in shelters had complete dentures that were not found to be clean. They showed that these elderly patients did not have a habit of cleaning dentures, and their reason for not cleaning was that they would have had to expend effort. They were difficult to stimulate to clean their dentures, and the investigators were not successful in increasing the percentage of clean dentures.

It is well accepted that chemical disinfectants have some advantages over mechanical cleaning, such as effective disinfection and ease of use[10,15,21]. However, some studies showed that not all of the disinfectants are effective on *Candida albicans* at dentures[15]. The alternative and economically more acceptable solution would decontaminate dentures and the results of this study showed that CloSYSII can produce such desired effects: it has highly effective antimicrobial disinfection properties against 4 test pathogenic microorganisms that represent a broad of spectrum with relevance of oral bacteria under a range of *in vitro* conditions. It is possible that the development of biofilm *in vivo* on the denture may reduce the effectiveness of CloSYSII; therefore, further *in vivo* studies were planned to investigate efficacy of the chemical cleansers. The *in vitro* part of the study could show different results from the *in vivo* study because of the variation in soaking temperature, time, and variation of the operators[22]. In this *in vitro* study, after even 3 minutes, the effect of CloSYSII was seen; but in *in vivo* study, the same effect could be seen different times. Therefore, further studies should focus on both the *in vivo* and *in vitro* studies, to explain the noted variations.

There have been few studies using CloSYSII oral spray as a denture cleanser in the literature. The effectiveness of topical chlorine dioxide (0.8%) in the management of chronic atrophic candidiasis was demonstrated by Mohammad et al[23]; they stated that ClO_2 provided a safe and clinically effective option in the management of chronic atrophic candidiasis. The results of this study show that CloSYSII can produce such adesired effect: it has highly effective antimicrobial disinfection properties against 5 test pathogenic microorganisms that represent a broad spectrum with relevance to oral bacteria under a range of *in vitro* conditions.

The significant reduction in the number of *C. albicans* in this study suggests that the use of mouth spray is a suitable method for cleaning dentures. Further studies are needed to determine if daily use of mouth spray can reduce the high prevalence of denture stomatitis patients.

Conclusion

Within the limitations of these experiments, it was found that CloSYSII mouth spray is easy to use and effectively influence denture microorganisms; therefore, it can be used as a denture disinfectant.

References

1. *Arendorf TM, Walker DM.* The prevalence and intra-oral distribution of *Candida albicans* in man. *Arch Oral Biol,* 1980; 25:1-10.
2. *Budtz-Jorgensen E, Stenderup A, Grabowski M.* An epidemiologic study of yeasts in elderly denture wearers. *Community Dent Oral Epidemiol,* 1975; 3:15-19.
3. *Padilha DMP, Hugo FN, Hilgert JB, et al.* Hand function and oral hygiene in older institutionalized Brazilians. *J Am Geriatr Soc*, 2007; 55:1333-1338.
4. *Pietrokovski J, Azuelos J, Tau S, et al.* Oral findings in elderly nursing home residents in selected countries: oral hygiene conditions and plaque accumulation on denture. *J Prosthet Dent*, 1995; 73:136-141.
5. *Budtz-Jorgensen E.* The significance of Candida albicans in denture stomatitis. *Scand J Dent Res,* 1974; 82:151-190.
6. *Chan EC, Iugovaz I, Sboo R, et al.* Comparison of two popular methods for removal and killing of bacteria from dentures. *J Can Dent Assoc*, 1991; 57:937-939.
7. *Davies RM, Jensen SB, Schiot CR, Loe H.* The effect of topical application of chlorhexidine on the bacterial colonization of the teeth and gingiva. *J Periodont Res, 1*970; 5:96-101.
8. *Pietrokovski J, Azuelos J, Tau S, et al.* Oral findings in elderly nursing home residents in selected countries: oral hygiene conditions and plaque accumulation on denture. *J Prosthet Dent*, 1995; 73:136-141.
9. *Schou L, Wight C, Cumming C.* Oral hygiene habits, denture plaque, presence of yeasts and stomatitis in institutionalized elderly in Lothian, Scotland. *Community Dent Oral Epidemiol*, 1987; 15:85-89.
10. *Padilha DMP, Hugo FN, Hilgert JB, Dal Moro RG.* Hand function and oral hygiene in older institutionalized Brazilians. *J Am Geriatr Soc*, 2007; 55:1333-1338.
11. *Nakamoto K, Tamamoto M, Hamada T.* Evaluation of denture cleansers with and without enzymes against *Candida albicans. J Prosthet Dent,* 1991; 66:792-795.
12. *Ödman PA.* The effectiveness of an enzyme-containing denture cleanser. *Quint Int,* 1992; 23:187-190.
13. *Palenic CJ, Miller CH.* In vitro testing of three denture-cleaning systems. *J Prosthet Dent*, 1984; 51:751-754.
14. *Budtz-Jorgensen E, Loe H.* Chlorhexidine as a denture disinfectant in the treatment of denture stomatitis. *Scan J Dent Res,* 1972; 80:457-464.
15. *Drake D, Wells J, Ettinger R.* Efficacy of denture cleansing agents in an in vitro bacteria-yeast colonization model. *Int J Prosthodont,* 1992; 5:214-220.
16. *Ghalichebaf M, Graser GN, Zander HA.* The efficacy of denture cleansing agents. *J Prosthet Dent,* 1982; 48:515-520.
17. *Rudd RW, Senia ES, Mc Cleskey FK, Adams ED.* Sterilization of complete dentures with sodium hypochlorite. *J Prosthet Dent,* 1984; 51:318-321.
18. *Moore TC, Smith DE, Kenny GE.* Sanitization of dentures by several hygiene methods. *J Prosthet Dent*, 1984; 52:158-163.

19. *Kulak Y, Arıkan A, Kazazoglu E. Existence of C.* albicans and microorganisms in denture stomatitis patients. *J Oral Rehab*, 1997; 24:788-790.
20. *Neal PR, Rippin JW.* The efficacy of a toothbrush disinfectant spray - an *in vitro* study. *J Dent,* 2003; 31:153-157.
21. *Minagi S, Tsunoda T, Yoshida K, et al.* Objective testing of the efficiency of denture-cleansing agents. *J Prosthet Dent,* 1987; 58:595-598.
22. *Nikawa H, Hamada T, et al.* A review of in vitro and in vivo methods to evaluate the efficacy of denture cleansers. *Int J Prosthodont,* 1999; 12:153-159.
23. *Mohammad AR, Giannini PJ, Preshaw PM, et al.* Clinical and microbiological efficacy of chlorine dioxide in the management of chronic atrophic candidiasis: an open study. *Int Dent J,* 2004; 54:154-158.

Correspondence and request for offprints to:

Dr. Altay Uludamar
Filistin cad. Kader sok. No: 6
Kat: 1, D: 1
Gaziosmanpaşa/ Ankara/ TURKEY
E-mail: a.uludamar@superonline.com