Fungal Genomics & Biology
ISSN: 2165-8056

Buergers, Fungal Genom Biol 2011, 1:1
DOI: 10.4172/2165-8056.1000101

Rapid Communication Open Access

Effect of Application Times on *Candida albicans* for Denture Disinfection

Ralf Buergers*

Department of Prosthetic Dentistry, University Medical Center Regensburg, Germany

Abstract

Study background: This *in vitro* study aimed to evaluate the minimum application times of three denture disinfection procedures to significantly reduce initial *Candida albicans* colonization.

Methods: Specimens of a soft denture relining material were incubated with *Candida albicans* for 2.5 h and allotted to three different disinfection regimes (sodium hypochlorite 1%, effervescent cleansing tabs, and microwaving immersed in water) with application times ranging from 30 to 600 s. Adhering fungi were quantified using a bioluminescence assay in combination with an automated plate reader for cell quantification.

Results: Luminescence at baseline varied between 409 and 472 relative luminescence units (rlu). A significant reduction of luminescence was found after 120 s of soaking in sodium hypochlorite (31 rlu) and effervescent cleansing tabs (35 rlu), and after 60 s of microwave irradiation (26 rlu).

Conclusions: Threshold minimum application times of 120 s for sodium hypochlorite and cleansing tabs, and 60 s for microwaving were determined. A further extension of application times did not have additional antifungal effects.

Keywords: Denture relining; Luminescence; Microwave irradiation; Sodium hypochlorite

Introduction

C. albicans has been considered as the most prevalent fungus in the human oral cavity and major microbiological factor in the pathogenesis of denture-related stomatitis, in which dentures may function as a reservoir of infection [1]. Up to 65% of all denture wearers are affected by *Candida* infections [1]. In general, yeast cells are known to form pathogenic biofilms on artificial devices such as pacemakers, shunts, implants, and prostheses [2]. These fungal biofilms have increased resistance to host defenses and are also extremely resistant to many conventional disinfectant measures [3]. Elderly or handicapped patients, whose manual dexterity may be limited, often prefer chemical denture cleansers over mechanical brushing [4]. A great variety of cleansing agents and disinfection regimes with antifungal properties are available, but only few have proven to be effective against *C. albicans* [5].

Sodium hypochlorite (NaOCl) is frequently used as a disinfectant in many applications and it is known to be antifungal in low concentrations (< 2%) [6]. Effervescent cleansing products dissolved in water have become a frequent and effective alternative to conventional disinfectants such as sodium hypochlorite [7]. In order to avoid the application of cost-intensive antiseptics, the use of microwave energy has been suggested for the inactivation of pathogens on dentures [8]. As disinfectants have been found to influence the mechanical properties of dentures, application times of the various disinfection regimes should be reduced to a minimum [9].

The aim of the present *in vitro* study was to evaluate the minimum application times of three disinfection procedures (sodium hypochlorite 1%, Blend-a-dent tabs, and microwave irradiation) to eliminate initial *C. albicans* colonization on a soft denture relining material.

Materials and Methods

Circular specimens (diameter 8 mm) of soft denture lining material Mucopren E (Kettenbach, Germany) were prepared and aged in a thermal cycler (Regensburger Kausimulator, EGO, Germany) with 3000 cycles (5°C/55°C). *C. albicans* human isolate (strain 1386; DSMZ, Braunschweig, Germany) was cultured overnight in yeast broth. Cells were harvested by centrifugation at 2300 rpm for 5 min at 18°C, and the optical density of the suspension was adjusted to 0.3 at 540 nm.

The *Candida* suspension was incubated with the specimens for 2.5 h at 37°C. To remove non-adhering fungi, all specimens were carefully washed three times with phosphate buffered saline (PBS) and randomly subjected to three different disinfection procedures: sodium hypochlorite 1% (Pharmacy, Regensburg University Medical Center, Germany), application of effervescent Blend-a-dent tabs (Procter & Gamble, Schwalbach, Germany; active ingredients: potassium monopersulphate and sodium perborate monohydrate), and microwave irradiation. Disinfection solutions were added for 0, 30, 60, 120, 240, 360, 480, and 600 s and removed before specimens were carefully washed with PBS. For microwaving, 2 ml distilled water was added to each specimen. Polystyrene 24 well-plates (Sarstedt, Newton, NC, USA) were covered and immediately irradiated in an unmodified domestic microwave oven (KOG-6D07, Daewoo Electronics, Butzbach, Germany; 800 W/2450 MHz). The adhering fungi have been quantified using the bioluminescence assay VIA Light AMR (Cambrex Bio Science, Rockland, USA) in combination with an automated multi-detection reader (Fluostar optima; BMG, Offenburg, Germany) as described previously [5].

Continuous data were summarized by using means and SD. The

***Corresponding author:** Ralf Buergers, DDS., PhD, Department of Prosthetic Dentistry, University Medical Center Regensburg, 93042 Regensburg, Germany, Tel: +49-941-944-6059; Fax: +49-941-944-6171; E-mail: ralf.buergers@klinik.uni-regensburg.de

Received October 29, 2011; **Accepted** December 04, 2011; **Published** December 06, 2011

Citation: Buergers R (2011) Effect of Application Times on *Candida albicans* for Denture Disinfection. Fungal Genom Biol 1:101. doi:10.4172/2165-8056.1000101

Disinfection procedures	Application time [s]							
	0	30	60	120	240	360	480	600
Sodium hypochlorite 1%	**438** (250)[a]	**556** (412)[a]	**577** (416)[a]	31 (64)[b]	23 (71)[b]	1 (1)[b]	47 (140)[b]	5 (16)[b]
Blend-a-dent cleansing tabs	**472** (264)[a]	**307** (81)[a]	**389** (497)[a]	35 (43)[b]	40 (35)[b]	25 (17)[b]	44 (43)[b]	22 (31)[b]
Microwave irradiation	**409** (108)[a]	**239** (107)	26 (16)[b]	5 (5)[b]	11 (8)[b]	9 (10)[b]	8 (14)[b]	4 (8)[b]

[a] and [b] indicate no statistical differences (p>0.05)

Table 1: Relative luminescence intensities [rlu] after application of three disinfection regimes (means; standard deviations).

detection of differences was performed by one-way ANOVA and the Tukey-Kramer multiple comparison test for post-hoc analysis (α=0.05). Statistical software (SPSS 15.0; SPSS Corp, Chicago, USA) was used for all calculations.

Results

Table 1 displays the comparative adherence of *C. albicans*. Post-hocs revealed no statistically significant differences (p>0.05 for all comparisons) between the three relative luminescence intensities (rli) at the baseline. A significant decrease of adhering fungi, indicated by a decrease of rli, was found after 120 s of soaking in sodium hypochlorite and Blend-a-dent tabs. A stepwise decrease of luminescence could be observed for successive microwave irradiation. The baseline rli (409 rlu) was nearly halved in value after 30 s (239 rlu), followed by a significant decrease of rli after 60 s. A further extension of the application times (sodium hypochlorite and Blend-a-dent tabs > 120 s; microwave irradiation > 60 s) did not lead to an additional significant reduction of luminescence values.

Discussion

Chemical denture cleaning procedures are recommended for elderly or handicapped patients, whose limited manual dexterity often results in insufficient mechanical brushing of the denture [4]. Although application times have a crucial influence on the efficacy of the disinfection procedure, they should be reduced to a minimum, because disinfectants have been found to influence the mechanical properties and color stability of dentures. The tested disinfection procedures have been proven to be effective in reducing *C. albicans* in previous investigations [4,5,10]. In this study, a bioluminescence kit based on the determination of cellular adenosine triphosphate (ATP) was used for simple, highly sensitive, and reproducible quantification of adhering fungal cells [5,11]. The present study showed that there is no linear correlation between application time and quantity of adhering fungi. For sodium hypochlorite and Blend-a-dent tabs, a definite minimum threshold application time of 120 s could be determined. In contrast, a stepwise reduction of adhering fungi was observed for microwave irradiation up to the threshold of 60 s, where no additional reduction of adhering *C. albicans* could be observed. In general, an extension of these threshold application times did not result in additional significant reduction of *C. albicans* adhesion.

Within the limitations of this study, it can be concluded that definite threshold application times (120 s for sodium hypochlorite 1% and Blend-a-dent tabs; 60 s for microwave irradiation) are required for the effective reduction of adhering *C. albicans*. A further extension of these minimum application times does not have an additional antifungal effect and is therefore dispensable.

Acknowledgements

The technical assistance of Gerlinde Held and Anja Dirscherl is appreciated. I am grateful to Maria and Hanna Richter for the linguistic revision of the manuscript.

References

1. Arendorf TM, Walker DM (1987) Denture stomatitis: a review. J Oral Rehabil 14: 217-227.
2. Ramage G, Martinez JP, Lopez-Ribot JL (2006) Candida biofilms on implanted biomaterials: a clinically significant problem. FEMS Yeast Res 6: 979-986.
3. Blankenship JR, Mitchell AP (2006) How to build a biofilm: a fungal perspective. Curr Opin Microbiol 9: 588-594.
4. Baysan A, Whiley R, Wright PS (1998) Use of microwave energy to disinfect a long-term soft lining material contaminated with Candida albicans or Staphylococcus aureus. J Prosthet Dent79: 454-458.
5. Buergers R, Rosentritt M, Schneider-Brachert W, Behr M, Handel G, et al.(2008) Efficacy of denture disinfection methods in controlling Candida albicans colonization in vitro. Acta Odontol Scand 66: 174-180.
6. Barnabe W, de Mendonca NT, Pimenta FC, Pegoraro LF, Scolaro JM (2004) Efficacy of sodium hypochlorite and coconut soap used as disinfecting agents in the reduction of denture stomatitis, *Streptococcus mutans* and *Candida albicans*. J Oral Rehabil 31: 453-459.
7. Dills SS, Olshan AM, Goldner S, Brogdon C (1988) Comparison of the antimicrobial capability of an abrasive paste and chemical-soak denture cleaners. J Prosthet Dent 60: 467-470.
8. Campanha NH, Pavarina AC, Brunetti IL, Vergani CE, Machado AL, et al. (2007) Candida albicans inactivation and cell membrane integrity damage by microwave irradiation. Mycoses 50: 140-147.
9. Asad T, Watkinson AC, Huggett R (1993) The effects of various disinfectant solutions on the surface hardness of an acrylic resin denture base material. Int J Prosthodont 6: 9-12.
10. Polyzois GL, Zissis AJ, Yannikakis SA (1995) The effect of glutaraldehyde and microwave disinfection on some properties of acrylic denture resin. Int J Prosthodont 8: 150-154.
11. Dexter SJ, Camara M, Davies M, Shakesheff KM (2003) Development of a bioluminescent ATP assay to quantify mammalian and bacterial cell number from a mixed population. Biomaterials 24: 27-34.