SHEILA RODRIGUES DE SOUSA PORTA

AVALIAÇÃO DO HIPOCLORITO DE SÓDIO A 0,5% COMO LIMPADOR DE PRÓTESE: ESTUDO CLÍNICO

PIRACICABA
2012

UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA

SHEILA RODRIGUES DE SOUSA PORTA

AVALIAÇÃO DO HIPOCLORITO DE SÓDIO A 0,5% COMO LIMPADOR DE PRÓTESE: ESTUDO CLÍNICO

Orientador: Profa. Dra. Altair Antoninha Del Bel Cury

TESE DE DOUTORADO APRESENTADA À FACULDADE DE ODONTOLOGIA DE PIRACICABA DA UNICAMP PARA OBTENÇÃO DO TÍTULO DE DOUTORA EM CLÍNICA ODONTOLÓGICA, NA ÁREA DE PRÓTESE DENTAL

Este exemplar corresponde à versão final da Tese defendida pela aluna, e orientada pela Profa. Dra. Altair Antoninha Del Bel Cury.

_____
Assinatura do Orientador

PIRACICABA, 2012

**FICHA CATALOGRÁFICA ELABORADA POR
MARILENE GIRELLO – CRB8/6159 - BIBLIOTECA DA
FACULDADE DE ODONTOLOGIA DE PIRACICABA DA UNICAMP**

P83a

Porta, Sheila Rodrigues de Sousa, 1962-
Avaliação do hipoclorito de sódio a 0,5% como limpador de prótese: estudo clínico / Sheila Rodrigues de Sousa Porta. -- Piracicaba, SP : [s.n.], 2012.

Orientador: Altair Antoninha Del Bel Cury.

Tese (doutorado) - Universidade Estadual de Campinas, Faculdade de Odontologia de Piracicaba.

1. Prótese total. 2. Higienizadores de dentadura. 3. Biofilme. 4. Cor. 5. Propriedades de superfície. I. Del Bel Cury, Altair Antoninha, 1948- II. Universidade Estadual de Campinas. Faculdade de Odontologia de Piracicaba. III. Título.

Informações para a Biblioteca Digital
Título em Inglês: Evaluation of 0.5% sodium hypochlorite as a denture cleanser: a clinical study
Palavras-chave em Inglês:
Denture, complete
Denture cleansers
Biofilm
Color
Surface properties
Área de concentração: Prótese Dental
Titulação: Doutora em Clínica Odontológica
Banca examinadora:
Altair Antoninha Del Bel Cury [Orientador]
Francisco José Guimarães de Freitas
Fernanda Faot
Carlos José Soares
Wander José da Silva
Data da defesa: 02-08-2012
Programa de Pós-Graduação: Clínica Odontológica

UNICAMP

UNIVERSIDADE ESTADUAL DE CAMPINAS
Faculdade de Odontologia de Piracicaba

A Comissão Julgadora dos trabalhos de Defesa de Tese de Doutorado, em sessão pública realizada em 02 de Agosto de 2012, considerou a candidata SHEILA RODRIGUES DE SOUSA PORTA aprovada.

Profa. Dra. ALTAIR ANTONINHA DEL BEL CURY

Prof. Dr. FRANCISCO JOSÉ GUIMARÃES DE FREITAS

Profa. Dra. FERNANDA FAOT

Prof. Dr. CARLOS JOSÉ SOARES

Prof. Dr. WANDER JOSÉ DA SILVA

Dedico este trabalho,

À **Deus**, por me guiar, amparar, carregar... e por colocar anjos em meu caminho, na forma de família e amigos.

Ao meu marido **Renato** e minhas filhas **Mariana** e **Marcela**, pelo carinho, estímulo e compreensão nos momentos de ausência. Sem o apoio de vocês eu não teria conseguido chegar até aqui.

Aos melhores pais que alguém pôde ter, **Ragosino** e **Maria**, pelo amor incondicional e por nunca medirem esforços para o meu crescimento pessoal e profissional.

Aos meus irmãos **José Carlos**, **Inês** e **Swame**, por estarem ao meu lado, sempre!

AGRADECIMENTO ESPECIAL

À minha orientadora, ***Profa. Dra. Altair Antoninha Del Bel Cury***, a minha gratidão pela oportunidade e confiança em mim depositada. É um orgulho e, ao mesmo tempo, uma grande responsabilidade tê-la como orientadora. Seu amor pela pesquisa, sua dedicação profissional e sua busca permanente pelo aperfeiçoamento são exemplos a serem seguidos.

AGRADECIMENTOS

À Universidade Estadual de Campinas por meio do seu Magnífico Reitor, **Prof. Dr. Fernando Ferreira Costa**.

À Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas, na pessoa de seu Diretor **Prof. Dr. Jacks Jorge Júnior.**

À Coordenadora dos Cursos de Pós-Graduação da Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas, **Profa. Dra. Renata Cunha Matheus Rodrigues Garcia.**

Ao Coordenador do Programa de Pós-Graduação em Clínica Odontológica da Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas, **Prof. Dr. Márcio de Moraes**.

À Universidade Federal de Uberlândia, na pessoa de seu Reitor **Prof. Dr. Alfredo Júlio Fernandes Neto** e, em especial à Escola Técnica de Saúde, pela liberação de minhas atividades.

Aos Professores do Programa de Pós-Graduação em Clínica Odontológica, Área Prótese Dental, da Faculdade de Odontologia de Piracicaba pelo convívio e aprendizado.

À **Profa. Dra. Lívia Maria Andaló Tenuta** do Departamento de Ciências Fisiológicas da Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas, pela permissão de uso do laboratório de Bioquímica Oral.

Ao **Prof. Dr. Mario Alexandre Coelho Sinhoreti** da Área de Materiais Dentários do Departamento de Odontologia Restauradora da Faculdade de

Odontologia de Piracicaba da Universidade Estadual de Campinas, pela permissão do uso do rugosímetro no laboratório de Materiais Dentários.

À **Profa. Dra. Débora Alves Nunes Leite Lima** da Área de Dentística do Departamento de Odontologia Restauradora da Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas, por permitir o uso do espectrofotômetro.

À **Maria Beatriz Freitas D'arce** pela ajuda e treinamento no uso do espectrofotômetro.

Ao **Prof. Dr. Wander José da Silva** pela contribuição na idealização e desenvolvimento deste trabalho.

Aos **Voluntários** pela confiança, paciência e comprometimento indispensáveis para o desenvolvimento deste estudo.

À **Sílvia Carneiro de Lucena** pela amizade sincera, pelo apoio, disponibilidade e dedicação a esta pesquisa. Sua ajuda foi imprescindível para a conclusão deste trabalho.

Aos amigos e colegas de curso, **Alfonso Ayala, Ana Paula Martins, Andréa Vasconcelos, Antônio Pedro, Arcelino Neto, Bruno Maior, Camila Campos, Carolina Meloto, Cindy Goes, Frederico Fernandes, Germana Camargos, Gisele Ribeiro, Indira Cavalcanti, Larissa Vilanova, Letícia Gonçalves, Lívia Forster, Luana Aquino, Marcele Pimentel, Plínio Senna, Priscila Gomes, Priscilla Lazari, Thaís Gonçalves e William Custódio**, pela convivência, pelas contribuições, pelas conversas jogadas fora, por tornarem o dia a dia mais fácil de ser vivido.

A **Sra. Gislaine,** técnica pelo Laboratório de Prótese Removível, pela atenção e carinho.

Aos técnicos do Laboratório de Bioquímica Oral **Waldomiro Vieira Filho** e **José Alfredo da Silva**, pela ajuda e agradável convivência.

Às **Sras. Érica Alessandra Pinho Sinhoreti**, secretária, **Roberta Clares Morales dos Santos**, **Raquel Q. Marcondes Cesar Sacchi** e **Priscilla Zuzi Boldrin,** assessoras da Coordenadoria Geral dos Programas de Pós-graduação da Faculdade de Odontologia de Piracicaba; e à **Sra. Eliete Aparecida Ferreira Lima Marim**, secretária do Departamento de Prótese e Periodontia da Faculdade de Odontologia de Piracicaba pela atenção desde o início do curso de pós-graduação.

A todos que, direta ou indiretamente, contribuíram para a realização deste trabalho, meu sincero agradecimento.

RESUMO

Estratégias que visem prevenir e reduzir a formação de biofilmes sobre próteses são necessárias, pois estas podem atuar como reservatório de micro-organismos. Outro aspecto importante é o estabelecimento de um protocolo de higienização que além de eficiente também seja bem aceito pelos pacientes. Assim, objetivou-se avaliar o efeito do hipoclorito de sódio (NaOCl) a 0,5% sobre o biofilme, estabilidade de cor e rugosidade de superfície (Ra) de próteses totais removíveis e a satisfação do paciente com o tratamento. Foram selecionados 15 voluntários que, após aceitar e assinar as condições do Termo de Consentimento Livre e Esclarecido aprovado pelo Comitê de Ética em Pesquisa da FOP/UNICAMP, foram orientados a complementar a higiene de suas próteses com a imersão em NaOCl 0,5%, durante 3 minutos, uma vez ao dia. O período experimental foi de 90 dias e as variáveis resposta foram mensuradas antes do início do uso do NaOCl e após 30, 60 e 90 dias. A avaliação microbiológica foi realizada no biofilme da prótese e na saliva do voluntário. Toda a superfície da prótese era percorrida por um cotonete de algodão que, em seguida, era individualmente acondicionado em tubo estéril contendo 3 mL de PBS. Amostras da saliva foram acondicionadas em tubos estéreis. Todos os tubos foram sonicados (7W, 30 s), as soluções iniciais serialmente diluídas e plaqueadas, em triplicata, em CHROMagar e ágar sangue. Após um período de incubação de 48 h a 37°C, o número de unidades formadoras de colônia foi determinado. A estabilidade de cor foi avaliada com o uso de um espectrofotômetro de refletância, mensurada no sistema CIELab e correlacionada para o ambiente clínico de acordo com as unidades da National Bureau of Standards (NBS). Para avaliação da Ra, foi realizado um delineamento *in situ* e espécimes (5 x 5 x 2mm) (n = 90) de resina acrílica termo-polimerizável foram confeccionados e colados na superfície vestibular das próteses inferiores. A rugosidade de superfície foi mensurada com o auxílio de um rugosímetro de contato. Para avaliar a aceitação do paciente com relação ao protocolo, foi pedido aos voluntários que traduzissem em valores o seu

grau de satisfação com o tratamento em um intervalo variando de 0 (totalmente insatisfeito) a 10 (totalmente satisfeito). Uma redução significativa no número de micro-organismos totais (ANOVA; $p< 0,05$) e *Candida* spp foi observada ao longo do tratamento. Embora tenham sido observadas alterações nos valores de L*, a* e b* nas mensurações de cor, não houve diferença significativa na cor das bases das próteses (Friedman; $p> 0,05$). Não houve diferença significante na rugosidade de superfície (Kruskal Wallis; $p > 0,05$). A satisfação do voluntário com o método de limpeza aumentou durante o período avaliado, chegando a 87% de indivíduos totalmente satisfeitos. Conclui-se que o método de higienização proposto com NaOCl teve ampla aceitação pelos voluntários; além de ser efetivo na redução de micro-organismos, a alteração de cor exibida foi clinicamente aceitável, bem como não houve alteração significativa na rugosidade de superfície.

Palavras-chave: resina acrílica, limpadores de prótese, biofilme, estabilidade de cor, rugosidade superficial

ABSTRACT

Strategies to prevent and to reduce biofilm formation on dentures are necessary, since they may become a reservoir of microorganisms. Additionally, it is important, in establishing a protocol for denture cleaning, to evaluate the effect of the cleaning agent on the prosthetic material and the patients' acceptability. Therefore, the objective of this study was to evaluate the effect of sodium hypochlorite (NaOCl) 0.5% on biofilm, color stability and surface roughness (Ra) of removable dentures besides the patients' satisfaction with the treatment. Fifteen volunteers were recruited and, after accepting the conditions of the Term of Consent approved by the Ethics Committee of FOP / UNICAMP, were instructed to daily immerse their dentures in a 0.5% NaOCl solution for 3 minutes. The follow-up time was 90 days and outcome variables were measured on baseline and on days 30, 60 and 90. For microbiological evaluation, samples were obtained from dentures and saliva. Swabs were taken from the whole surface of the dentures and, then, individually placed in a sterile tube containing 3 ml of PBS. Next, each volunteer was asked to spit into an empty sterile tube. All tubes were sonicated (7W, 30 s), the initial solutions serially diluted and plated in triplicate on blood agar and CHROMagar and, after an incubation period of 48 h at 37°C, the number of colony forming units (cfu/mL) was determined. Color stability was measured with a reflectance spectrophotometer and evaluated using the CIELab system. It was also quantified in accordance with units of the National Bureau of Standards (NBS). To Ra assessment, an *in situ* design was developed. Specimens (5 x 5 x 2 mm) (n = 90) of heat-polymerized acrylic resin were fabricated and fixed in the buccal posterior surface of lower dentures. The surface roughness was measured using a profilometer. To evaluate the acceptability of the cleaning protocol, each volunteer was asked to translate in values their degree of satisfaction with the treatment at an interval ranging from 0 (totally dissatisfied) to 10 (totally satisfied). A significant reduction in the number of total micro-organisms (ANOVA; $p < 0.05$) and *Candida spp* was observed throughout the treatment. Although changes had been observed

in L *, a * b * values, there were no significant color changes (Friedman; $p > 0.05$). The surface roughness did not present significant changes after the evaluation period (Kruskal Wallis; $p > 0.05$). The volunteers' satisfaction increased throughout the experimental period, reaching 87% of individuals totally satisfied. Data revealed that the 0.5% NaOCl immersion protocol was effective in reducing microorganisms, color changes exhibited for all dentures can be considered clinically acceptable and the surface roughness did not show significant changes. Also, the cleaning method was well accepted by volunteers.

Key words: acrylic resin, denture cleansers, biofilm, color stability, surface roughness

SUMÁRIO

INTRODUÇÃO

Nas últimas décadas tem-se observado um crescimento na expectativa de vida das pessoas com consequente aumento da população idosa. Embora a perda dental não seja um processo natural do envelhecimento, dados epidemiológicos revelam que aproximadamente 67% dos idosos brasileiros são portadores de próteses totais, enquanto outros 19% têm necessidade de uso deste tipo de aparelho (Ministério da Saúde, 2011). O edentulismo normalmente coexiste com um quadro precário de saúde oral (Petersen & Yamamoto, 2005; Polzer *et al.* 2010) e, aliado a uma redução da autonomia decorrente do processo do envelhecimento, pode afetar seriamente a saúde geral do idoso (Tramini *et al*., 2007). A reabilitação oral, por meio de prótese fixa ou removível, restabelece as funções do sistema estomatognático e contribui significativamente para o bem-estar, autoconfiança, nutrição, saúde geral (Mc Grath & Bedi, 2001; Polzer *et al.* 2010) e estética do paciente edêntulo.

O material comumente utilizado para a confecção de bases de próteses removíveis é a resina acrílica à base de poli (metil) metacrilato (PMMA), ativada termicamente (Coulthwaite & Verran, 2007). Este material propicia uma estética satisfatória, possui boa resistência, é de fácil manipulação e de baixo custo (Anusavice, 2005). No entanto, a resina da base das próteses pode funcionar como um nicho bastante favorável à proliferação de micro-organismos, onde estes, organizados em biofilme, estão mais protegidos da ação antimicrobiana e física da saliva e de outros agentes químicos (Pereira-Cenci *et al*., 2007; Pasternak, 2009; Tanaka *et al*., 2009; von Fraunhofer & Loewy, 2009; Glass *et al*., 2010). Estes micro-organismos, além de aderirem à superfície da prótese, penetram no interior da mesma via um complexo de poros e fendas formados pela liberação de gases durante a polimerização da PMMA (Glass *et al*., 2001). Assim, o uso de prótese, aliado a uma higiene oral deficiente, pode afetar negativamente a saúde geral do indivíduo, podendo contribuir para a disseminação sistêmica de

micro-organismos potencialmente patógenos (Coulthwaite & Verran, 2007; Glass *et al*., 2010).

O tipo de micro-organismo que pode ser disseminado pela resina acrílica varia de acordo com a composição do biofilme presente na superfície (Neppelenbroek *et al*., 2009) ou nos poros das próteses (Glass *et al*., 2010). Tem sido demonstrada a relação entre bactérias orais e endocardite bacteriana, pneumonia por aspiração, infecção gastrointestinal e doença pulmonar obstrutiva crônica (Coulthwaite & Verran, 2007; von Fraunhofer e Loewy, 2009). Patógenos periodontais com forte associação com várias doenças sistêmicas, como *Aggregatibacter actinomycetemcomitans* e *Porphyromonas gingivalis,* também já foram detectados na microbiota de pacientes edêntulos portadores de prótese total (Sachdeo *et al*., 2008).

Os micro-organismos presentes no ambiente oral interagem entre si de diversas maneiras, tais como a utilização de produtos metabólicos uns dos outros através de comunicação via moléculas sinalizadoras, ajudando no processo de adesão e consequente colonização e formação de biofilme. Esta interação pode ser exemplificada com relação à formação de biofilmes de *Candida,* onde a presença do *S. mutans* favorece a formação de hifas de *Candida albicans (*Pereira-Cenci *et al.,* 2010). A colonização de fungos e bactérias nas bases de resina acrílica é um dos fatores responsáveis pela estomatite induzida por prótese (candidose), uma das lesões mais prevalentes da mucosa bucal (Sesma *et al*., 2005; Coulthwaite & Verran, 2007; Lima *et al*., 2007; von Fraunhofer & Loewy, 2009; Pereira-Cenci *et al*., 2010; De Freitas Fernandes *et al*., 2011). Micro-organismos oportunistas ou patogênicos podem penetrar na resina acrílica da base das próteses e sobreviver a uma profundidade que varia de 1,0 a 2,0 µm, podendo causar reinfecção da mucosa do paciente via prótese (Nepplelenbroek *et al*., 2009). Assim, o tratamento da candidose associada ao uso de próteses removíveis deve ser direcionado, primeiramente, para o controle do biofilme acumulado sobre estas (Webb *et al*., 1998).

Próteses mal higienizadas seriam, ainda, um dos fatores causadores do mau hálito (Spielman *et al*., 1996; von Fraunhofer & Loewy, 2009) resultado da degradação do biofilme que fica depositado na língua, dentes e próteses dentais (Ayers e Colquhoun, 1998). Um regime efetivo de higiene oral é fundamental para controlar a formação do biofilme sobre a superfície da prótese e contribuir para a prevenção e controle das doenças orais e sistêmicas associadas (Coulthwaite & Verran, 2007; Pasternak, 2009). No entanto, muitos pacientes idosos têm dificuldade em higienizar adequadamente suas próteses, quer seja por falta de orientação (Jagger e Harrison, 1995), diminuição da destreza manual, acuidade visual ou deficiência física ou mental (Coulthwaite & Verran, 2007).

A limpeza das próteses pode ser realizada por método mecânico e ou imersão em agente químico, sendo o melhor resultado obtido com uma associação dos dois métodos (Paranhos *et al*., 2007). A escovação, isoladamente, não consegue remover o biofilme dos poros (Barnabé *et al*., 2004; Glass *et al*., 2010) e, embora estudos atestem a eficácia de agentes químicos na limpeza de próteses (Gornitsky *et al*., 2002), essas substâncias, quando utilizadas isoladamente, não são efetivas no controle do biofilme sobre as próteses (Paranhos *et al*., 2007; Vieira *et al*., 2010). O agente químico ideal deve ser efetivo na remoção de depósitos orgânicos, inorgânicos e possuir propriedades bactericidas e fungicidas. Além disso, deve ser compatível com todos os materiais da prótese e deve ter baixo custo, que possibilite seu uso regular (Abelson, 1985; Jagger e Harrison, 1995). Os agentes químicos disponíveis comercialmente podem ser hipocloritos, peróxidos, enzimas, ácidos e enxaguantes bucais. Cada um destes limpadores tem modo de ação e eficácia diferentes (Felton *et al*., 2011).

Em um estudo *in situ*, Lima *et al*. (2007) compararam o efeito do uso de uma solução enzimática e do hipoclorito de sódio a 0,5% na rugosidade de superfície e no acumulo de biofilme na superfície da resina acrílica. Em um estudo cruzado, os voluntários foram divididos em três grupos: controle negativo, uso de solução enzimática e uso de hipoclorito. Houve aumento da rugosidade nos três grupos, sem diferença significante. Já com relação à habilidade em reduzir o

acumulo de biofilme, o hipoclorito foi considerado mais eficaz. Em outro estudo, De Freitas Fernandes *et al*. (2011) avaliou *in vitro* o efeito de limpadores químicos sobre o biofilme mono e multiespécie de *Candida,* formado sobre a superfície de materiais para confecção de próteses removíveis. Os limpadores químicos, contendo ou não enzimas, reduziram significantemente os níveis de *Candida*, entretanto o NaOCl a 0,5% foi mais eficaz, na medida em que resultou na ausência de células viáveis.

Os hipocloritos possuem ação bactericida e fungicida, sendo altamente eficazes na redução do biofilme formado sobre a superfície de materiais para confecção de próteses removíveis (Lima *et al*., 2007; De Freitas Fernandes *et al*., 2011) e na remoção de manchas leves (Abelson, 1985). Apesar de não dissolverem o cálculo, inibem sua formação, pois atuam sobre a matriz do biofilme (Budtz-Jörgensen, 1979; Jagger & Harrison, 1995). O hipoclorito de sódio está disponível comercialmente em diferentes concentrações. Concentração e tempo de imersão são fatores críticos, sendo que o uso de soluções concentradas por um tempo prolongado deve ser evitado, uma vez que pode afetar a coloração da resina (Anusavice, 2005).

A estabilidade de cor pode ser considerada um dos fatores mais importantes na aceitação do trabalho protético pelo paciente, sendo influenciada pelo método de polimerização da resina acrílica e o tipo de limpador utilizado. Para avaliar diferenças cromáticas, a Associação Dentária Americana (ADA) recomenda o sistema de cores CIELAB, proposto pela Commission Internationale de l'Eclairage (CIE) (Hong *et al*., 2009). Este sistema permite traduzir diferentes combinações cromáticas em dados numéricos. Um aspecto importante do sistema CIELAB é que a diferença de cor entre os tempos pode ser dada usando o parâmetro ΔE (Nimeroff, 1968).

Entre os efeitos deletérios que os agentes de limpeza podem causar sobre os componentes da prótese está o aumento da rugosidade de superfície, apontado um dos fatores contribuintes para alteração de cor da resina acrílica e acumulo de biofilme (Pereira-Cenci *et al*., 2010). A rugosidade de superfície pode

ser numericamente expressa por vários parâmetros, sendo o mais comum, a média dos valores de rugosidade, obtido pela média aritmética entre as distâncias pico-vale ao longo do comprimento da amostra, quando um estilete é movido sobre sua superfície (Lima *et al*., 2007). Uma superfície considerada clinicamente lisa pode apresentar micro-retenções que promovem a colonização microbiana. Embora a porosidade interna das próteses resulte de vários fatores e tende a variar, é possível que a microporosidade da base da prótese desempenhe um importante papel na formação do biofilme (von Fraunhofer & Loewy, 2009). A rugosidade de superfície da resina acrílica da prótese parece ter relação direta com o desenvolvimento do biofilme e sua colonização por *C. albicans* e outros micro-organismos. Ainda, tem sido observado que as regiões posteriores e o interior das próteses apresentam-se mais contaminados (Paranhos *et al*., 2007; von Fraunhofer e Loewy, 2009; Neppelenbroek *et al*., 2009).

Assim, considerando a indicação do uso de agentes químicos na higienização das próteses, a possibilidade desses agentes afetarem propriedades da resina acrílica, a necessidade de se estabelecer estratégias viáveis que visem prevenir e reduzir a formação de biofilmes, a eficácia apresentada pelo hipoclorito de sódio quando comparado a outros agentes químicos, propôs-se analisar o efeito do uso diário de uma solução de hipoclorito de sódio a 0,5% no biofilme, na estabilidade de cor e na rugosidade de superfície de próteses removíveis. Avaliou-se ainda, a satisfação do paciente com o uso deste desinfetante.

CAPÍTULO[1]

Evaluation of sodium hypochlorite as a denture cleanser: a clinical study

Sheila Rodrigues de Sousa Porta, DDS[a], MSc; Sílvia Carneiro de Lucena, DDS, MSc[a]; Wander José da Silva, DDS, MSc, PhD[b]; Altair Antoninha Del Bel Cury, DDS, MSc, PhD[b],

Department of Prosthodontics and Periodontology, Piracicaba Dental School, State University of Campinas – UNICAMP, Piracicaba, Sao Paulo, Brazil

[a] Graduate student, Department of Prosthodontics and Periodontology, Piracicaba Dental School, State University of Campinas.

[b] Professor, Department of Prosthodontics and Periodontology, Piracicaba Dental School, State University of Campinas.

Corresponding author:

Altair Antoninha Del Bel Cury
Faculdade de Odontologia de Piracicaba – UNICAMP
Avenida Limeira, 901 – Bairrro Areião
Piracicaba, São Paulo 13414-903, Brazil
E-mail: altcury@fop.unicamp.br

1 Este trabalho foi realizado no formato alternativo conforme Deliberação CCPG/002/06 da Comissão Central de Pós-Graduação (CCPG) da Universidade Estadual de Campinas, sendo submetido ao periódico *Clinical Oral Investigations* (Anexo 4).

ABSTRACT

Objectives: The aim of this study was to evaluate the effect of sodium hypochlorite (NaOCl) on biofilms, color stability and surface roughness (Ra) of complete dentures and the patients' acceptability.

Materials and Methods: Fifteen volunteers were instructed to keep their dentures daily immersed in a 0.5% NaOCl solution for 3 minutes, over 90 days. To evaluate the effect of NaOCl in controlling denture biofilm, swabs were taken from the dentures, diluted in PBS and inoculated on CHROMagar^{TM} and blood agar. The number of colony-forming units (cfu) was counted after a 48h incubation period. Color changes (ΔE) were assessed with a spectrophotometer using the CIEL*a*b* system. Ra was measured using a profilometer. The patients' acceptability of the cleaning method was checked based on a degree of satisfaction. Cell counts were analyzed using ANOVA. Data of color and surface roughness were evaluated with Friedman test and Kruskal Wallis, respectively.

Results: A significant reduction of the total number of microorganisms ($p<0.05$) and *Candida spp*. was noticed after the experimental time. No significant differences were found for ΔE and for Ra after 90 days ($p>0.05$). The level of patients' satisfaction increased throughout the follow-up time.

Conclusion: The 0.5% NaOCl was effective in reducing microorganisms without causing significant color and roughness changes. The volunteers reported great satisfaction with the cleaning results.

Clinical Relevance: The 0.5% NaOCl daily immersion can be indicated as an effective auxiliary method for denture hygiene.

Keywords: complete dentures; denture cleansers; biofilm; surface roughness; color stability.

INTRODUCTION

The rehabilitation of geriatric patients with complete dentures is expected to be a larger part of dental care in the next few decades[1]. It is critical for dentists and healthcare providers to pay attention to the oral hygiene needs of those patients, that usually have difficult to keep their dentures cleaned[2,3].

As a consequence of improper oral hygiene, denture may become a “reservoir” of microorganisms[4,5]. A roughened topography and, primarily, the microporous surfaces of an acrylic resin provide a wide range of environments to support potentially pathogenic microorganisms than can threaten the health of a physically vulnerable patient [6-8]. These microorganisms can be responsible for the development of local and systemic infections such as denture stomatitis, halitosis, respiratory airways diseases, bacterial endocarditis and gastrointestinal infections [4,5,8,9].

Therefore, careful daily removal of the biofilm present in the oral cavity and on complete dentures is of paramount importance to minimize opportunistic infections, to contribute to good oral and overall systemic health[10-12] and to maintain an esthetic, odor-free appliance[8,13]. Mechanical, chemical and a combination of mechanical and chemical strategies are available to patients to facilitate the removal of denture deposits. Denture daily brushing is recommended for all wearers[14], but brushing alone, in some conditions, can be an ineffective method of denture disinfection[3,6]. Since many elderly individuals have difficulty in maintaining oral and denture hygiene in consequence of diminished manual dexterity, impaired vision, or associated debilitating conditions[15], it is recommended to combine mechanical and chemical denture cleaning[5,8,12,16-19].

An ideal denture cleanser should be able to reduce biofilm accumulation, be bactericidal and fungicidal, without affect the physical and mechanical properties of denture base or prosthetic teeth (no whitening or abrading). It should be nontoxic, short acting, easy to use, clean tough stains, control denture odors and be cost effective[8,12]. Commercial denture cleansers use various active

agents: hypochlorite, peroxides, enzymes and acids or a combination of them[20]. Each immersion cleanser has a different mode of action and a different rate of efficacy for removal of adherent denture biofilms. Hypochlorite soaking solution has been demonstrated superior cleansing properties when compared to other types of commercially available denture cleansers [12,13,19,21-25]. However, there is a discussion if NaOCl can degrade the acrylic resin components, causing color changes (lightening)[12] and an increase on surface roughness[23].

There are few published guidelines on the daily care of complete denture prostheses[8] and, unfortunately, evidence-based guidelines do not exist[9,12]. Considering that it is necessary to stipulate a simple and effective routine protocol for denture cleaning[23,26,27] the aim of this study was to evaluate, *in vivo,* the efficacy of 0.5% sodium hypochlorite (NaOCl) as a denture cleanser and its effect on color stability and surface roughness of complete dentures. The patients' satisfaction with the denture cleaning method was also assessed.

MATERIALS AND METHODS

Experimental design

This clinical study evaluated the daily use of a 0.5% NaOCl solution as a denture cleanser. The response variables were number of total microorganisms and *Candida spp*, color stability and surface roughness of denture base and patients' satisfaction. The sample was consisted of fifteen complete denture wearers that were instructed to keep their dentures immersed in a 0.5% NaOCl solution for 3 minutes, once a day, after the nocturnal brushing. The follow-up time was 90 days and outcome variables were measured on baseline and on days 30, 60 and 90. The efficacy of NaOCl was analyzed by the number of total microorganisms and *Candida* spp on dentures surfaces and saliva. Color stability was evaluated with a portable spectrophotometer and surface roughness was measured using a profilometer. Additionally, patients' acceptability of the cleaning

method was assessed based on their degree of satisfaction. Data collected at each time-point were compared.

Volunteers' Selection

Twenty eight volunteers were recruited from a previous research of the Department of Prosthodontics and Periodontology of Piracicaba Dental School, State University of Campinas (FOP/UNICAMP), all wearing both complete dentures made from heat-polymerized acrylic resin for a mean period of 2 years. Subjects that presented adequate general health conditions and ability to comply with the experimental protocol were invited to participate. The inclusion criteria were do not using antibiotics, antifungal agents or antiseptic mouthwashes at pre-experimental and or during experimental period. The final sample was consisted of fifteen subjects, 3 men and 12 women, mean age of 69 ± 7.2 years. This study was approved by the local Research and Ethics Committee and all volunteers signed an informed and written consent.

Treating protocol

Volunteers were asked to keep cleaning their dentures by brushing as they have been doing. Additionally, during the experimental period, they were instructed to immerse their dentures daily in a 0.5% NaOCl solution (Proderma Pharmacy, Piracicaba, Brazil) for 3 minutes. After that, dentures should be thoroughly rinsed in running water before reinsertion into the oral cavity.

Also, volunteers were instructed to not use chemical cleansers and mouthwashes for at least one week before baseline data collection and during the experimental period.

Data Collection

Microbiological analysis

To determine the efficacy of the 0.5% NaOCl immersion protocol, samples for culture of *Candida* spp and total microorganisms were taken from

volunteers' dentures and saliva. One at a time, upper and lower dentures were removed from the mouth of the volunteers, gently rinsed under water and swabs were taken from the whole surface of the dentures. Then, each swab was individually placed in a sterile tube containing 3mL of phosphate buffered solution (PBS). Next, each volunteer was asked to spit into an empty sterile tube. The tubes were sonicated (7W for 30s) and the samples were serially diluted in PBS. These were vortexed for 30s between dilutions to maximize the homogeneity of the suspensions. Then, 20µL of each diluted solution were plated in triplicate on CHROMagar^{TM} and blood agar. The plates were incubated for 48h at 37°C in aerobiosis (CHROMagar^{TM}) and 10% carbon dioxide (blood agar) and the number of colony-forming units (cfu) was counted using a stereomicroscope. The use of CHROMagar also allowed a presumptive identification of the *Candida* spp isolated.

Color stability evaluation

To analyze the influence of 0.5% NaOCl on the color stability of base acrylic resin, the color of the 15 upper dentures was measured with a portable spectrophotometer (CM-700d; Konica Minolta Sensing Inc; Japan). The equipment was calibrated according to the manufacturer's instructions and it was used the standard illumination of D65. The dentures were washed under water and gently dried with a tissue paper prior to color evaluation. A device, in high-viscosity silicone (Zetalabor, Zhermack S.p.A, Badia Polesine, Rovigo, Italy), was fabricated for each denture and it was used to ensure that the same area was located in the spectrophotometer for color measurement at different time points. Also, this device had been used as a background, to avoid the influence of surface clarity on color determination.

The color differences were evaluated using the CIE L*a*b* color scale. This system is based on 3 parameters for defining color: L*, a* and b*, where L* represents lightness, from 0 (black) to 100 (perfect white), a* represents the red (positive value) or green chroma (negative value), while b* represents yellow (positive value) and blue chroma (negative value). The total color difference, ΔE*,

was calculated using the following formula:$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$, where delta values represent the differences measured in L*, a*, and b* values between the baseline coordinates and those measured at the time points. A limit of $\Delta E \leq 3.7$ was considered as clinically acceptable[28,29].

The levels of ΔE* were also quantified by the National Bureau of Standards (NBS) with the NBS units of color difference (table 1). NBS units are expressed by the following formula: NBS unit = ΔE x 0.92. NBS values lower than 3.0 (appreciable) are not clinically perceptible[29].

Table 1 Color difference according to the National Bureau of Standards

Color Difference	NBS Units
Trace	0.0 – 0.5
Slight	0.5 – 1.5
Noticeable	1.5 – 3.0
Appreciable	3.0 – 6.0
Much	6.0 – 12.0
Very much	12.0 - +

Preparation of specimens

To surface roughness assessment an in situ design was developed. Rectangular aluminum matrices (5 x 5 x 70 mm) were invested in metallic flasks. After plaster setting, the matrices were removed and heat-polymerized acrylic resin (Classico, Artigos Odontologicos Classico Ltda, Sao Paulo, Brazil) was packed and processed in a hot water bath at 74°C during 9h. After that, all flasks were allowed to bench cool for at least 2h. The specimens were then removed and immersed in distilled water at 37°C for 24h for residual monomer release. After that, they were sectioned at a metallographic machine (2 mm thick) and 90 specimens, measuring

5 x 5 x 2 mm, were obtained. They were polished using progressively smoother aluminum oxide papers (320-, 400- and 600 grit) in a horizontal polisher. Then, the specimens were ultrasonically cleansed in deionised water for 20 minutes.

Surface Roughness assessment

The surface roughness was measured using a profilometer (Surfcorder SE 1700; Kosaka Laboratory Ltd., Kosaka, Japan) with a 0.01 mm resolution, calibrated at a cut-off length of 0.8 mm, 2.4 mm percussion of measure and stylus speed of 0.5 mm/s. Three readings were made for each specimen, and the mean value of the Ra (µm) of the 90 specimens was 0.47± 0.09.

After the baseline measurement, six specimens were randomly glued, with light-cured resin, to the buccal posterior surface of each lower denture (three specimens on each side). On days 30, 60 and 90, two specimens (one from each side) were removed for surface roughness reassessment. Specimens were not reinserted.

Patients' satisfaction

The acceptability of the hygiene protocol was checked in every measurement session. Each volunteer was asked to translate in values their degree of satisfaction with the treatment using numerical rating scale[30] in a range from 0 (totally unsatisfied) to 10 (totally satisfied).

Statistical analysis

All analyses were performed using the SAS software (SAS Institute Inc., version 9.0, Cary, USA) with the level of significance set at 5%. The normality of error distribution and degree of non-constant variance were checked for the response variables. To normalize data, the cell count was transformed by logarithm ($\log_{10} x$) and theses data were analyzed using one-way ANOVA, followed by Tukey Test. Data of roughness and color changes were analyzed by Kruskal Wallis and by Friedman Test, respectively.

RESULTS

Figure 1 shows the flow diagram of the progress through the phases of this trial[31]. All the 15 volunteers allocated in this study were analyzed.

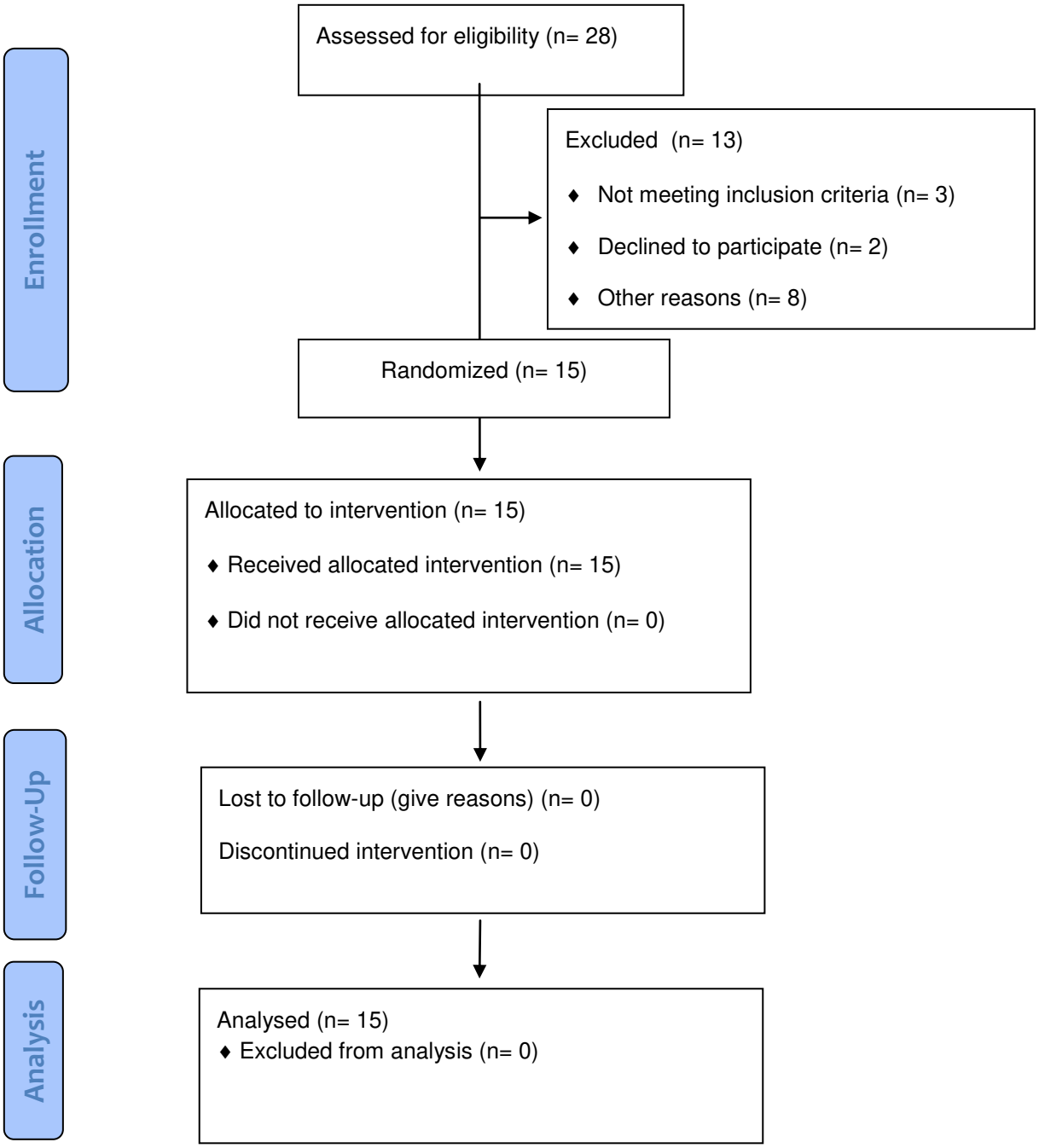


Fig. 1 – Flow diagram of study participants (adapted from the CONSORT Declaration).

In the beginning (baseline) all volunteers (15) presented biofilm on dentures and saliva. However as the treatment with 0.5% NaOCl began the total microorganisms decreased in all studied sites (Table 2). In contrast, only 10 volunteers (66.67%) had positive culture for *Candida spp.* in their upper dentures being two of them also positive in their lower prostheses as well in the saliva. After 30 days using NaOCl only three volunteers (20%) were positive for *Candida* and after 60 days only two volunteers (13%) still presented *Candida* spp (table 3). It is important to highlighted that were the same volunteers that showed *Candida* spp in all sites. Presumptive identification showed that *C.albicans* was the predominant isolate in all the studied period and *C. glabrata* remaining cells were not observed after the experimental time.

Table 2 Total microorganisms (CFU x 10^6 , mean ± SD, n = 15)

Time Point	Upper denture	Lower denture	Saliva
Baseline	69.1 ± 67.4a	54.9 ± 57.6a	69.5 ± 50.4a
30 days	15.9 ± 28.0b	7.6 ± 18.4b	48.7 ± 50.5ab
60 days	3.6 ± 6.6 bc	3.5 ± 9.3b	22.5 ± 24.2ab
90 days	0.79 ± 0.6 c	0.9 ± 1.6b	17.4 ± 12.3b

Different letters represent statistical differences among time points.

Table 3 Volunteers presenting *Candida* spp on upper and lower dentures and in saliva (n = 15)

Time Point	Upper	Lower	Saliva
Baseline	10	02	02
30 days	03	01	03
60 days	02	01	02
90 days	02	01	02

Regarding color stability, it was noticed slight changes in L*, a* and b* values throughout the experimental time, but no significant differences were found for ΔL*, Δa* and Δb* values. The same way, ΔE values did not show significant differences after denture daily immersion for 90 days. Using NBS units to measure color changes, 14 dentures demonstrated “trace” or “slight” changes and one denture demonstrated “noticeable” change (Table 4). A noticeable change is considered visually perceivable by 50% of human observers under controlled conditions, thus it is clinically acceptable[32].

Table 4 Color changes of upper base acrylic resin after experimental time

Volunteer	ΔL	Δa	Δb	ΔE	Color difference
1	0.69	-0.69	0.09	0.98	Slight
2	0.02	-0.96	-0.31	1.01	Slight
3	-0.33	-0.03	0.09	0.37	Trace
4	0.08	0.69	0.38	0.78	Slight
5	-0.4	0.14	-0.31	0.53	Trace
6	-0.73	-0.6	-0.23	0.98	Slight
7	0.04	0.01	-0.02	0.05	Trace
8	0.36	0.07	0.42	0.56	Slight
9	-0.56	0.17	0.65	0.88	Slight
10	-0.36	0.14	0.29	0.48	Trace
11	-1.61	-0.9	-0.9	2.05	Noticeable
12	-0.09	-0.18	0.48	0.53	Trace
13	0.16	-0.54	-0.21	0.61	Slight
14	0.3	0.29	0.37	0.56	Slight
15	-0.07	0.11	0.01	0.14	Trace

The roughness (Ra) baseline mean was 0.47 ± 0.09 and no statistically significant differences were observed between baseline and the evaluated time points ($p>0.05$).

The volunteers' satisfaction with the denture hygiene protocol increased from 53% of patients completely satisfied after 30 days of using hypochlorite for approximately 87% after 90 days. All volunteers reported cleaner and smoother dentures, a pleasant breath and an improvement in taste.

DISCUSSION

To the authors' knowledge, this clinical study was the first to evaluate the daily immersion in a 0.5% NaOCl solution, in a short-time of 3 minutes, as a routine protocol for denture cleaning. The objective of immersing a denture in a disinfectant is to remove biofilm, decontaminate the surface by decreasing microorganisms and prevent recolonization since dentures may function as a reservoir of pathogens [19,22,23]. Furthermore, the disinfectant should not affect the physical and mechanical properties of denture base or prosthetic teeth[8,12]. Although hypochlorite solutions have been demonstrated excellent cleansing properties [12,13,17,22,23,25] some authors affirm that soaking dentures in that disinfectant may degrade the acrylic resin components, depending on the concentration and immersion time. However, the immersion time evaluated in those investigations was 10 min or more. Herein, it was evaluated the effect of a low concentrated (0.5%) hypochlorite solution for a short time immersion of 3 minutes daily on reducing microorganisms, on color stability and on surface roughness of complete dentures. The experimental time was 90 days. Patients' satisfaction, as well as adverse effects which could influence the acceptability of this treatment, was also assessed.

A significant reduction of the total number of microorganisms ($p<0.001$) was observed after the soaking period for both dentures and in saliva. This could be attributed to NaOCl antimicrobial activity with action on bacterial essential enzymatic sites, promoting irreversible inactivation originated by hydroxyl ions and

chloramination action[33]. NaOCl also acts dissolving mucin and other organic substances, such as extracellular polymeric matrix[34] what may have facilitated denture biofilm removal. The microbiota of saliva in denture wearers may be derived from dentures as well as from oral mucous membranes, mainly the tongue, and it may be the proportions of pathogens present that cause the change from health to disease, rather than the presence or absence of particular species[8].

The results of this study showed that soaking dentures in 0.5% NaOCl was an effective treatment against *Candida spp* which is in accordance with the findings of previous *in vitro* studies [21-24,35]. Before the treatment with NaOCl, 66.67% (n=10) of the volunteers were positive for *Candida* spp. in their upper dentures. Repeated sampling, at an interval of 30 days, indicated that this contamination had decreased to 20% of the individuals. After 90 days, only two dentures, 13.33% of the initial number, showed yeast counts remaining. The significant reduction on *Candida* counts is an important finding considering that denture wearing and deficient oral hygiene are recognized as two local factors predisposing to *Candida* overgrowth as well as to oral infections, such as denture stomatitis [10,36,37]. Throughout the experimental time, *C. albicans* was the predominant specie isolated, as related by literature[38].

Although satisfactory results were found for NaOCl, dental literature has shown that this product has the potential to bleach denture-base[34,39]. Color change in acrylic resins can result from extrinsic and intrinsic factors[28] such as solubility, water sorption, surface roughness and chemical degradation[29]. All the dentures evaluated in this study presented similar chemical structure (polymethyl methacrylate), were processed with the conventional water bath method and had been installed 2 years before beginning the cleaning protocol.

Color evaluation can be performed visually or by using a spectrophotometer or a colorimeter. In the present study, a spectrophotometer was used to measure the color of 15 denture base acrylic resins. Color of the surface, surface clarity, thickness and smoothness of the specimen surface may influence

color determination[28]. Therefore, a silicone device was used as a background to avoid variations on color proceedings.

Color changes were evaluated using the CIEL*a*b* colorimetric system, a uniform 3-dimensional system that has been widely used for the determination of chromatic differences by translating combinations of differences into mathematical data[29]. In this system, color change (ΔE) is defined as relative color change between repeated color evaluations. A color difference below 3.7 has been reported to be clinically acceptable [28,29,40]. In this study, the highest ΔE value observed was 2.05 (volunteer 11, table 04), representing a lack of visible color change. In fact, it was observed slight changes, with increase or decrease in L*, a* and b* values throughout the experimental time. However, no significant differences were found among ΔE values which mean that, after immersion for 90 days, the influence of 0.5% NaOCl on the color of the dentures was not significant.

NBS units were also used in this study since it is an important parameter for color comparison. In a clinical scenario, because the color of the entire base changes, it is difficult to detect a change in color. Therefore, most patients would probably not detect color changes in the denture base that were below “much change” (NBS unit less than 6.0) if they occurred over a long time period[29]. After the experimental time, one denture underwent “noticeable” change (NBS = 1.9) and 14 dentures underwent “trace” or “slight” changes. All these changes are clinically acceptable. Important to clarify that a noticeable change is only detected by 50% of human observers and under controlled conditions [32].

In this study, a not significant rougher surface of the specimens was found after the 0.5% NaOCl soaking treatment ($p > 0.05$), which could contribute for no differences in color perception [26,28,41].

Patients’ satisfaction could influence the acceptability and effectiveness of the denture cleaning method in a daily use setting[42]. In this study, the level of satisfaction increased from 53% of patients completely satisfied after 30 days of the use of hypochlorite for approximately 87% after 90 days. Initially there were complaints, from three volunteers, about the smell and residual taste of

hypochlorite. It was reminded them that dentures should be thoroughly rinsed in running water, after soaking in the disinfectant and before reinsertation into the mouth. Therefore, after 60 days, all volunteers reported cleaner and smooth dentures and also a pleasant breath. Besides it, after 90 days all of them also reported an improvement in taste.

Although the influence of the investigation on the volunteers' hygiene behavior is one limitation that has to be taken into account[36], the present study tried to integrate all aspects of denture care: patient satisfaction with the treatment, denture decontamination and base material defects after decontamination processing. The results revealed that soaking complete dentures in 0.5% NaOCl for three minutes daily, in conjunction with brushing, could be suggested as a simple and effective denture cleaning protocol. Nevertheless, further clinical evaluation is required to investigate the long-term effectiveness of this cleaning method.

CONCLUSIONS

The 0.5% NaOCl, used as co-adjuvant for denture cleaning, was effective in reducing microorganisms, without causing color or significant roughness changes and the volunteers reported great satisfaction with the cleaning results.

REFERENCES

1. Sachdeo A, Haffajee AD, Socransky SS (2008) Biofilms in the edentulous oral cavity. J Prosthodont 17:348-356

2. Haumschild MS, Haumschild RJ (2009) The importance of oral health in long-term care. J Am Med Dir Assoc 10:667-671

3. Gornitsky M, Paradis J, Zandaverde G, Malo AM, Velly AM (2002) A clinical and microbiological evaluation of denture cleansers for geriatric patients in long-term care institutions. J Can Dent Assoc 68:39-45

4. Glass RT, Conrad RS, Bullard JW, Goodson LB, Mehta A, Stanley JL, Loewy ZG (2010) Evaluation of microbial flora found in previously worn prostheses from the northeast and southwest regions of the United States. J Prosthet Dent 103:384-389

5. Strajnic L, Dokic M, Vucinic P (2011) Contemporary methods and mobile denture cleansers and theirs significance for older population. Med Pregl 64 (9-10):497-502

6. Shay K (2000) Denture hygiene: a review and update. J Contemp Dent Pract 1 (2):28-41

7. Imsand M, Janssens JP, Auckenthaler R, Mojon P, Budtz-Jörgensen E (2002) Bronchopneumonia and oral health in hospitalized older patients. A pilot study. Gerodontology 19 (2):66-72

8. Coulthwaite L, Verran J (2007) Potential pathogenic aspects of denture plaque. Br J Biomed Sci 64 (4):180-189

9. Glass RT, Conrad RS, Bullard JW, Goodson LB, Mehta A, Lech SJ, Loewy ZG (2011) Evaluation of cleansing methods for previously worn prostheses. Compend Contin Educ Dent 32 (3):68-73

10. Coco BJ, Bagg J, Cross LJ, A. J, Cross J, Ramage G (2008) Mixed *Candida albicans* and *Candida glabrata* populations associated with the pathogenesis of denture stomatitis. Oral Microbiol Immunol 23:377-383

11. Pereira-Cenci T, Da Silva WJ, Cenci MS, Del Bel Cury AA (2010) Temporal changes of denture plaque microbiologic composition evaluated in situ. Int J Prosthodont 23:239-242

12. Felton DA (2011) Edentulism and Comorbid Factors. J Prosthodont 18:88-96

13. Lima EMCS, Moura JS, Del Bel Cury AA, R. GRCM, Cury JA (2007) Effect of enzymatic and NaOCl treatments on acrylic roughness and on biofilm accumulation. J Oral Rehabil 33:356-362

14. Ahariz M, Loeb I, Courtois P (2010) Oral candidiasis and dentures. Rev Stomatol Chir Maxillofac 111 (2):74-78

15. Akar GC, Ergul S (2008) The oral hygiene and denture status among residential home residents. Clin Oral Investig 12:61-65

16. Nishi Y, Seto K, Kamashita Y, Take C, Kurono A, Nagaoka E (2012) Examination of denture-cleaning methods based on the quantity of microorganisms adhering to a denture. Gerodontology 29(2): 259-266. doi:10.1111/j.1741-2358.2011.00461.x

17. Barnabé W, De Mendonça Neto T, Pimenta FC, Pegoraro LF, Scolaro JM (2004) Efficacy of sodium hypochlorite and coconut soap used as disinfecting agents in reduction of denture stomatitis, *Streptococcus mutans* and *Candida albicans*. J Oral Rehabil 31:453-459

18. Paranhos HFO, Silva-Lovato CH, Souza RF, Cruz PC, Freitas KM, Peracini A (2007) Effects of mechanical and chemical methods on denture biofilm accumulation. J Oral Rehabil 34:606-612

19. Jose A, Coco BJ, Milligan S, Young B, Lappin DF, Bagg J, Murray C, Ramage G (2010) Reducing the incidence of denture stomatitis: are denture cleansers sufficienty? J Prosthodont 19:252-257

20. Budtz-Jörgensen E (1979) Materials and methods for cleaning dentures. J Prosthet Dent 42 (6):619-623

21. Da Silva FC, Kimpara ET, Mancini MNG, Balducci I, Jorge AOC, Koga-Ito CY (2008) Effectiveness of six different disinfectants on removing five microbial species and effects on the topographic characteristics of acrylic resin. J Prosthodont 17:627-633

22. Ferreira MAF, Pereira-Cenci T, Vasconcelos LMR, Rodrigues-Garcia RCM, Del Bel Cury AA (2009) Efficacy of denture cleansers on denture liners contamined with *Candida* species. Clin Oral Investig 13:237-242

23. Vieira APC, Senna PM, Da Silva WJ, Del Bel Cury AA (2010) Long-term efficacy os denture cleansers in preventing Candida spp. biofilm recolonization on liner surface. Braz Oral Res 24 (3):342-348

24. Da Silva PM, Acosta EJ, De Rezende Pinto L, Graeff M, Spolidorio DM, Almeida RS, Porto VC (2011) Microscopical analysis of *Candida albicans* biofilms on heat-polymerized acrylic resin after chlorhexidine gluconate and sodium hypochlorite treatments. Mycoses 54 (6):712-717. doi:10.1111/j.1439-0507.2010.02005.x.

25. Rossato MB, Unfer B, May LG, Braun KO (2011) Analysis of the effectiveness of different hygiene procedures used in dental prostheses. Oral Health Prev Dent 9 (3):221-227

26. Hashiguchi M, Nishi Y, Kanie T, Ban S, Nagaoka E (2009) Bactericidal efficacy of glycine-type amphoteric surfactant as a denture cleaner and its influence on properties of denture base resins. Dent Mater J 28 (3):307-314

27. Srinivasan M, Gulabani M (2010) A microbiological evaluation of the use of denture cleansers in combination with an oral rinse in complete denture patients. Indian J Dent Res 21:353-356

28. Assunção WG, Barão VAR, Pita MS, Goiato MC (2009) Effect of polymerization methods and thermal cycling on color stability of acrylic resin denture teeth. J Prosthet Dent 102:385-392

29. Hong G, Murata H, Li Y, Sadamori S, Hamada T (2009) Influence of denture cleansers on the color stability of three types of denture base acrylic resin. J Prosthet Dent 101:205-213

30. Paul-Dauphin A, Guillemin F, Virion JM, Briançon S (1999) Bias and precision in Visual Analogue Scales: a randomized controlled trial. Am J Epidemiol 150:1117-1127

31. Schulz KF, Altman DG, Moher D (2010) CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. Trials 11 (32)

32. Imirzalioglu P, Karacaer O, Yilmaz B, Ozmen I (2010) Color stability of denture acrylic resins and a soft lining material against tea, coffee and nicotine. J Prosthodont 19:118-124. doi:10.1111/j.1532-849X.2009.00535.x

33. Estrela C, Estrela RAC, Barbin EL, Spano JC, Marchesan MA, Pécora JD (2002) Mechanism of action of sodium hypochlorite. Braz Dent J 13 (2):113-117

34. Nikawa H, Hamada T, Yamashiro H, Kumagai H (1999) A review of *in vitro* and *in vivo* methods to evaluate the efficacy of denture cleansers. Int J Prosthodont 12 (2):153-159

35. Pereira-Cenci T, Del Bel Cury AA, Cenci MS, Rodrigues-Garcia RCM (2007) In vitro Candida colonization on acrylic resin and denture liners: influence of surface free energy, roughness, saliva and adhering bacteria. Int J Prosthodont 20:308-310

36. Abbeele AV, Meel H, Ahariz M, Perraudin J, Beyer I, Courtois P (2008) Denture contamination by yeasts in the elderly. Gerodontology 25(4):222-228. doi:doi:10.1111/j.1741-2358.2007.00247.x

37. Gendreau L, Loewy ZG (2011) Epidemiology and etiology of denture stomatitis. J Prosthodont 20(4): 251-260. doi: 10.1111/j.1532-849X.2011.00698.x

38. Marcos-Arias C, Vicente JL, Sahand IH, Eguia A, De-Juan A, Madariaga L, Aguirre JM, Eraso E, Quinós G (2009) Isolation of *Candida dubliniensis* in denture stomatitis. Arch Oral Biol 54:127-131. doi:10.1016/j.archoralbio.2008.09.005

39. McNeme SJ, von Gonten AS, Woolsey GD (1991) Effects of laboratory disinfecting agents on color stability of denture acrylic resins. J Prosthet Dent 66:132-136

40. Imamura S, Takahashi H, Hayakawa I, Rendon-Loyaga PG, Minakuchi S (2008) Effect of filler type and polishing on the discoloration of composite resin artificial teeth. Dent Mater J 27 (6):802-808

41. Berger JC, Driscoll CF, Romberg E, Luo Q, Thompson G (2006) Surface roughness of denture base acrylic resins after processing and after polishing. J Prosthodont 15 (3):180-186. doi: 10.1111/j.1532-849X.2006.00098.x

42. de Souza RF, de Freitas OPH, Lovato da Silva CH, Abu-Naba'a L, Fedorowicz Z, Gurgam CA (2009) Interventions for cleaning dentures in adults. Cochrane Database Syst Rev (4). doi:10.1002/14651858.CD007395.pub2

CONCLUSÃO

O hipoclorito de sódio a 0,5% se mostrou efetivo na redução de micro-organismos e não provocou alterações significativas na cor e rugosidade das bases das próteses. A satisfação dos voluntários sugere que o protocolo de higienização foi considerado simples e efetivo.

REFERÊNCIAS*

1. Abelson DC. Denture plaque and denture cleansers: review of literature. Gerodont. 1985; 1(5): 202-6.
2. Anusavice KJ. Phillips Materiais Dentários. 11 ed. Rio de Janeiro: Elsevier Editora Ltda; 2005.
3. Ayers KMS, Colquhoun ANK. Halitosis: causes, diagnosis and treatment. N Z Dent J. 1998; 94(418): 156-6.
4. De Freitas Fernandes FS, Pereira-Cenci T, Da Silva WJ, Filho AP, Straioto FG, Del Bel Cury AA. Efficacy of denture cleansers on Candida spp. biofilm formed on polyamide and polymethyl methacrylate resins. J Prosthet Dent. 2011; 105(1): 51-8
5. Douglas CW, Watson AJ. Future needs for fixed and removable partial dentures in the United States. J Prosthet Dent. 2002; 87: 9-14.
6. Glass RT, Bullard JW, Hadley CS, Mix EW, Conrad RS. Partial spectrum of microorganisms found in dentures and possible disease implications. J Am Osteopath Assoc. 2001; 101(2): 92-4.
7. Jagger DC, Harrison A. Denture cleaning - the best approach. Br Dent J. 1995; 178(11): 413-7.
8. Karamouzos A, Papadopoulos MA, Kolokithas G, Athanasiou AE. Precision of in vivo spectrophotometric colour evaluation of natural teeth. J Oral Rehabil. 2007; 34(8): 613-21.
9. McGrath C, Bedi R. Can dentures improve the quality of life of those who have experienced considerable tooth loss? J Dent 2001; 29(4): 243-6.
10. Ministério da Saúde. Projeto SBBrasil 2010: Pesquisa nacional de saúde bucal – Resultados principais. Brasília: Ministério da Saúde; 2011.

* De acordo com a norma utilizada na FOP/Unicamp, baseada no modelo Vancouver. Abreviatura dos periódicos em conformidade com o Medline.

11. Neppelenbroek KM, Pinto ECT, Pavarina AC, Vergani CE, Jorge JH, Almihatti HJ. Aderência de microorganismos em materiais para base de próteses. Rev Odontol Univ São Paulo. 2009; 21(2): 126-36.
12. Nimeroff I. Colorimetry. Natl Bureau Stand Monogr. 1968; 104: 4-32.
13. Pasternak J. Biofilmes: um inimigo (in) visível. SBCC. 2009; 36-8
14. Petersen PO, Yamamoto T. Improving the oral health of older people: the approach of the WHO Global Oral Health Programme. Community Dent Oral Epidemiol. 2005; 33: 81-92.
15. Polzer I, Schimmel M, Muller F, Biffar R. Edentulism and general health problems of the elderly. Int Dent J. 2010; 60(3): 143-5.
16. Sahyoun NR, Krall E. Low dietary quality among older adults with self-perceived ill-fitting dentures. J Am Diet Assoc. 2003; 103: 1494-9.
17. Sesma N, Laganá DC, Gil C, Morimoto S. Capacidade de remoção do biofilme por meio de um produto enzimático para higienização de bases protéticas. RPG Rev Pós Grad. 2005; 12(4): 417-22.
18. Shinkai RSA, Hatch JP, Rugh JD, Sakai S, Mobley CC, Saunders MJ. Dietary intake in edentulous subjects. J Oral Rehabil. 1993;20:159-76.
19. Spielman AI, Bivona P, Rifkin BR. A common oral problem. N Y State Dent J. 1996; 62(10): 36-42.
20. Tanaka J, Tanaka M, T. K. Longitudinal research on the oral environment of elderly wearing fixed or removable prostheses. J Prosthodont Res. 2009; 53: 83-8.
21. Tramini P, Montal S, Valcarcel J. Tooth loss and associated factors in long-term institutionalised elderly patients. Gerodont. 2007; 24(4): 196-203.
22. Von Fraunhofer JA, Loewy Z. Factors involved in microbial colonization of oral prostheses. Gen Dent. 2009; 57(2): 136-43.
23. Webb BC, Thomas CJ, Willcox MD, Harty DW, Knox KW. Candida-associated denture stomatitis. Aetiology and management: a review. Part 2. Oral diseases caused by Candida species. Aust Dent J. 1998; 43: 160-6.

ANEXO 1 – Certificado de aprovação do Comitê de Ética em Pesquisa

COMITÊ DE ÉTICA EM PESQUISA
FACULDADE DE ODONTOLOGIA DE PIRACICABA
UNIVERSIDADE ESTADUAL DE CAMPINAS

CERTIFICADO

O Comitê de Ética em Pesquisa da FOP-UNICAMP certifica que o projeto de pesquisa **"Condição de próteses dentais removíveis: Influência sobre a performance mastigatória, compostos sulfurados voláteis e a presença de biofilme"**, protocolo nº 068/2008, dos pesquisadores Altair Antoninha Del Bel Cury, Renata Cunha Matheus Rodrigues Garcia, Sheila Rodrigues de Sousa Porta, Sílvia Carneiro de Lucena, Simone Guimarães Farias Gomes e Wander José da Silva, satisfaz as exigências do Conselho Nacional de Saúde - Ministério da Saúde para as pesquisas em seres humanos e foi aprovado por este comitê em 15/11/2010.

The Ethics Committee in Research of the School of Dentistry of Piracicaba - State University of Campinas, certify that the project **"Denture condition: Influency on masticatory performance, volatile sulfur compounds and presence of biofilm"**, register number 068/2008, of Altair Antoninha Del Bel Cury, Renata Cunha Matheus Rodrigues Garcia, Sheila Rodrigues de Sousa Porta, Sílvia Carneiro de Lucena, Simone Guimarães Farias Gomes and Wander José da Silva, comply with the recommendations of the National Health Council - Ministry of Health of Brazil for research in human subjects and therefore was approved by this committee at 11/15/2010.

Prof. Dr. Pablo Agustin Vargas
Secretário
CEP/FOP/UNICAMP

Prof. Dr. Jacks Jorge Junior
Coordenador
CEP/FOP/UNICAMP

Nota: O título do protocolo aparece como fornecido pelos pesquisadores, sem qualquer edição.
Notice: The title of the project appears as provided by the authors, without editing.

ANEXO 2 – Protocolo de Registro: ClinicalTrials

ClinicalTrials.gov
Protocol Registration System

Protocol Registration Receipt
06/08/2012

Evaluation of Sodium Hypochlorite as a Denture Cleanser

This study has been completed.

Sponsor:	University of Campinas, Brazil
Collaborators:	
Information provided by (Responsible Party):	Sheila Rodrigues de Sousa Porta, University of Campinas, Brazil
ClinicalTrials.gov Identifier:	NCT01616355

Purpose

The present study tried to integrate all aspects of denture care: patient satisfaction with the treatment, denture decontamination and base material defects after decontamination processing.

Condition	Intervention	Phase
Problems With Dentures	Sodium hypochlorite immersion	N/A

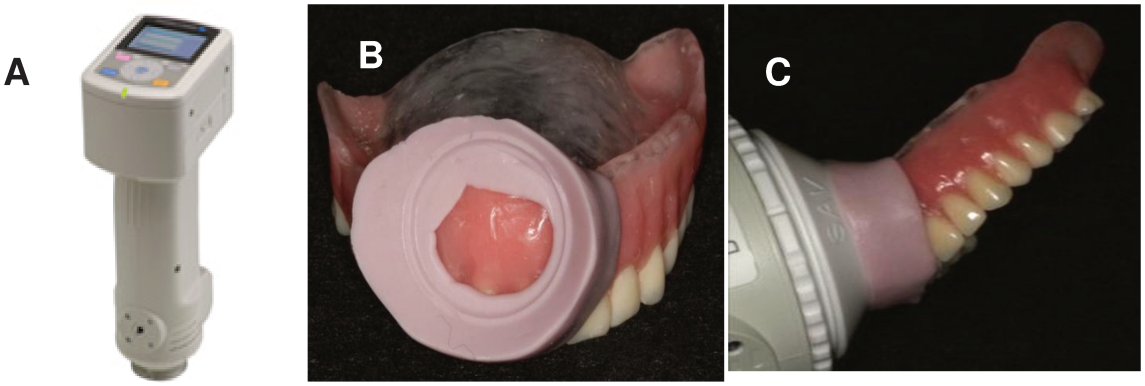
Study Type: Interventional
Study Design: Basic Science, Single Group Assignment, Open Label, N/A, Efficacy Study
Official Title: Denture Condition: Influence on Masticatory Performance, Volatile Sulfur Compounds and Presence of Biofilm

Further study details as provided by Sheila Rodrigues de Sousa Porta, University of Campinas, Brazil:

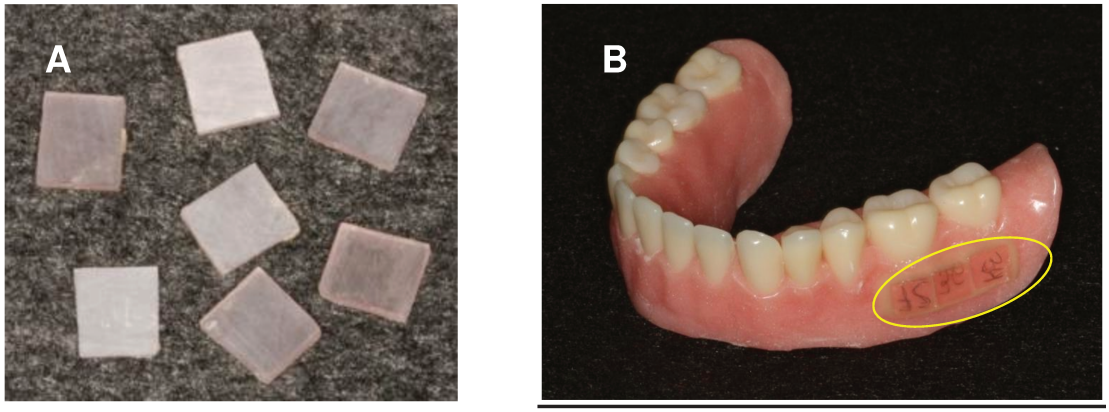
Primary Outcome Measure:

- Microorganisms reduction [Time Frame: Baseline, 30, 60 and 90 days] [Designated as safety issue: Yes]
  Quantitative analysis of total microorganisms and Candida spp. Samples for culture were collected from dentures and saliva.

ANEXO 3 – Ilustrações Materiais e Métodos



Avaliação da estabilidade de cor. A) Espectrofotômetro Konica Minolta CM-700d. B) Dispositivo em silicone para padronização da posição do aparelho. C) Leitura da cor.



Avaliação da rugosidade de superfície. A) Espécimes em resina acrílica termopolimerizável (5 x 5 x 2 mm). B) Espécimes fixados na prótese total inferior.

ANEXO 4 – Comprovante de submissão do artigo

Clinical Oral Investigations

Evaluation of sodium hypochlorite as a denture cleanser: a clinical study

--Manuscript Draft--

Manuscript Number:	CLOI-D-12-00499
Full Title:	Evaluation of sodium hypochlorite as a denture cleanser: a clinical study
Article Type:	Original Article
Corresponding Author:	Altair Antoninha Del Bel Cury, DDS; MSc; PhD UNICAMP Piracicaba,, São Paulo BRAZIL
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	UNICAMP
Corresponding Author's Secondary Institution:	
First Author:	Sheila Rodrigues de Sousa Porta, MSc
First Author Secondary Information:	
Order of Authors:	Sheila Rodrigues de Sousa Porta, MSc
	Silvia Carneiro de Lucena, DDS; MSc
	Wander José da Silva, DDS, MSc, PhD
	Altair Antoninha Del Bel Cury, DDS; MSc; PhD
Order of Authors Secondary Information:	
Abstract:	Objectives: The aim of this study was to evaluate the effect of sodium hypochlorite (NaOCl) on biofilms, color stability and surface roughness (Ra) of complete dentures and the patients' acceptability. Materials and Methods: Fifteen volunteers were instructed to keep their dentures daily immersed in a 0.5% NaOCl solution for 3 minutes, over 90 days. To evaluate the effect of NaOCl in controlling denture biofilm, swabs were taken from the dentures, diluted in PBS and inoculated on CHROMagarTM and blood agar. The number of colony-forming units (cfu) was counted after a 48h incubation period. Color changes (ΔE) were assessed with a spectrophotometer using the CIEL*a*b* system. Ra was measured using a profilometer. The patients' acceptability of the cleaning method was checked based on a degree of satisfaction. Cell counts were analyzed using ANOVA. Data of color and surface roughness were evaluated with Friedman test and Kruskal Wallis, respectively. Results: A significant reduction of the total number of microorganisms ($p<0.05$) and Candida spp. was noticed after the experimental time. No significant differences were found for ΔE and for Ra after 90 days ($p>0.05$). The level of patients' satisfaction increased throughout the follow-up time. Conclusion: The 0.5% NaOCl was effective in reducing microorganisms without causing significant color and roughness changes. The volunteers reported great satisfaction with the cleaning results. Clinical Relevance: The 0.5% NaOCl daily immersion can be indicated as an effective auxiliary method for denture hygiene. Keywords: complete dentures; denture cleansers; biofilm; surface roughness; color stability.
Suggested Reviewers:	