UNIVERSIDADE DE UBERABA
MESTRADO ACADÊMICO EM ODONTOLOGIA
MARCELA SILVA COSTA

ASPECTOS MICROBIOLÓGICOS E FÍSICO–MECÂNICOS ASSOCIADOS AO USO DE DIFERENTES FORMAS COMERCIAIS DE ADESIVOS PARA PRÓTESES DENTÁRIAS

UBERABA – MG
2019

MARCELA SILVA COSTA

ASPECTOS MICROBIOLÓGICOS E FÍSICO–MECÂNICOS ASSOCIADOS AO USO DE DIFERENTES FORMAS COMERCIAIS DE ADESIVOS PARA PRÓTESES DENTÁRIAS

Dissertação apresentada ao Programa de Pós-Graduação em Odontologia – Mestrado Acadêmico da Universidade de Uberaba, como requisito parcial para a obtenção do título de Mestre em Odontologia, na área de concentração em Clínica Odontológica Integrada.

Orientadora: Profa. Dra. Denise Tornavoi de Castro

UBERABA – MG

2019

Catalogação elaborada pelo Setor de Referência da Biblioteca Central UNIUBE

Costa, Marcela Silva.
C823a Aspectos microbiológicos e físico-mecânicos associados ao uso de diferentes formas comerciais de adesivos para próteses dentárias / Marcela Silva Costa. – Uberaba, 2019.
63 f. : il. color.

Dissertação (mestrado) – Universidade de Uberaba. Programa de Mestrado em Odontologia. Área Clínica Odontológica Integrada.
Orientadora: Profa. Dra. Denise Tornavoi de Castro.

1. Prótese dentária. 2. Adesivos dentários. 3. Biofilme. 4. Higiene bucal. I. Castro, Denise Tornavoi de. II. Universidade de Uberaba. Programa de Mestrado em Odontologia. Área Clínica Odontológica Integrada. III. Título.

CDD 617.69

MARCELA SILVA COSTA

ASPECTOS MICROBIOLÓGICOS E FÍSICO-MECÂNICOS ASSOCIADOS AO USO DE DIFERENTES FORMAS COMERCIAIS DE ADESIVOS PARA PRÓTESES DENTÁRIAS

Dissertação apresentada como parte dos requisitos para obtenção do título de Mestre em Odontologia do Programa de Pós-Graduação em Odontologia - Mestrado da Universidade de Uberaba.

Área de concentração: Clínica Odontológica Integrada

Aprovado (a) em: 17/12/2019

BANCA EXAMINADORA:

Profª. Drª. Denise Tornavoi de Castro
Orientadora
Universidade de Uberaba

Prof. Dr. Cesar Penazzo Lepri
Universidade de Uberaba

Profª. Drª. Andrea Candido dos Reis
Faculdade de Odontologia de Ribeirão Preto

DEDICATÓRIA

À Deus, que iluminou o meu caminho durante esta caminhada, me dando forças, coragem, sendo o meu Guia durante minha jornada.

Ao meu pai, Márcio Ernane da Costa, por todo seu amor, sua dedicação ao meu futuro, com quem aprendo diariamente a ser uma pessoa melhor, com seus exemplos de humildade, persistência, responsabilidade e acima de tudo, pelo apoio em todos os meus sonhos, me impulsionando na busca por novos conhecimentos.

À minha mãe, Márcia Teodora da Silva Costa, pelo cuidado, preocupação e dedicação em todos os momentos, por ser a mulher mais incrível, por todo amor do mundo, pelo cuidado incansável, por ser o esteio da nossa família e o meu exemplo.

Às minhas irmãs, Marina Vitória e Maria Clara, por acreditarem sempre no meu potencial, por estarem sempre ao meu lado, pelo carinho e companheirismo, por ser referência para mim.

Ao Lucas, pelo companheirismo e apoio, por ser paciente e compreensivo, me incentivando em todos os momentos.

À toda minha família, que está sempre presente, pelos ensinamentos e orações durante essa trajetória.

AGRADECIMENTOS ESPECIAIS

Primeiramente a Deus, por ser essencial em minha vida, autor do meu destino.

Aos meus pais Márcio Ernane da Costa e Márcia Teodora da Silva Costa, por serem meus principais exemplos, pelo trabalho diário investido na minha educação. Por confiarem, pelo amor incondicional, pelos incentivos, pelas palavras, pela ajuda e por ser colo em qualquer momento. Às minhas irmãs, Maria Vitória e Maria Clara, pelo companheirismo, carinho, amor, por acreditarem na realização dos meus sonhos. Ao Lucas, pela paciência e incentivo. Devo essa vitória a cada um de vocês.

À minha querida orientadora professora Denise Tornavoi de Castro, que desde o princípio, me acolheu com muita atenção e carinho, com uma serenidade inigualável, com muito conhecimento para oferecer e sempre disposta a ajudar. Pela disposição, pela atenção, pelo cuidado e paciência com que teve todas as vezes em que eu precisei de ajuda, obrigada pela compreensão. Pela oportunidade de conhecer o laboratório da FORP-USP, pelos congressos, pelos ensinamentos e pela sabedoria transmitidos. Agradeço à Deus por ter te conhecido, por ter tido a honra de ser sua orientada, uma pessoa de um coração bondoso, humilde e disposto a ajudar. Com toda certeza, seu caminho será tão brilhante quanto você, torço pelo seu sucesso e pela sua felicidade. Eterna gratidão por tudo.

AGRADECIMENTOS

À Universidade de Uberaba, por meio do Reitor Prof. Dr. Marcelo Palmério.

A Pró-reitoria de Pesquisa, Pós-graduação e Extensão por meio do Pró-Reitor Prof. Dr. André Luís Teixeira Fernandes.

Ao coordenador do Mestrado em Odontologia, Prof. Dr. César Penazzo Lepri, pela dedicação para com todos do mestrado.

Aos Professores do Programa de Mestrado, pelos ensinamentos em todas as aulas ministradas e pelo apoio.

Aos Professores convidados para qualificação, Maria Angélica Hueb de Menezes Oliveira, Vinícius Rangel Geraldo Martins e César Penazzo Lepri pelos ensinamentos e por contribuírem com este trabalho.

À Flávia, sempre disposta a ajudar, meu muito obrigado.

À FORP-USP pela parceria durante a fase laboratorial, em especial à técnica Viviane de Cássia Oliveira pela ajuda durante os experimentos microbiológicos.

Aos amigos que fiz e aos amigos de graduação que permaneceram juntos no mestrado.

À Larissa, pela amizade desde a primeira vez que nos vimos, pelo companheirismo, alegria e parceria em todos os momentos. À Isabela, amiga de graduação que me acompanhou durante o mestrado, pela ajuda e amizade. À Janaína, pelo companheirismo durante esse tempo.

Ao Marcelo, Martins e Antônio, por estarem sempre dispostos a ajudar, que foram fundamentais para a conclusão deste trabalho, onde me ajudaram no Laboratório de Ensaios Mecânicos Odontológicos. À Camila, pela ajuda durante a pesquisa.

RESUMO

O objetivo desse estudo foi avaliar a influência de diferentes formas comerciais de adesivos para prótese dentária na formação de biofilmes multiespécies e na força adesiva, bem como a eficácia de diferentes protocolos de higienização para a remoção dos mesmos. Amostras em resina acrílica termopolimerizável foram confeccionadas nas dimensões de 6 mm de largura x 10 mm de comprimento e 3 mm de espessura para a análise microbiológica e eficácia dos protocolos de higienização, e com 25 mm de diâmetro x 35 mm de altura para a análise da força adesiva. Estas foram divididas em quatro grupos: Controle (Sem Adesivo), Ultra Corega® Creme, Corega® Fita Adesiva e Ultra Corega® Pó. A formação de biofilme multiespécies (*Candida albicans, Staphylococcus aureus e Pseudomonas aeruginosa*) foi avaliada pela contagem das unidades formadoras de colônias (n=10) e por microscopia de fluorescência (n=2). Para avaliar a eficácia dos protocolos de higienização, as amostras foram divididas em cinco subgrupos (n=10): Escovação com água destilada; Escovação com sabonete líquido Protex®; Escovação com dentifrício convencional Colgate®; Imersão em Corega Tabs® e Imersão em Corega Tabs® seguida da escovação com a própria solução. O adesivo remanescente foi quantificado com o software ImageJ. A força adesiva foi testada em 5 minutos, 3 horas, 6 horas, 12 horas e 24 horas após a aplicação do adesivo. Os dados foram avaliados pelo teste de Kruskal-Wallis e pós teste de Dunn ou ANOVA de dois fatores e pós teste de Bonferroni, a depender da distribuição e das medidas resumo, com nível de significância de 5%. *C. albicans* formou mais biofilme em Corega® Fita Adesiva ($p=0,007$) e Ultra Corega® Pó ($p=0,001$), *P. aeruginosa* em Ultra Corega® Creme ($p<0,001$) e Ultra Corega® Pó ($p<0,001$) e *S. aureus* em Corega® Fita Adesiva ($p<0,001$). Todas as formas comerciais dos adesivos promoveram maior formação de biofilme em relação ao grupo sem adesivo ($p=0,003$). A escovação com Colgate® e Protex® foi mais eficaz na remoção dos adesivos ($p<0,05$). Considerando-se a forma comercial, independente do tempo, o Ultra Corega® Pó apresentou a maior força adesiva ($p<0,05$). Apenas o Corega® Fita Adesiva apresentou alteração na força adesiva em função do tempo, sendo esta maior em 3 horas ($p=0,004$). O uso de materiais adesivos favorece o acúmulo de biofilme, e a escovação a remoção do adesivo. A força adesiva varia dependendo do tipo comercial.

Palavras chave: Prótese dentária; Adesivos para prótese; Biofilme; Higiene; Força adesiva.

ABSTRACT

The aim of this study was to evaluate the influence of different commercial forms of denture adhesives on the formation of multispecies biofilms and adhesive strength, as well as the effectiveness of different hygiene protocols for their removal. Thermopolymerizable acrylic resin samples were made in dimensions 6 mm wide x 10 mm long and 3 mm thick for microbiological analysis and efficacy of hygiene protocols, and 25 mm diameter x 35 mm high for the analysis of adhesive strength. These were divided into four groups: Control (No Adhesive), Ultra Corega® Cream, Corega® Strip Adhesive and Ultra Corega® Powder. The formation of multispecies biofilm (*Candida albicans*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*) was evaluated by counting colony forming units (n=10) and fluorescence microscopy (n=2). To evaluate the effectiveness of the hygiene protocols, the samples were divided into five subgroups (n=10): Brushing with distilled water; Brushing with Protex® liquid soap; Brushing with Colgate® conventional toothpaste; Immersion in Corega Tabs® and Immersion in Corega Tabs® followed by brushing with the solution itself. The remaining adhesive was quantified with ImageJ software. The adhesive strength was tested at 5 minutes, 3 hours, 6 hours, 12 hours and 24 hours after adhesive application. Data were evaluated by Kruskal-Wallis test and Dunn post hoc test or 2-way ANOVA and Bonferroni post hoc test, depending on distribution and summary measures, with a significance level of 5%. *C. albicans* formed more biofilm in Corega® Strip ($p=0.007$) and Ultra Corega® Powder ($p=0.001$), *P. aeruginosa* in Ultra Corega® Cream ($p<0.001$) and Ultra Corega® Powder ($p<0.001$) and *S. aureus* in Corega® Strip ($p<0.001$). All commercial forms of the adhesives promoted higher biofilm formation compared to the group without adhesive ($p=0.003$). Brushing with Colgate® and Protex® was most effective at removing the adhesives ($p<0.05$). Considering comercial form, independently of time, Ultra Corega® Powder had the highest adhesive strength ($p<0.05$). Only Corega® Strip showed a change in adhesive strength as a function of time, which was greater in 3 hours ($p=0.004$). The use of adhesive materials favors biofilm accumulation, and brushing adhesive removal. The adhesive strength varies depending on the commercial type.

Keywords: Dental prosthesis; Prosthetic adhesives; Biofilm; Hygiene; Adhesive strength.

SUMÁRIO

1. INTRODUÇÃO

Os avanços no campo da saúde refletem na melhoria da qualidade e aumento da expectativa de vida. A quantidade de pessoas com sessenta anos ou mais deverá atingir dois bilhões em 2050 (22% da população global) (HARADA-HADA *et al*., 2016). Dessa forma, problemas de saúde e adaptações ao novo estilo de vida tornaram-se uma realidade para a qual os profissionais de saúde devem estar preparados (MANGER *et al*., 2017; DIETRICH *et al*., 2017).

A preocupação com esse grupo populacional torna-se evidente também por parte dos profissionais da odontologia. Embora medidas preventivas tenham levado à redução do número de dentes perdidos em indivíduos adultos, ainda é grande o número de edentados total ou parcial, assim, a demanda por aparelhos protéticos deverá aumentar devido ao rápido crescimento da população idosa (FELTON *et al*., 2011; CARDOSO *et al*., 2016).

As próteses implantossuportadas são uma alternativa viável para o tratamento de pacientes edêntulos, entretanto, as próteses totais convencionais ainda representam a principal opção de tratamento devido ao baixo custo, limitações sistêmicas ou escolha individual (NICOLAS e VEYRUNE, 2010). Porém, são comuns queixas relacionadas com a falta de retenção, instabilidade, dificuldades de mastigação, baixa autoestima, redução da qualidade de vida, do convívio social e da satisfação (CARDOSO *et al*., 2016; SHAMSOLKETABI; NILI, 2018).

Materiais adesivos são reconhecidos como agentes auxiliares na retenção, estabilidade e função destas próteses (ALMEIDA *et al*., 2018). Propostos no final do século XVIII, foram relatados cientificamente pela primeira vez em 1935 e, quando indicados adequadamente, podem melhorar a tensão superficial interfacial entre as bases de prótese e os tecidos moles subjacentes melhorando a retenção das próteses, com impacto significativo na qualidade de vida dos usuários (PRADÍES *et al*., 2009; OLIVEIRA-JUNIOR *et al*., 2014). Além disso, podem ser usados para estabilizar bases de prótese durante o registro dos relacionamentos maxilomandibulares e servir como importante via para entrega de fármacos aos tecidos orais (ELLIS; PELEKIS; THOMASON, 2007; EMAMI *et al*., 2009; KORE *et al*., 2013; ALMEIDA *et al*., 2018).

Diferentes formas de adesivos para próteses dentárias são amplamente utilizadas pelos pacientes com edentulismo (POLYZOIS; BAAT, 2012; RAJARAM; MANOJ, 2017), devendo estes ser biocompatíveis, de fácil aplicação e remoção, e capazes de

manter a força de adesão por 12 a 16 horas (FALLAHI *et al*., 2018). Estes materiais podem ser divididos em insolúveis e solúveis, com composições variadas (OLIVEIRA-JUNIOR *et al*., 2014). Faz parte do grupo insolúvel a forma de fita e estes produtos são geralmente impregnados a um componente ativado pela saliva, como o alginato de sódio ou polímero de óxido de etileno, tornando-se pegajosos quando absorvem saliva. As formas de creme e pó consistem em produtos solúveis, compostos por ingredientes ativos, tais como sais de polímeros com rápida e baixa solubilidade, dentre eles carboximetilcelulose (CMC) e polivinil éter metilcelulose (PVM-MA), e não ativos, como petrolato, óleo mineral, corantes e borato de sódio que são adicionados como aglutinantes, corantes ou conservantes. Os adesivos para próteses são, portanto, um complemento na reabilitação oral (POLYZOIS; BAAT, 2012).

Estima-se que nos EUA cerca de 22% dos pacientes completamente desdentados usam adesivos regularmente e aproximadamente 75% dos dentistas recomendam o uso aos pacientes de próteses totais (GRASSO, 1996; OLIVEIRA *et al*., 2010) (OLIVEIRA-JUNIOR *et al*., 2018). Entretanto, a higiene inadequada da superfície da prótese pode fazer dos adesivos um substrato adicional ao crescimento de micro-organismos (NUNES *et al*., 2016; CARTAGENA *et al.*, 2017), favorecendo o desenvolvimento de problemas locais, incluindo candidoses crônicas e subsequente estomatite protética (EP) (EMAMI *et al*., 2009) caracterizada por ser uma doença com etiologia multifatorial, porém, independente dos fatores de contribuição como idade, doença sistêmica, tabagismo, uso da prótese durante o sono, redução do fluxo salivar, trauma causado pela falta de retenção e estabilidade da prótese, a *Candida albicans* é reconhecida como o principal agente causador (GENDREAU; LOEWY, 2011).

Embora a maioria da literatura discuta unicamente esta condição, há evidências de tratar-se de uma doença polimicrobiana, com a associação de diversas espécies bacterianas patogênicas encontradas na cavidade oral (PEREIRA *et al*., 2013; O'DONNEL *et al*., 2015; SHI *et al*., 2016). Além disso, a proliferação de algumas bactérias orais relacionada à uma pobre higienização tem sido associada a doenças sistêmicas tais como endocardite bacteriana, pneumonia aspirativa, doença pulmonar obstrutiva crônica, infecções generalizadas do trato respiratório, principalmente em idosos dependentes (COULTHWAITE; VERRAN, 2007; O'DONNEL, *et al*., 2016). No entanto, o efeito dos adesivos no crescimento de biofilmes multiespécies é pouco conhecido, embora saiba-se que as cooperações inter-reino favorecem a adesão e colonização, bem como a resistência a agentes antimicrobianos.

Diante disso, este estudo avaliou a influência do uso de diferentes formas comerciais de adesivos para prótese dentária na formação de biofilmes multiespécies e na força adesiva, bem como a eficácia de diferentes protocolos de higienização para a remoção dos mesmos. A hipótese nula foi a de que não existe influência do tipo de adesivo na formação de biofilme, na força adesiva, nem mesmo diferença na eficácia dos protocolos de higienização para remoção dos adesivos.

2. PROPOSIÇÃO

O objetivo desse estudo foi avaliar a influência do uso de diferentes formas comerciais de adesivos para prótese dentária na formação de biofilmes multiespécies e na força adesiva, bem como a eficácia de diferentes protocolos de higienização para a remoção dos mesmos.

3. CAPÍTULO 1

Microbiological and physical-mechanical aspects associated with different commercial forms of denture adhesives

Marcela S. Costa, MD, [a] Cláudia H. Lovato da Silva, PhD, [b] Viviane C. Oliveira, MD, [c] Evandro Watanabe, PhD, [d] Andréa C. dos Reis, PhD, [e] César P. Lepri, PhD, [f] Denise T. de Castro, PhD, [g]

[a] Graduate student, Department of Biomaterials, University of Uberaba, Uberaba (MG), Brazil.

[b] Associated Professor, Department of Dental Materials and Prosthodontics, Ribeirão Preto Dental School, University of São Paulo, Ribeirão Preto (SP), Brazil.

[c] Laboratory technique, Department of Dental Materials and Prosthodontics, Ribeirão Preto Dental School, University of São Paulo, Ribeirão Preto (SP), Brazil.

[d] Associated Professor, Department of Restorative Dentistry, Ribeirão Preto Dental School, University of São Paulo, Ribeirão Preto (SP), Brazil.

[e] Associated Professor, Department of Dental Materials and Prosthodontics, Ribeirão Preto Dental School, University of São Paulo, Ribeirão Preto (SP), Brazil.

[f] Associated Professor, Department of Biomaterials, University of Uberaba, Uberaba (MG), Brazil.

[g] Associated Professor, Department of Biomaterials, University of Uberaba, Uberaba (MG), Brazil.

Corresponding author: Denise Tornavoi de Castro

Av. Nenê Sabino 1801, Bairro Universitário, CEP 38.055-500; Campus Aeroporto; Uberaba MG, Brazil; Phone numbers +55 34 339-8913

E-mail address: dctornavoi@hotmail.com

The authors thank that this study was funded in part by the Higher Education Personnel Improvement Coordination - Brazil (CAPES) - Financial Code 001 and the Institutional Research Support Program (PAPE-UNIUBE).

ABSTRACT

Statement of problem. Denture adhesives are widely used worldwide and the dentist should know the advantages and disadvantages in order to refer them to patients. **Objective.** This study evaluated the influence of different forms of denture adhesives on the formation of multispecies biofilms and adhesive strength, as well the effectiveness of hygiene protocols for their removal.

Materials and methods. Thermopolymerizable acrylic resin samples were made for microbiological and efficacy of hygiene protocols analysis (6x10x3mm), and for the analysis of adhesive strength (25x35mm) and divided into four groups: Control (No Adhesive), Ultra Corega Cream, Corega Strip Adhesive and Ultra Corega Powder. The formation of multispecies biofilm was evaluated by counting colony forming units (n=10) and fluorescence microscopy (n=2). To evaluate the effectiveness of the hygiene protocols, the samples were divided into five subgroups (n=10): Brushing with distilled water; Brushing with Protex liquid soap; Brushing with Colgate conventional toothpaste; Immersion in Corega Tabs and Immersion in Corega Tabs followed by brushing with the solution itself. The remaining adhesive was quantified with ImageJ software. The adhesive strength was tested at different times after adhesive application. Data were evaluated by Kruskal-Wallis test and Dunn post hoc test or 2-way ANOVA and Bonferroni post hoc test, depending on distribution and summary measures (α=.05).

Results: *Candida albicans* formed more biofilm in Strip (P=.007) and Powder (P=.001), *Pseudomonas aeruginosa* in Cream (P<.001) and Powder (P<.001) and *Staphylococcus. aureus* Strip (P<.001). All commercial forms of the adhesives promoted higher biofilm formation compared to the group without adhesive (P=.003). Brushing with Colgate and Protex was most effective at removing the adhesives (P<.05). Independently, Powder had the highest adhesive strength (P<.05). Only Strip showed a change in adhesive strength as a function of time, which was greater in 3 hours (P=.004).

Conclusion. The use of adhesive materials favors biofilm accumulation, and brushing favor adhesive removal. The adhesive strength may vary depending on the commercial type.

CLINICAL IMPLICATIONS. The use of different commercial forms of adhesive is effective in retaining dental prosthesis for an adequate period of time, but may increase the risk of opportunistic infections as it favors the accumulation of multispecies biofilm,

and the dentist should emphasize the importance of removal of this material during cleaning.

4. INTRODUCTION

Advances in the field of health reflect in improved quality and increased life expectancy. The number of people aged sixty is expected to reach two billion by 2050 (22% of the global population).[1] Thus, health problems and adaptations to the new lifestyle have become a reality for which health professionals must be prepared.[2,3]

The concern with this population group becomes evident also by the dental professionals. Although preventive measures have led to a reduction in the number of missing teeth in adult individuals, the number of total or partial edentulous teeth is still high, so the demand for prosthetic appliances is expected to increase due to the fast growth of the elderly population.[4,5]

Implant-supported prosthesis are a viable alternative for treating edentulous patients; however, conventional full dentures still represent the main treatment option due to low cost, systemic limitations or individual choice.[6] Nevertheless, complaints related to lack of retention, instability, chewing difficulties, low self-esteem, reduced quality of life, social life and satisfaction are common.[5,7]

Adhesive materials are recognized as auxiliary agents in the retention, stability and function of these prosthesis.[8] Proposals in the late 18th century were first reported scientifically in 1935, and when appropriately indicated, can improve interfacial surface tension between the prosthesis bases and underlying soft tissues by improving users' quality of life.[9,10] In addition, they can be used to stabilize prosthetic bases during the registration of maxillomandibular relationships and serve as an important route for drug delivery to oral tissues. [8,11-13]

It is estimated that in the USA about 22% of completely edentulous patients regularly use adhesives and approximately 75% of dentists recommend to full denture users. [14-16] However, inadequate hygiene of the prosthesis surface can make adhesives an additional substrate for the growth of microorganisms,[17,18] favoring the development of local problems, including chronic candidosis and subsequent denture stomatitis (DS), characterized by being a disease with a multifactorial etiology, but regardless of the contributing factors such as age, systemic disease, smoking, use of the prosthesis during sleep, reduced salivary flow, trauma caused by lack of retention and stability of the prosthesis, *Candida albicans* is recognized as the principal etiological agent.[19,20]

Although most of the literature discusses only this condition, there is evidence that it is a polymicrobial disease, with the association of several pathogenic bacterial species found in the oral cavity.[21-23] In addition, the proliferation of some oral bacteria related to a poor hygiene has been associated with systemic diseases such as bacterial endocarditis, aspiration pneumonia, chronic obstructive pulmonary disease, widespread respiratory tract infections, especially in dependent elderly.[24,25] However, the effect of adhesives on the growth of multispecies biofilms is little known, although it is known that interkingdom cooperation favors adhesion and colonization as well as resistance to antimicrobial agents.

Different forms of denture adhesives are widely used by edentulous patients,[26,27] which should be biocompatible, easy to apply and remove, and capable of maintaining adhesive strength for 12 to 16 hours.[28]

Therefore, this study evaluated the influence of the use of different commercial forms of denture adhesives on the formation of multispecies biofilms and adhesive strength, as well as the effectiveness of different hygiene protocols for their removal. The null hypothesis was that there is no influence of adhesive type on biofilm formation, adhesive strength, or even difference in the effectiveness of hygiene protocols for adhesive removal.

5. MATERIALS AND METHODS

5.1 Preparation of Acrylic Resin Specimen

The thermopolymerizable acrylic resin (Classic; Classic Dental Articles) was used. The specimens used for microbiological analysis and analysis of the efficacy of hygiene protocols were made in dimensions of 6 mm wide x 10 mm long and 3 mm thick, and for the analysis of the adhesive strength, with dimensions of 25 mm in diameter x 35 mm in height, from the inclusion of matrices in conventional metal muffle (OGP; Produtos Odontológica Ltda).

During the plastic phase, the resin was placed in the molds prepared in the metal muffles and then placed in hydraulic presses (Protecni Hydraulic Press; Protecni Equipamentos Médicos) with a load of 1000 Kgf for 60 minutes. The specimen were polymerized by conventional heating in an electric thermal cycler (Thermocycler T100; Oficina de Precisão Universidade de São Paulo), and after the cooling of the muffles at room temperature, the specimens were disinfected and finished. The specimens were immersed for 24 hours in distilled water at 37ºC to eliminate residual monomer.

The surface roughness of the specimens was standardized by sandpaper polishing and the use of the Rugosimeter (Surftest SJ 201P; Mitutoyo Corporation), reproducing the average roughness of the internal surface of the prosthesis bases. The specimens used in the present study had an average surface roughness (Ra) value of approximately 3.0 μm.[16,29]

5.2 Microbiological Analysis

The microorganisms, *Staphylococcus aureus* (ATCC 25923), *Candida albicans* (ATCC 10231) and *Pseudomonas aeroginosa* (ATCC 27853), were used in the present study. The evaluation of microbial colonization included the formation of multispecies biofilm in the substrates. The substrates consisted of non-adhesive acrylic resin specimens, and specimens with denture adhesives Ultra Corega Cream, Corega Strip Adhesive and Ultra Corega Powder (GlaxoSmithKline Brasil Ltda).

The process of applying the adhesives to specimens previously sterilized by hydrogen peroxide[30] (Multilav Sterilization) was performed according to aseptic principles in a class II biological safety cabinet (Pachane; Pa 400-ECO). The quantity of products (Ultra Corega Cream and Ultra Corega Powder) in each sample was

standardized to 0.025 g, using precision balance. The adhesives were applied and spread evenly directly on the surface of the specimen, with a spatula, forming a thin layer. Corega Strip Adhesive was cut to a compatible size to cover the entire surface of the specimen. After application, all samples were exposed to ultraviolet light for 20 minutes to disinfect the applied adhesives.[16]

A static multispecies biofilm model on 24 well plate was used. Cellular concentrations were adjusted according to the methodology of Kart *et al*., 2014.[31] Inoculum suspensions containing ~ 10^6 CFU mL $^{-1}$ of *S. aureus*, 10^6 CFU mL $^{-1}$ of *P. aeruginosa* and 10^5 CFU mL $^{-1}$ of exponentially growing *C. albicans* were made in BHI - Brain heart infusion (HiMedia Laboratories; Pvt. Ltd.). For *C. albicans*, due to the variable morphology of the genus, the counting in a Neubauer chamber (HBG; Giessen) was performed by optical microscope (Axio Observer A1; Carl Zeiss). To prevent the death of *S. aureus* and *C. albicans* by *P. aeruginosa*, BHI was supplemented with bovine serum albumin. In the class II biological safety cabinet (Pachane; Pa 400-ECO), specimen from each group were individually inserted into each well of the 24-well plate (TPP; Trasadingen) and 1 mL of the culture medium with microbial inoculum was transferred.

The plates were incubated in a microbiological oven (Shaker Incubator; Mod. CE-320; CienLab) at 37ºC with 75 rpm agitation, in order to generate stress and promote correct microbial adhesion and not just sedimentation. After 4 hours, the initial period of adhesion of the microorganisms, the culture medium was removed from each well and each specimen washed twice with 1 mL of phosphate buffered saline (PBS) in order to remove non-adherent cells. In each well was added 1 mL of sterile BHI and the plates were incubated for 20 hours.

After the biofilm formation period, each specimen was washed with 1 mL PBS, inserted into a polypropylene tube (TPP; Trasadingen) with 3 mL PBS and sonicated in an ultrasonic vat (Altsonic; Clean 9CA) (200 watts/40 Hz) for 20 minutes for detachment of the biofilm.

Then, 25 µL aliquots of decimal dilutions (10^{-1} to 10^{-4}) of the resulting suspension were sown in selective growth culture medium. Salty mannitol agar (HiMedia Laboratories; Pvt. Ltd.) supplemented with 200 UI/mL Nystatin (Homeocenter; Handling pharmacy) was used for *S. aureus*, Sabouraud Dextrose Agar (HiMedia Laboratories; Pvt. Ltd.) supplemented with 5 µg/mL of Chloramphenicol for *C. albicans* and Cetrimide Agar (HiMedia Laboratories; Pvt. Ltd.) supplemented with 200 UI/mL of Nystatin and 5% of glycerol for *P. aeruginosa*. The samples were incubated at 37ºC for 24 hours.

After the incubation period, the number of viable cells was quantified in terms of colony forming units per milliliter (CFU/mL) (n=10). The number of colonies from each dilution was counted, and the CFU value obtained, based on the dilution that promoted between 1-300 colonies, as follows: CFU/mL = number of colonies x 10 n/q, where: n = absolute value of dilution, q = amount of plated suspension (0.025 mL). The CFU/mL value was converted to $\log^{10}$.

Qualitative analysis of the biofilm was performed by fluorescence microscopy. Biofilms formed on the specimen surface (n=2) were stained with the FilmTracer™ LIVE/DEAD (Molecular Probes) cell viability kit according to the manufacturer's recommendations. After rinsing, the specimens were transferred to a new 24-well plate and each sample stained with 1 mL of the 0.3% solution of Syto 9 and Propidium Iodide dyes and incubated at room temperature in the dark for 15 minutes.

After incubation, the specimens were rinsed with PBS, mounted on 0.14 mm thick glass coverslips (24x60 mm) and observed under inverted microscope with filters at excitation wavelengths of 490 nm and 546 nm (Axio Observer A1; Carl Zeiss Microscopy Ltd.) at 63x magnification. Images were captured and analyzed using ZEN 2.3 lite software (Carl Zeiss; Microscopy Ltd.).

5.3 Removal of adhesive analysis

For the analysis of the effectiveness of different hygiene protocols to removal of adhesives from the surface of acrylic resin, the adhesives were applied in the same way as microbiological analysis. The protocols used (n=10) were: Brushing for 1 minute with distilled water; Brushing for 1 minute with liquid soap Protex; Brushing for 1 minute with conventional toothpaste Colgate; Immersion for 5 minutes in 250 mL of warm water (38ºC) and Corega Tabs tablete; and Immersion for 5 minutes in 250 mL of warm water and Corega Tabs tablet followed by brushing with the solution itself for 1 minute.

For the brushing groups was used the electric brush (Oral-B Pro Health Power; Oral B), coupled in a standardized fixed support, with a force of 190 g, associated with a solution of the respective (soap or toothpaste), in the proportion of 1 :1.

After hygiene protocols, the specimens were rinsed with distilled water, immersed in 1% dye (Neutral Red; Gold Lab) for 5 minutes and then photographed. The camera was placed on a stand with the objective facing the upper surface of the specimen at 90 degrees in order to image undercut areas. The same focusing distance was standardized to all specimens. The quantification of adhesive remaining on the surface of the samples

was performed on the images, with the aid of Image J Software by which the area of the specimen covered with adhesive (%) was calculated.

5.4 Adhesive strength analysis

The adhesive force measurement was performed according to the method described by Cartagena *et al*., 2017[18], using two cylinders of thermopolymerizable acrylic resin, so that for each product 10 repetitions were performed. For the test, one of the cylinder pairs was moistened with tap water. Then 0.3 g of the adhesives (Ultra Corega Cream and Ultra Corega Powder) were applied to each sample so that the entire surface of the cylinder was coated.

Corega Adhesive Strip was cut to cover the entire surface of the cylinder.The specimens were then immersed in distilled water at 37°C for 5 minutes, 3 hours, 6 hours, 12 hours and 24 hours. Subsequently, the other specimen in the set was humidified with a thin layer of artificial saliva and then the cylinders were aligned on the Universal Testing Machine (Emic 1000), and a 12 N compression force was initially applied for 30 seconds simulating a slight force occlusion.[32] Finally, the tensile test was performed at a speed of 1mm / min, and the maximum force calculated (N).

5.5 Statistical analysis

Statistical analysis was performed using SPSS version 22.0 software. Data were analyzed for distribution (Levene test) and homogeneity (Shapiro-Wills test); for the microbiological and removal adhesives analysis, Kruskal-Wallis test and Dunn post hoc test were used; for the analysis of the adhesive strength the data were submitted to 2-way ANOVA test and Bonferroni post hoc test. The adopted significance level was 5%.

6. RESULTS

6.1 Microbiological Analysis

The CFU/mL count of each microorganism alone varied according to the type of product (Table 1). The Strip (*P*=.007) and Powder (*P*=.001) adhesives provided an increase in *C. albicans* biofilm formation, in relation to the control group and the adhesives in the form of Cream (*P*<.001) and Powder (*P*<.001) favored the formation of *P. aeruginosa* biofilm. There is an increase in the formation of *S. aureus* biofilm when using the Strip adhesive (*P*<.001).

When considering total biofilm, it is noted that all forms of adhesive favored biofilm formation over the control group (*P*<.05).

Fluorescence microscopy proved the results obtained by counting colony forming units, since in the groups in which the different commercial forms of denture adhesives were applied there was a high density of viable cells (in green) in relation to the control group, demonstrating that the use of these materials favors the formation of multispecies biofilm (Figure 1).

6.2 Removal of adhesive analysis

When considering the factor "*Hygiene protocol"*, a statistically significant difference was observed regarding the effectiveness of removing adhesives from the specimen surface (*P*<.05) (Table 2).

Specimens that were subjected to brushing with neutral soap Protex and conventional toothpaste Colgate showed smaller area covered by adhesive than the other groups, with no statistically significant difference between them (*P*=1.00). The immersion in Corega Tabs resulted in the smallest efficacy (*P*<.05), with larger area of remaining adhesive observed.

When considering the factor "Commercial form of denture adhesive", there was no significant difference (*P*=.977) (Table 3).

When considering the interaction "Hygiene protocol x Commercial form of denture adhesive", a significant difference was observed (*P*<.05) (Table 4). In general, it should be noted that brushing with Colgate and Protex, and Corega Tabs immersion associated with brushing promoted better removal of all comercial forms of denture adhesives tested (*P*<.05).

Brushing with distilled water was more effective for removing Corega Strip Adhesive compared to others (P<.05) and immersion in Corega Tabs was less effective for removing Corega Strip Adhesive compared to Ultra Corega Cream (P=.011).

6.3 Adhesive strength analysis

There was a significant difference in adhesive strength when considering the "Commercial form of denture adhesive" factor (P=.002) independently, as well as in the interaction between the two factors (P=.045) (Table 5).

The Ultra Corega Powder adhesive had the highest adhesive strength compared to the others (P<.05) (Figure 2).

The "time" factor, independently, did not promote observed statistical difference in the adhesive strength (P=.072) (Figure 3).

The commercial forms of denture adhesive presented different strength only in the first 5 minutes and 3 hours. In 5 minutes, Corega Strip presented the lowest adhesive capacity (P <.05) and in 3 hours Ultra Corega Cream (P<.05) (Figure 4).

Only Corega Strip showed a change in adhesive strength as a function of time, which is greater at 3 hours compared to 5 minutes (P=.011) and 24 hours (P=.034) (Figure 5).

7. DISCUSSION

The results of this study rejected the null hypothesis, as significant differences were found in biofilm formation, the effectiveness of hygiene protocols in the removal of adhesives and the adhesive strength presented by different types of adhesives.

Topography and surface roughness of dental materials are critical factors for microorganism adhesion and biofilm formation in the oral cavity.[33] The use of denture adhesives alters the surface topography of acrylic resin,[15] which may explain the microbiological results of this study.

The emergence and spread of microbial resistance worldwide is compromising the effectiveness of treatments.[34] The threat includes the spread of multiresistant bacteria and infections without treatment options,[35] with widespread social and economic effects, requiring action at the national and global levels. More investment in basic science is needed especially for critical priority pathogens like *P. aeruginosa* and high priority such as *S. aureus*, as new antibiotics alone will not be sufficient to eliminate these microorganisms. Actions should address infection prevention and control activities.[36] Therefore, in the present study, for the microbiological analysis, we used a multispecies biofilm model composed of *C. albicans* (yeast), *S. aureus* (gram-positive cocci) and *P. aeruginosa* (gram-negative bacillus), representing oral pathogens commonly isolated from dental prosthesis surface.

The results of the present study indicated that the growth of each species of the microorganisms that composed the biofilm varied according to the type of adhesive. *C. albicans* and *S. aureus* showed higher growth when Corega Strip was used. Strip denture adhesives are composed of insoluble polypropylene and cellulose slides with the addition of ethylene oxide and / or sodium alginate, which become viscous when absorbing water.[37] These findings corroborate the study by Oliveira Junior *et al*., 2018[16], where a greater adhesion of *C. albicans* was observed, both in single and mixed species than in the group without adhesive and Ultra Corega Cream. The present study and Oliveira Junior *et al*., 2018[16] contrast with the work of Rajaram *et al.*, 2017[27], in which antifungal effects of three commercial forms of denture adhesives were observed, but the presence of antimicrobial agents in the composition explains the discrepancy of the results.

P. aeruginosa had the growth favored by the use of Ultra Corega Powder and Cream, while *C. albicans* showed higher growth in Ultra Corega Cream. Therefore, in general, the three commercial forms of denture adhesives tested increased the adherence

of multispecies biofilm compared to the non-adhesive group, as evidenced by fluorescence microscopy images, although they showed a standard "blurry" probably due to adhesive componentes.[16] These results reinforce the recommendation to the manufacturers of these products regarding the inclusion of antimicrobial components in order to prevent the occurrence of local problems, such as prosthetic and systemic stomatitis.[8, 18, 38]

When in the oral cavity, the adhesives become viscous due to the absorption of saliva and spread between the alveolar crest and the prosthesis surface, and this phenomenon is responsible for its adhesive capacity; however, when removing prostheses for hygiene, they can leave residues that are difficult to remove, [1] which may limit the effectiveness of daily cleaning. This fact is important because adhesive residues, presence of extracellular matrix or cellular debris can provide greater accumulation of pathogenic microorganisms, favoring the recolonization of the prosthesis surface.

Thus, the main strategies to avoid these problems should focus on hygiene education, which can be done mechanically, chemically or by a combination of both.[39] In this study, we evaluated the effectiveness of different hygiene protocols in the removal of prosthetic adhesives.

No difference was observed regarding the removal of the adhesive as a function of type, whereas in the study by Harada-Hada *et al*. 2016[1] the powdered adhesives were more easily removed, followed by cream and strip adhesives, respectively, after the use of 5 prosthesis hygiene solutions. However, the results of the present study indicated that brushing with conventional Colgate toothpaste, Protex neutral soap and Corega Tabs immersion associated with brushing with the solution itself promoted better removal of prosthetic adhesives. Immersion, alone, in Corega Tabs promoted the worst results, demonstrating that daily cleaning involving mechanical brushing is indispensable.

Taking into consideration the possibility of adverse effects to acrylic resins of chemical agents used for disinfecting or reducing biofilm in dentures, as well as using conventional toothpastes, brushing with neutral soap Protex may be a good choice, as it is a product with proven antimicrobial efficacy, low cost and easily accessible that does not promote adverse effects to acrylic resin.[39, 40]

The mechanism of adhesion of the prosthesis to the mucosa by the adhesives is almost always contradictory, as high adhesion is required for fixation and low adhesion to facilitate removal. Typically, prosthetic adhesives are expected to provide retention

and stability over a period of time so that there is a balance between fixation and removal possibility.

The test used in the present study to evaluate the adhesive strength of different commercial forms of denture adhesive was performed as suggested by Zhao *et al*., 2004[41] and Cartagena *et al.,* 2017[18], with the advantage of being simple, requiring no special equipment to perform. Acrylic resin cylinders are easily processed and their positioning on the testing machine is simple.

An adhesive interacts with the prosthesis surface on one side and the underlying oral mucosa on the other side over a period of time. A thin layer of material is applied to the inner surface of the prosthesis, which is then inserted into the oral cavity. Hydrophilic compounds absorb and maintain water to improve adhesion strength and hydrophobic compounds prevent excessive swelling and dissolution.[42,43]

Considering the commercial form factor alone, the data showed that the Powder-shaped adhesive presented higher bond strength, compared to other forms. These results may be associated with the fact that when in contact with water, the power becomes stickier, gum-shaped, which favors its adhesion to the prosthesis surface. [37]

Studies report higher bond strength immediately after application of the adhesive, with peak again within 3 to 6 hours of use, followed by loss of efficacy over time[13,44] due to breakage of the adhesive by oral fluids or gradual degradation.[45] In the present study, Corega Strip Adhesive showed change in adhesive strength as a function of time, which is greater in 3 hours, but in general, all commercial forms showed good adhesive strength within 24 hours of use, which may provide safety and patient comfort for longer than expected time (12 to 16 hours).[28]

The results of this study demonstrate that different commercial forms of adhesive are effective in retaining removable prosthesis for a satisfactory period of time, but may increase the risk of opportunistic infections as they favor the accumulation of multispecies biofilm. Therefore, the advantages and disadvantages related to use should be discussed with the patient before prescribing these materials.

8. CONCLUSION

Within the limitations of this study, the following conclusions were drawn:

1- The use of different commercial forms of denture adhesive favors the formation of multispecies biofilms.

2- Daily treatments with mechanical cleaning of the prosthesis is indispensable for the removal of adhesives.

3- The different commercial forms of denture adhesive tested have good adhesive strength as a function of time.

9. REFERENCES

1.Harada-Hada K, Hong G, Abekura H, Murata H. Evaluation of the efficiency of denture cleaners for removing denture adhesives. Gerodontology 2016; 33(4):453-460.

2. Manger D, Walshaw M, Fitzgerald R, Doughty J, Wanyonyi KL, White S, et al. Evidence summary: the relationship between oral health and pulmonary disease. Br Dent J 2017; 222: 527-533.

3. Dietrich T, Webb I, Stenhouse L, Patini A, Ready D, Wanyonyi KL, et al. Evidence summary: the relationship between oral and cardiovascular disease. Br Dent J 2017; 222: 381-385.

4. Felton D, Cooper L, Duqum I, Minsley G, Guckes A, Haug S, et al. Evidence-based guidelines for the care and maintenance of complete dentures: a publication of the American College of Prosthodontists. Int J Prosthodont 2011; Suppl 1: p.S1-S12.

5. Cardoso M, Balducci I, Telles DDEM, Lourenço EJ, Nogueira Junior L. Edentulism in Brazil: trends, projections and expectations until 2040. Ciênc Saúde Coletiva 2016; 21: 1239-1246.

6. Nicolas E, Veyrune J, Lassauzay C. A six-month assessment of oral health-related quality of life of complete denture wearers using denture adhesive: a pilot study. Int J Prosthodont. 2010; 19: 443–448.

7. Shamsolketabi S, Nili M. The effect of denture adhesive on the efficiency of complete denture in patients with different alveolar ridges. Dent Res J 2018; 15: 271-275.

8. Almeida NLM, Saldanha LL, da Silva RA, Pinke KH, da Costa EF, Porto VC, et al. Antimicrobial activity of denture adhesive associated with Equisetum giganteum- and Punica granatum-enriched fractions against Candida albicans biofilms on acrylic resin surfaces. Biofouling 2018; 34: 62-73.

9. Pradíes G, Sanz I, Evans O, Martínez F, Sanz M. Clinical study comparing the efficacy of two denture adhesives in complete denture patients. Int J Prosthodont 2009; 22: 361–367.

10. Oliveira Junior NM, Rodriguez LS, Mendoza Marin DO, Paleari AG, Pero AC, Compagnoni MA. Masticatory performance of complete denture wearers after using two adhesives: A crossover randomized clinical trial. J Prosthet Dent 2014; 112:1182–1187.

11. Ellis JS, Pelekis ND, Thomason JM. Conventional rehabilitation of edentulous patients: the impact on oral health-related quality of life and patient satisfaction. Int J Prosthodont 2007; 16: 37-72.

12. Emami E, Heydecke G, Rompré PH, De Grandmont P, Feine JS. Impact of implant support for mandibular dentures on satisfaction, oral and general health-related quality of life: a meta-analysis of randomized-controlled trials. Clin oral Imp Res 2009; 20: 533-544.

13. Kore DR, Kattadiyil MT, Hall DB, Bahjri K. In vitro comparison of the tensile bond strength of denture adhesives on denture bases. J Prosthet Dent 2013; 110: 488-493.

14. Grasso JE. Denture adhesives: changing attitudes. J Am Dent Assoc 1996; 127: 90–96.

15. Oliveira MC, Oliveira VM, Vieira AC, Rambob I. In vivo assessment of the effect of an adhesive for complete dentures on colonisation of Candida species. Gerodontology 2010; 27: 303-307.

16. de Oliveira Junior NM, Mendoza Marin DO, Leite ARP, Pero AC, Klein MI, Compagnoni MA. Influence of the use of complete denture adhesives on microbial adhesion and biofilm formation by single- and mixed-species. PloS One 2018 10;13(10):e0203951.

17. Nunes EM, Policastro VB, Scavassin PM, Leite AR, Medonza Marin DO, Giro G, et al. Crossover clinical trial of different methods of removing a denture adhesive and the influence on the oral microbiota. J Prosthet Dent 2016; 115: 462-468.

18. Cartagena AF, Esmerino LA, Polak-Junior R, Olivieri Parreiras S, Domingos Michél M, Farago PV, et al. New denture adhesive containing miconazole nitrate polymeric microparticles: Antifungal, adhesive force and toxicity properties. Dent Mater 2017; 33: 53-61.

19. Emami E, Taraf H, Grandmont P, Gauthier G, Koninck L, Lamarche C, et al. The association of denture stomatitis and partial removable dental prostheses: a systematic review. Int J Prosthodont 2012; 25: 113-119.

20. Gendreau L, Loewy ZG. Epidemiology and etiology of denture stomatitis. Int J Prosthodont 2011; 20: 251-260.

21. Pereira CA, Toledo BC, Santos CT, Pereira Costa AC, Back-Brito GN, Kaminagakura, et al. Opportunistic microorganisms in individuals with lesions of denture stomatitis. Diagnostic Microbiology And Infectious Disease 2013; 76: 419-424.

22. O'Donnell LE, Robertson D, Nile CJ, Cross LJ, Riggio M, Sherriff A, et al. The oral microbiome of denture wearers is influenced by levels of natural dentition. Plos One 2015; 10: p.e0137717.

23. Shi B, Wu T, Mclean J, Edlund A, Young Y, He X, et al. The Denture-Associated Oral Microbiome in Health and Stomatitis. mSphere 2016; 1: p.e00215-16.

24. Coulthwaite, Verran J. Potential pathogenic aspects of denture plaque. Br J Biomed Sci 2007; 64: 180-189.

25. O'Donnell LE, Smith K, Williams C, Nile CJ, Lappin DF, Bradshaw D, et al. Dentures are a Reservoir for Respiratory Pathogens. Int J Prosthodont 2016; 25: 99-104.

26. Polyzois GL, Baat C. Attitudes and usage of denture adhesives by complete denture wearers: a survey in Greece and Netherlands. Gerodontology 2012; 29: 807-814.

27. Rajaram A, Manoj SS. Influence of 3 different forms of a commercially available denture adhesive material on the growth of Candida species: An in vitro study. J Prosthet Dent 2017; 118: 379-385.

28. Fallahi A, Khadivi N, Roohpour N, Middleton AM, Kazemzadeh-Narbat M, Annabi N, et al. Characterization, mechanistic analysis and improving the properties of denture adhesives. Dent Mater 2018; 34: 120-131.

29. Zissis AJ, Polyzois GL, Yannikakis SA, Harrison A. Roughness of denture materials: a comparative study. Int J Prosthodont 2000; 13: 136-140.

30. Münker TJAG, van de Vijfeijken SECM, Mulder CS, Vespasiano V, Becking AG, Kleverlaan CJ. Effects of sterilization on the mechanical properties of poly (methil methacrylate) based personalized medical devices.J Mech Behav Biomed Mater 2018; 81:168-172.

31. Kart D, Tavenier S, Van Acker H, Nelis HJ, Coenye T. Activity of disinfectants against multispecies biofilms formed by Staphylococcus aureus, Candida albicans and Pseudomonas aeruginosa. Biofouling 2014; 30: 377-383.

32. Haraldson T, Karlsson U, Carlsson GE. Bite force and oral function in complete denture. J Oral Rehabil 1979; 1: 41-48.

33. Teughels, W; Van Assche, N; Sliepen, I; Quirynen, M. Effect of material characteristics and/or surface topography on biofilm development. Clin Oral Implants Res 2006; 17: 68-81.

34. World Health Organization: Antimicrobial resistance: global report on surveillance, 2014.

35. Unemo M, Nicholas RA: Emergence of multidrug-resistant, extensively drug-resistant and untreatable gonorrhea. Future Microbiol 2012;7:1401–1422.
36. World Health Organization: Antibacterial agents in clinical development: an analysis of the antibacterial clinical development pipeline, including tuberculosis, 2017.
37. Adisman IK. The use of denture adhesives as an aid to denture treatment. J Prosthet Dent 1989; 62:711–715.
38. Garaicoa JL, Fischer CL, Bates AM, Holloway J, Avila-Ortiz G, Guthmiller JM, et al. Promise of Combining Antifungal Agents in Denture Adhesives to Fight Candida Species Infections. Int J Prosthodont 2016; 8: 755-762.
39. Zoccolotti JO, Tasso CO, Arbeláez MIA, Malavolta IF, Pereira ECDS, Esteves CSG, et al. Properties of an acrylic resin after immersion in antiseptic soaps: Low-cost, easy-access procedure for the prevention of denture stomatitis. PLoS One. 2018; 13: e0203187.
40. Zoccolotti JO, Suzuki RB, Rinaldi TB, Pellissari CVG, Sanitá PV, Jorge JH. Physical properties of artificial teeth after immersion in liquid disinfectant soaps. Am J Dent 2019; 32: 14-20.
41. Zhao K, Cheng XR, Chao YL, Li ZA, Han GL. Laboratoryevaluation of a new denture adhesive. Dent Mater 2004; 20: 419–424.
42. Harada-Hada K, Mimura S, Hong G, Hashida T, Abekura H, Murata H, et al. Accelerating effects of cellulase in the removal of denture adhesives from acrylic denture bases. J Prosthodont 2017; 61: 185-192.
43.Yiran AN, Danyag LI, Roohpour N, Gautrot JE, Barber AH. Failure mechanisms in denture adhesives. Dent mater 2016; 32:615-623.
44. Chew CL. Retention of denture adhesives: an in vitro study. J Oral Rehabil 1990; 17: 425-434.
45. De Vengencie J, Ng MC, Ford P, Iacopino AM. In vitro evaluation of denture adhesives: possible efficacy of complex carbohydrates. Int J Prosthodont 1997; 10: 61-72.

TABLES

Table 1. Comparison of colony forming units count (CFU / mL) in log_{10} under different experimental conditions.

	C. albicans	***P. aeruginosa***	***S.aureus***	**Total microbiota**
Control – Without adhesive	3.22 [2.95;3.54] ^{A}	6.11 [5.79;6.48]^{A}	5.89 [5.66;6.21]^{A}	5.70 [4.58;5.63]^{A}
Ultra Corega Cream	3.55 [3.31;4.18]^{AB}	8.01 [7.81;8.25]^{B}	6.32 [6.04;6.72]^{AB}	6.32 [5.36;6.74]^{B}
Corega Strip	4.32 [3.89;4.49]^{B}	7.56 [7.08;7.76]^{AB}	7.01 [6.80;7.30]^{B}	6.81 [5.65;6.79]^{B}
Ultra Corega Powder	4.52 [3.91;4.90]^{B}	8.02 [7.39;8.15]^{B}	6.59 [6.14;6.73]^{AB}	6.59 [5.64;6.77]^{B}

Data are expressed as median [Confidence Interval] (n=10). * Different letters indicate significant difference between groups for the same microorganism. Kruskal-Wallis followed by Dunn's post hoc test. $P<.05$.

Table 2. Area (%) of specimens with surface adhesive remaining, according to different hygiene protocols.

Hygiene Protocols	Residual area (%)
Brushing with neutral soap Protex	2.14 [1.87; 3.36]^{A}
Brushing with conventional toothpaste Colgate	2.22 [1.84; 3.76]^{A}
Immersion in Corega Tabs + Brushing	6.00 [5.15; 10.75]^{B}
Brushing with destilled water	21.10 [17.12; 28.28]^{B}
Immersion in Corega Tabs	91.09 [86.28; 93.33]^{C}

Data are expressed as median [Confidence Interval] (n=10). * Different letters indicate significant difference between groups. Kruskal-Wallis followed by Dunn's post hoc test. $P<.05$.

Table 3. Remaining sample surface adhesive (%) according to commercial form.

Adhesive	Residual area (%)
Ultra Corega Cream	5.37 [15.40; 33.72] [A]
Corega Strip	5.21 [14.08; 34.62] [A]
Ultra Corega Powder	7.33 [16.61; 36.61] [A]

Data are expressed as median [Confidence Interval] (n=10).

Table 4. Remaining adhesive on sample surface (%) according to hygiene protocol and commercial form of denture adhesive.

	Ultra Corega Cream	**Corega Strip**	**Ultra Corega Powder**
Brushing with conventional toothpaste Colgate	2.3 [1.97; 4.05]^{Aa}	2.16 [1.37; 4.35]^{Aa}	1.02 [-0.19; 5.26]^{Aa}
Brushing with neutral soap Protex	2.18 [1.24; 2.98]^{Aa}	2.71 [1.48; 5.20]^{Aa}	1.96 [1.05; 3.76]^{Aa}
Immersion in Corega Tabs + Brushing	4.72 [2.07; 10.48]^{Aba}	5.68 [2.28; 18.45]^{Aa}	6.05 [5.11; 9.32]^{ABa}
Brushing with destilled water	22.29 [17.33; 40.00]^{BCa}	7.67 [4.72; 16.72]^{ABb}	28.06 [19.71; 33.72]^{BCa}
Immersion in Corega Tabs	85.04 [74.93; 91.09]^{Ca}	94.14 [92.00; 96.96]^{Bb}	93.79 [87.01; 97.37]^{Cab}

Data are expressed as median [Confidence Interval] (n=10). * Different capital letters indicate significant difference between lines; Different lowercase letters indicate significant difference between columns. Kruskal-Wallis followed by Dunn's post hoc test. $P<.05$.

Table 5. ANOVA for the effect of time and commercial form of denture adhesive on adhesive strength (N).

Cross-subject effect testing

Dependent variable: Resistance

Source of Variation	Sum of Squares	df	Medium square	F	*P*
Corrected Model	116.037[a]	14	8.288	2.715	.002
Intercept	18533.262	1	18533.262	6071.924	<.001
Adhesive	41.150	2	20.575	6.741	.002
Time	26.853	4	6.713	2.199	.072
Adhesive * Time	48.034	8	6.004	1.967	.045
Error	412.059	135	3.052		
Total	19061.358	150			
Corrected Total	528.096	149			

ILLUSTRATIONS

Figure 1. Fluorescence microscopy of the biofilm (63x). A. Control (acrylic resin without adhesive); B. Ultra Corega Cream, C. Corega Strip Adhesive and D. Ultra Corega Powder.

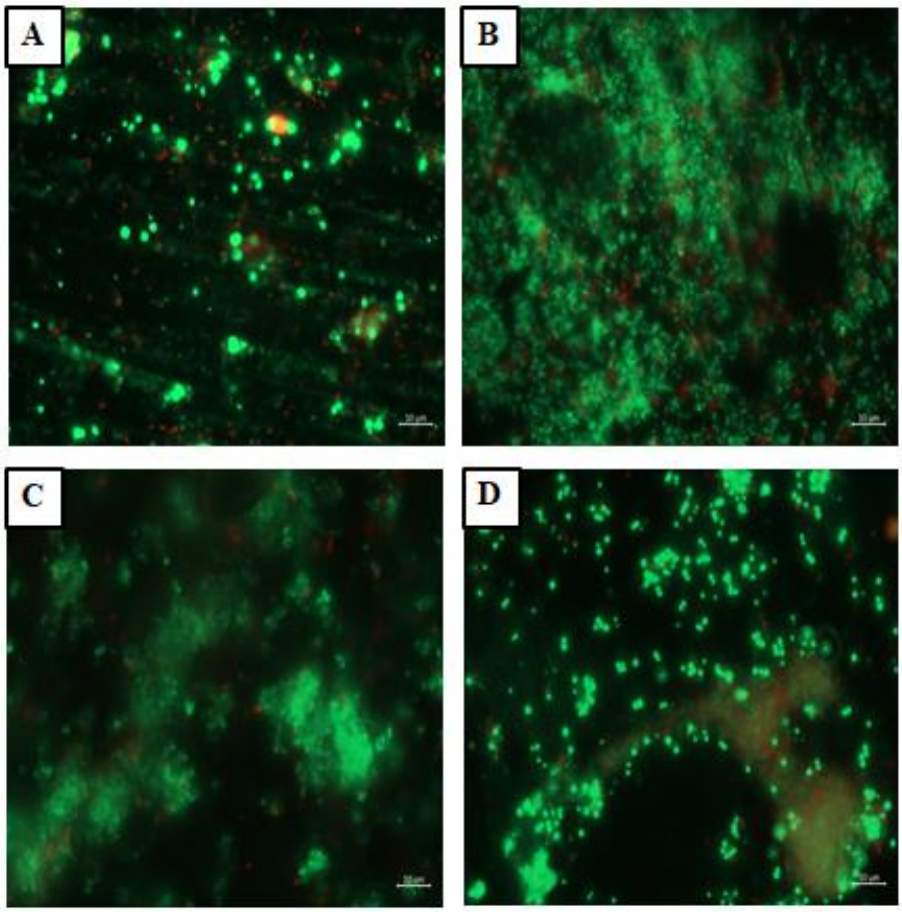


Figure 2. Adhesive Force (N) in different commercial forms of denture adhesives.

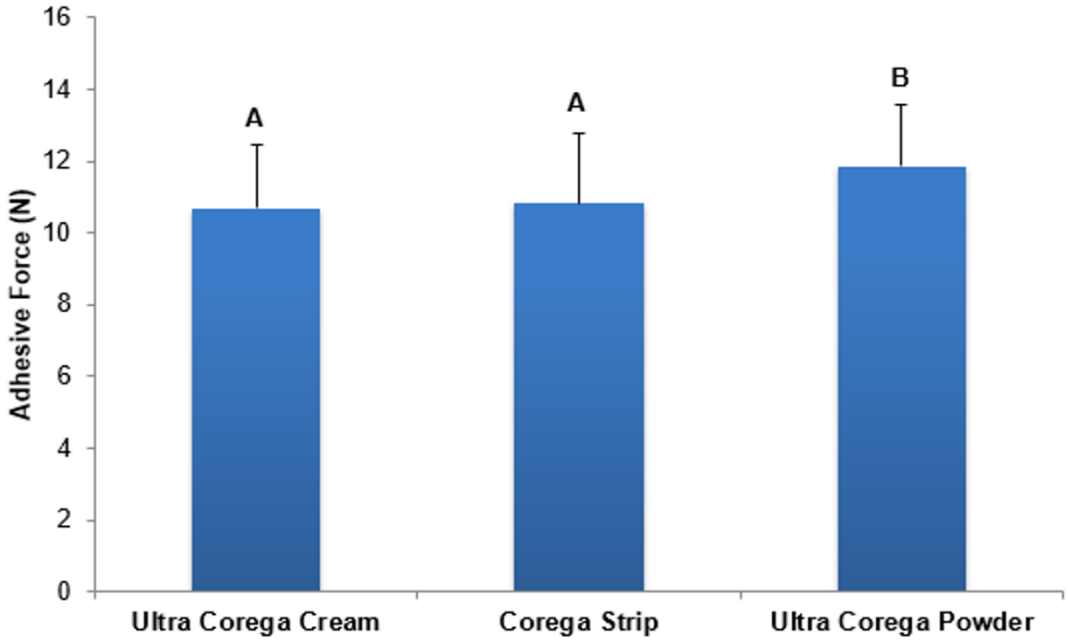


Figure 3. Adhesive Force (N) in function of time.

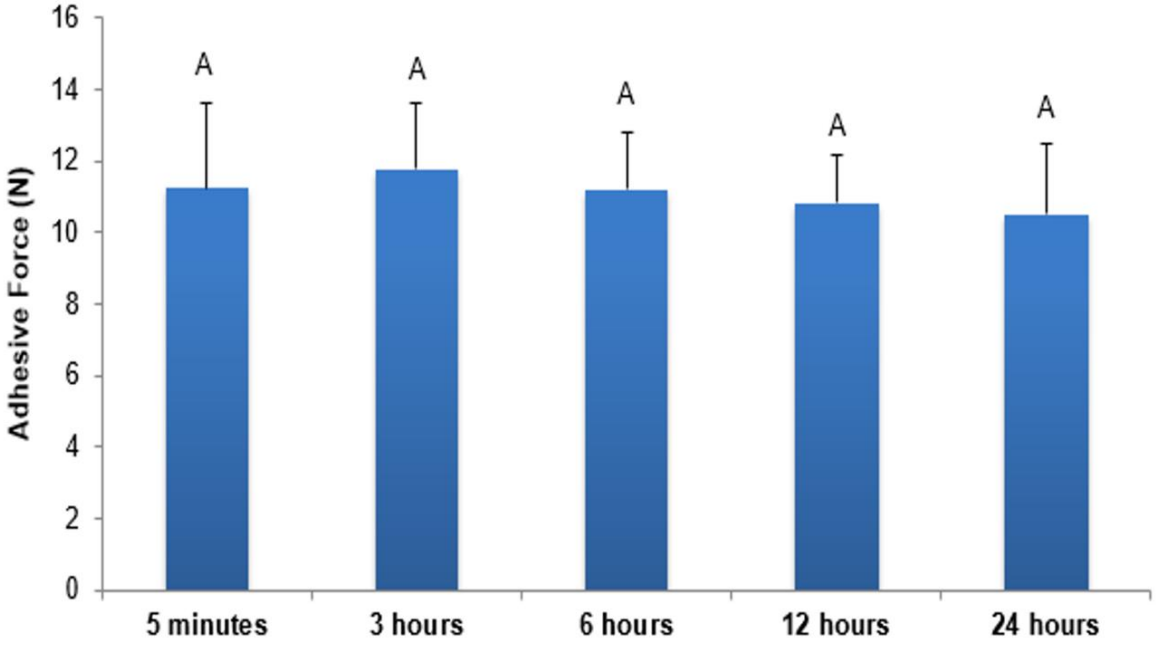


Figure 4. Comparison of the adhesive force (N) in different comercial forms of denture adhesives in a same time.

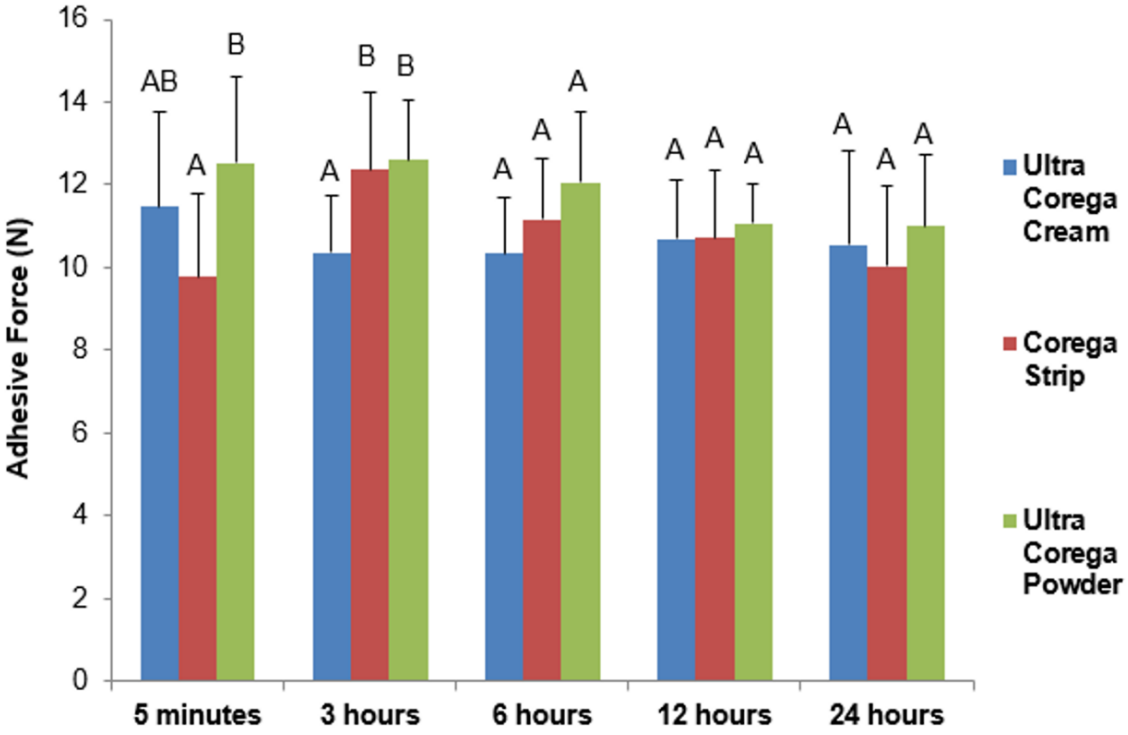
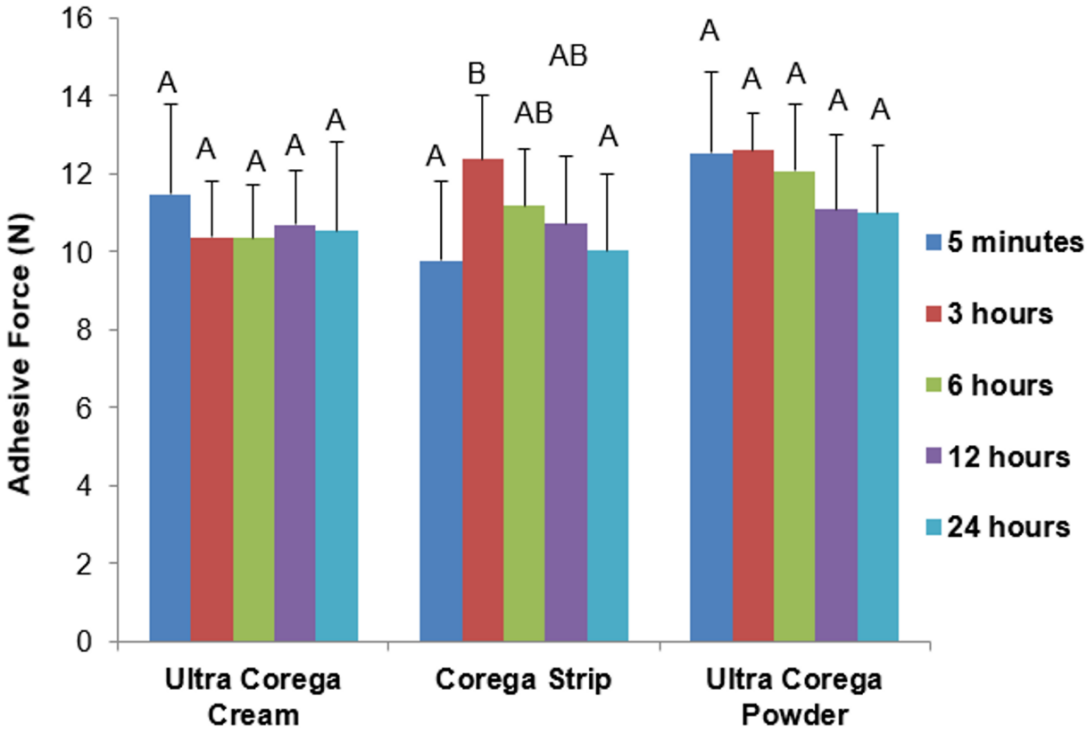


Figure 5. Comparison of the adhesive force (N) of each comercial forms of denture adhesives.



10. CONCLUSÃO

Dentro das limitações deste estudo, foram tiradas as seguintes conclusões:

1- O uso de diferentes formas comerciais de adesivo protético favorece a formação de biofilmes multiespécies.

2- Os tratamentos diários com a limpeza mecânica da prótese são indispensáveis para a remoção completa dos adesivos.

3- As diferentes formas comerciais de adesivo protético testadas apresentam boa força adesiva em função do tempo.

11. REFERÊNCIAS BIBLIOGRÁFICAS[1]

1. ADISMAN, I.K. The use of denture adhesives as an aid to denture treatment. **The Jounal of Prosthetic Dentistry,** v.62, n. 6, p. 711-5, 1989.

2. ALMEIDA, N.L.M. et al. Antimicrobial activity of denture adhesive associated with Equisetum giganteum and Punica granatum-enriched fractions against Candida albicans biofilms on acrylic resin surfaces. **Biofouling,** v. 34, n. 1, p. 62-73, 2018.

3. CARDOSO, M. et al. Edentulism in Brazil: trends, projections and expectations until 2040. **Ciência & Saúde Coletiva**, v.21, n.4, p.1239-1246, 2016.

4. CARTAGENA, A.F. et al. New denture adhesive containing miconazole nitrate polymeric microparticles: Antifungal, adhesive force and toxicity properties. **Dental Materials**, v.33, n.2, p.e53-e61, 2017.

5. CHEW, C.L. Retention of denture adhesives: an in vitro study. **Journal of Oral Rehabilitation**, v. 17, n. 5, p. 425-34, 1990.

6. COULTHWAITE, L,; VERRAN, J. Potential pathogenic aspects of denture plaque. **British journal of biomedical science,** v.64, p.180-189, 2007.

7. de OLIVEIRA JUNIOR, N.M. et al. Influence of the use of complete denture adhesives on microbial adhesion and biofilm formation by single- and mixed-species. **PloS One,** v. 13, n. 10, e0203951, 2018.

8. de VEGENICIE, J. et al. In vitro evaluation of denture adhesives: possible efficacy of complex carbohydrates. **The Journal of Prosthodontics,** v. 10, n. 1, p. 61-72, 1997.

9. DIETRICH, T. et al. Evidence summary: the relationship between oral and cardiovascular disease. **British Dental Journal**, v.222, n.5, p.381-385, 2017.

10. ELLIS, J.S.; PELEKIS, N.D.; THOMASON, J.M. Conventional rehabilitation of edentulous patients: the impact on oral health-related quality of life and patient satisfaction. **Journal of Prosthodontics**, v.16, n.2, p.37-72, 2007.

11. EMAMI, E. et al. The association of denture stomatitis and partial removable dental prostheses: a systematic review. **Journal of Prosthodontics**, v.25, p.113-119, 2009.

12. FALLAHI, A. et al. Characterization, mechanistic analysis and improving the properties of denture adhesives. **Dental Materials**, v. 34, n. 1, p.120-131, 2018.

13. FELTON, D. et al. Evidence-based for the care and maintenance of complete dentures: a publication of the American College of Prosthodontists. **Journal of Prosthodontics**, v.20, n.1, p. S1-S12, 2011.

[1] De acordo com a Associação Brasileira de Normas Técnicas. NBR 6023: Informação edocumentação: referências: elaboração. Rio de Janeiro, 2002.

14. GARAICOA, J. et al. Promise of Combining Antifungal Agents in Denture Adhesives to Fight Candida Species Infections. **Journal of Prosthodontics,** v.27, n.8, p. 755-762, 2016.

15. GENDREAU, L.; LOEWY, Z.G. Epidemiology and etiology of denture stomatitis. **Journal of Prosthodontics**, v.20, n. 4, p. 251-260, 2011.

16. GRASSO, J.E. Denture adhesives: changing attitudes. **Journal of American Dental Association,** v. 127, n. 1, p. 90–96, 1996.

17. HARADA-HADA, K. et al. Accelerating effects of cellulase in the removal of denture adhesives from acrylic denture bases. **Journal of Prosthodontics**, v. 61, n.2, p. 185-192, 2017.

18.HARADA-HADA, K. et al. Evaluation of the efficiency of denture cleaners for removing denture adhesives. **Gerodontology**, v. 33, n. 4, p. 453-460, 2016.

19. HARALDSON, T.; KARLSSON, U.; CARLSSON, G.E. Bite force and oral function in complete denture. **Journal of Oral Rehabilitation,** v.6, n.1, p. 41-8, 1979.

20. KART, D. et al. Activity of disinfectants against multispecies biofilms formed by Staphylococcus aureus, Candida albicans and Pseudomonas aeruginosa. **Biofouling**, v.30, n.3, p.377-383, 2014.

21. KORE, D.R. et al. In vitro comparison of the tensile bond strength of denture adhesives on denture bases. **Journal of Prosthetic Dentistry**, v.110, p.488-493, 2013.

22. MANGER, D. et al. Evidence summary: the relationship between oral health and pulmonary disease. **British Dental Journal**, v.222, n.7, p.527-533, 2017.

23. MUNKER T.J.A.G. et al. Effects of sterilization on the mechanical properties of poly (methil methacrylate) based personalized medical devices. **Journal of Mechanical Behavior of Biomedical Materials**, v. 81, p. 168-172, 2018.

24. NICOLAS, E.; VEYRUNE, J.; LASSAUZAY, C. A. six-month assessment of oral health-related quality of life of complete denture wearers using denture adhesive: a pilot study. **Journal of Prosthodontics**, v. 19, n. 6, p. 443–8, 2010.

25. NUNES, E.M. et al. Crossover clinical trial of different methods of removing a denture adhesive and the influence on the oral microbiota. **Journal of Prosthetic Dentistry,** v. 115, n. 4, p.462-468, 2016.

26. O'DONNELL, L.E. et al. Dentures are a Reservoir for Respiratory Pathogens. **Journal of Prosthodontics,** v. 25, n.2, p. 99-104, 2016.

27. ODONNELL, L.E. et al. The oral microbiome of denture wearers is influenced by levels of natural dentition. **Plos One**, v.10, p.e0137717, 2015.

28. OLIVEIRA JUNIOR. et al. Masticatory performance of complete denture wearers after using two adhesives: A crossover randomized clinical trial. **Journal of Prosthetic Dentistry**, v. 112, n. 5, p. 1182–7, 2014.

29. OLIVEIRA, M.C. et al. In vivo assessment of the effect of an adhesive for complete dentures on colonisation of Candida species. **Gerodontology**, v. 27, n. 4, p. 303-7, 2010.

30. PEREIRA, C.A. et al. Opportunistic microorganisms in individuals with lesions of denture stomatitis. **Diagnostic Microbiology And Infectious Disease**, v.76, p.419-424, 2013.

31. POLYZOIS, G.L.; BAAT, C. Attitudes and usage of denture adhesives by complete denture wearers: a survey in Greece and Netherlands. **Gerodontology**, v.29, p. 807-814, 2012.

32. PRADÍES, G. et al. Clinical study comparing the efficacy of two denture adhesives in complete denture patients. **International Journal of Prosthodontics**, v. 22, p. 361-7, 2009.

33. RAJARAM, A.; MANOJ, S.S. Influence of 3 different forms of a commercially available denture adhesive material on the growth of Candida species: An in vitro study. **Journal of Prosthetic Dentistry**, v.118, n.3, p.379-385, 2017.

34.SHAMSOLKETABI, S.; NILI, M. The effect of denture adhesive on the efficiency of complete denture in patients with different alveolar ridges. **Dental Research Journal**, v.15, n.4, p.271-275, 2018.

35. SHI, B. et al. The Denture Associated Oral Microbiome in Health and Stomatitis. **mSphere**, v.1, p.e00215-16, 2016.

36. TEUGHELS, W. et al. Effect of material characteristics and/or surface topography on biofilm development. **Clinical Oral Implants Research** v. 17, p. 68-81, 2006.

37. UNEMO, M.; NICHOLAS, R.A. Emergence of multidrug-resistant, extensively drug-resistant and untreatable gonorrhea. **Future Microbiology** v.7, n. 12, p. 1401-1412, 2012.

38. VENGENCIE, J.; N.G MAY, C.; FORD PHIL. In vitro evaluation of denthure adhesives: possible efficacy of complex carbohydrates. **International Journal of Prosthodontics,** v. 10, n. 1, p. 61-72, 1997.

39. WORLD HEALTH ORGANIZATION: Antibacterial agents in clinical development: an analysis of the antibacterial clinical development pipeline, including tuberculosis, 2017.

40. WORLD HEALTH ORGANIZATION: Antimicrobial resistance: global report on surveillance, 2014.

41. YIRAN, A.N. et al. Failure mechanisms in denture adhesives. **Dental materials,** v.32, n. 5, p. 615-23, 2016.

42. ZHAO, K. et al. Laboratory evaluation of a new denture adhesive. **Dental Materials** v. 20, n. 5, p. 419-24, 2004.

43. ZISSIS, A.J. et al. Roughness of denture materials: a comparative study. **The International Journal of Prosthodontics** v.13, n.2, p.136-140, 2000.

44. ZOCCOLOTTI, J.O.; SUZUKI, R.B.; RINALDI, T.B**.** et al. Physical properties of artificial teeth after immersion in liquid disinfectant soaps. **American Journal of Dentistry v. 32, n.1, p.**14-20, 2019.

45. ZOCCOLOTTI, J.O.; TASSO, C.O.; ARBELÁEZ, M.I.A. et al. Properties of an acrylic resin after immersion in antiseptic soaps: Low-cost, easy-access procedure for the prevention of denture stomatitis. **PLoS One** v. 13, n.8, e0203187, 2018.

12. APÊNDICE

Figura 1. Formas comerciais dos adesivos para prótese dentária. A- Corega Fita Adesiva, B- Ultra Corega Pó, C- Ultra Corega Creme.

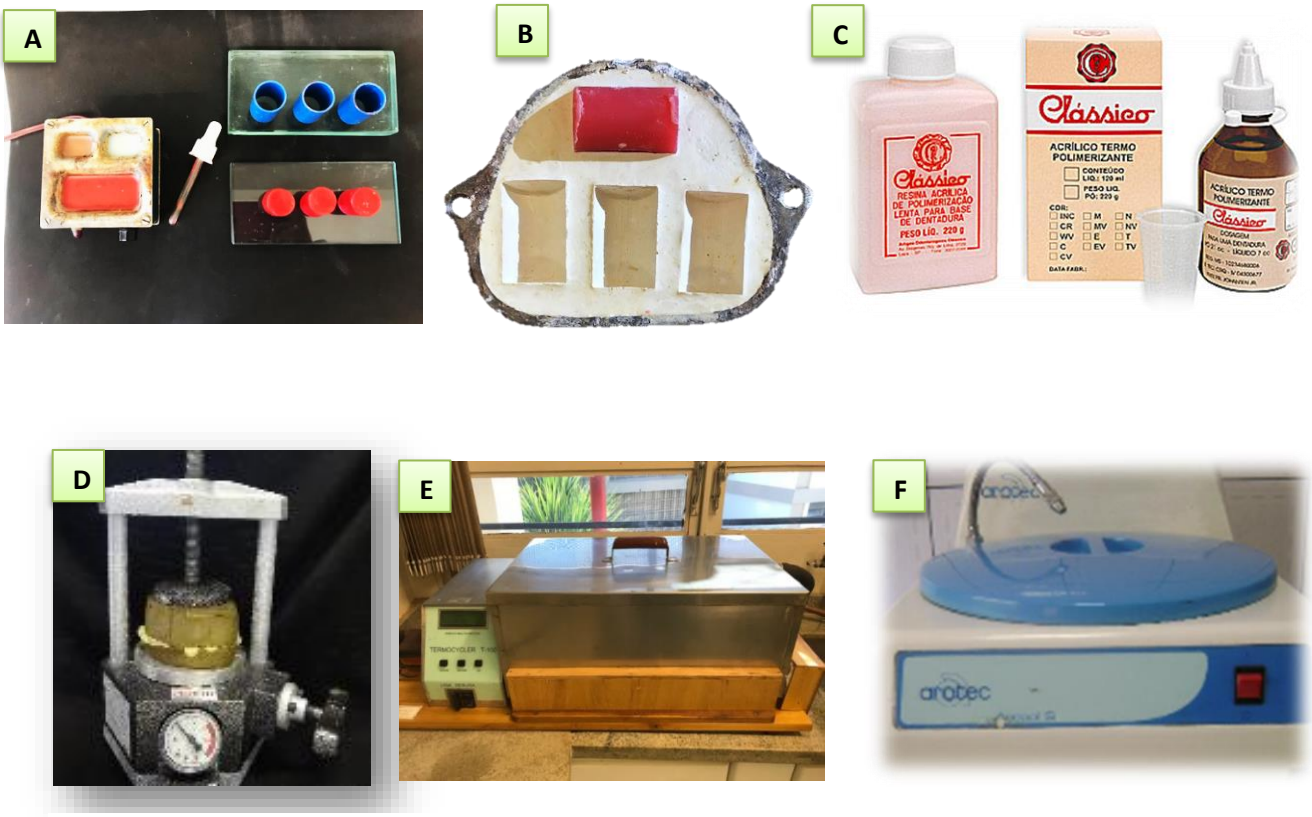


Figura 2. Confecção dos espécimes em resina acrílica. A- Preparação das matrizes em cera, B- Moldes dos espécimes prontos, C- Resina acrílica utilizada, D- Prensagem em prensa hidráulica, E- Termocicladora elétrica, F- Politriz utilizada para acabamento e polimento dos espécimes.

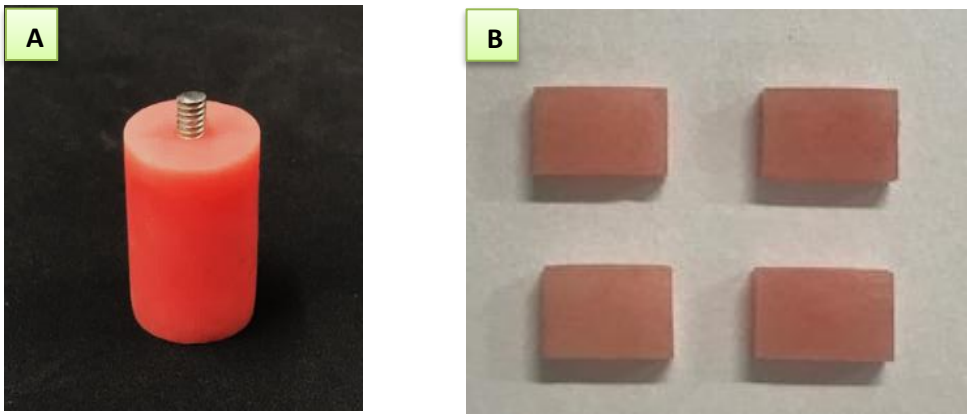


Figura 3. A- Espécime cilíndrico em resina acrílica; B- Espécimes retangulares em resina acrílica.

Figura 4. Análise da rugosidade superficial dos espécimes.

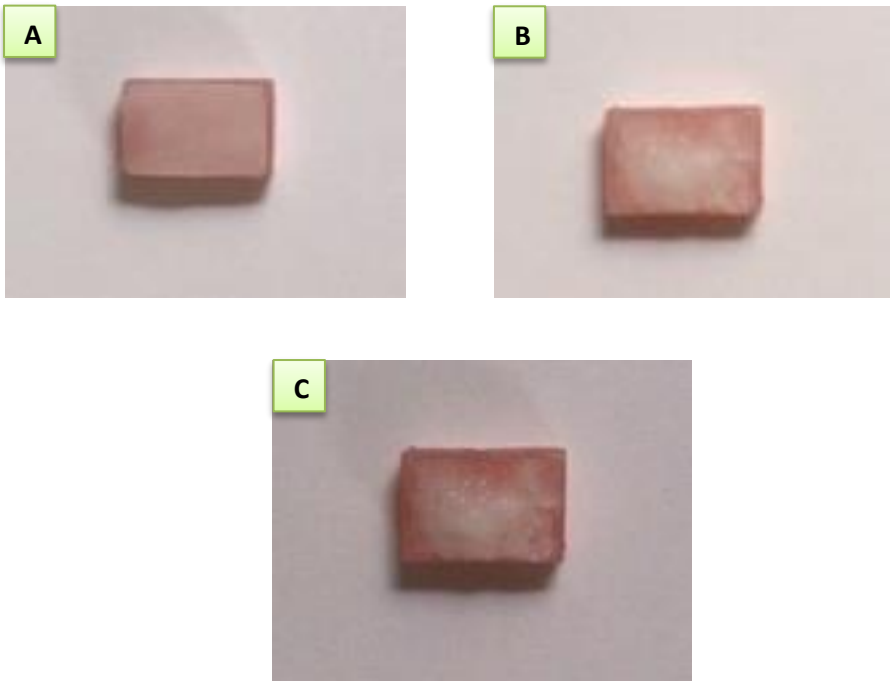


Figura 5. A- Aplicação do Corega® Fita no espécime, B- Aplicação do Ultra Corega® Creme no espécime, C- Aplicação do Ultra Corega® Pó no espécime.

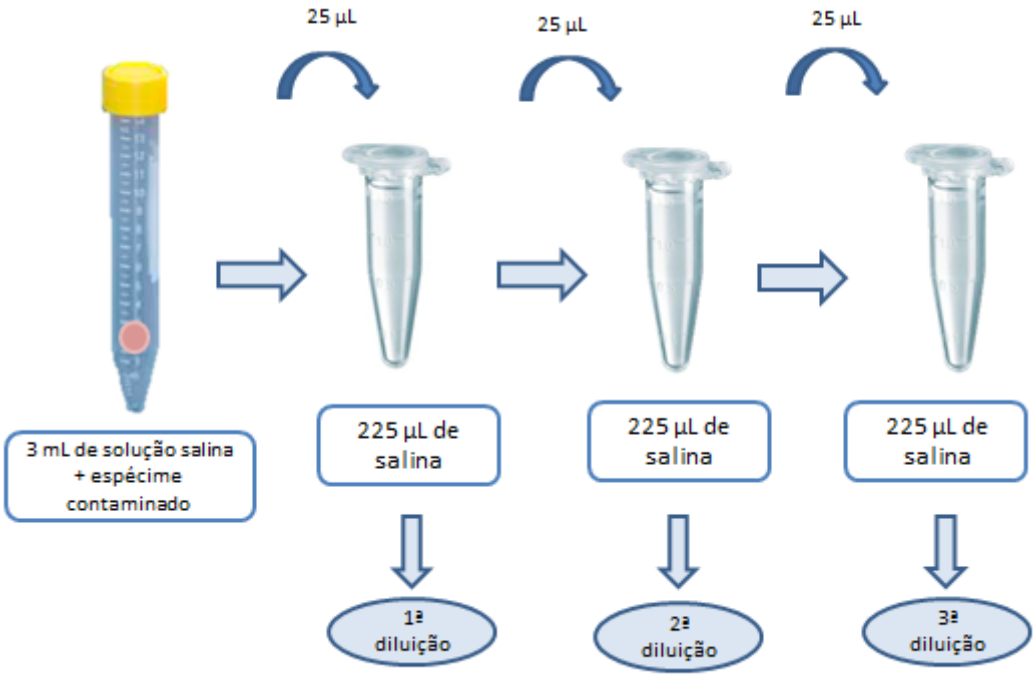


Figura 6. Esquema demonstrando as diferentes diluições para contagem de UFC.

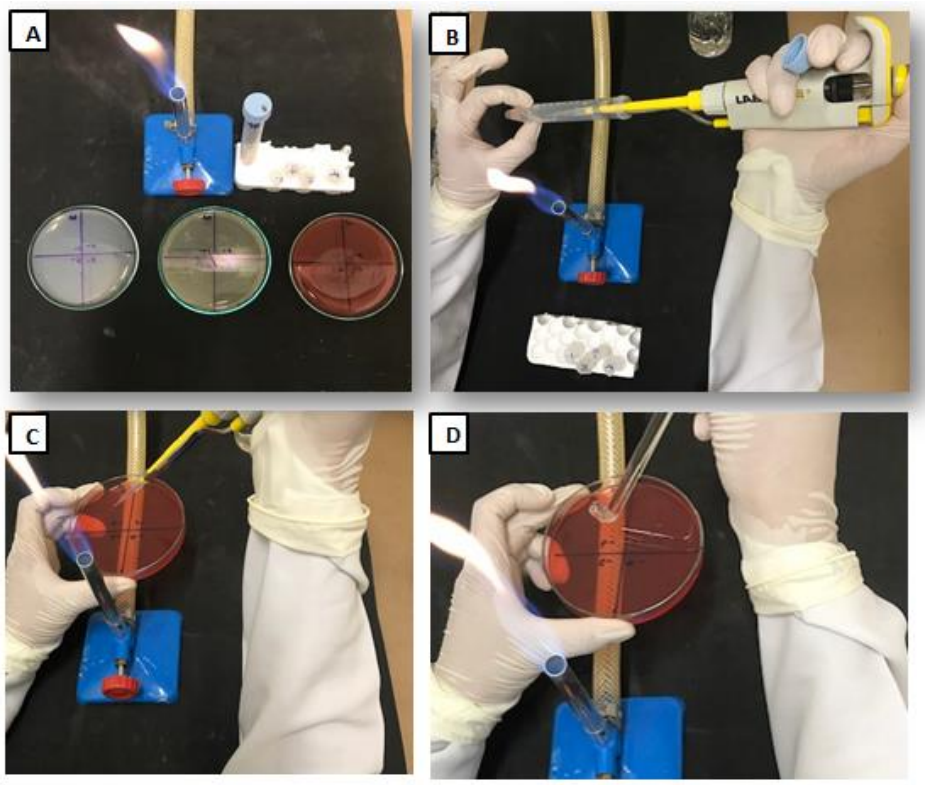


Figura 7. A- Placas de Petri com os diferentes meios de cultura, B- Corpo de prova contaminado, C- Aplicação no meio de cultura, D- Semeadura das alíquotas nas diferentes diluições.

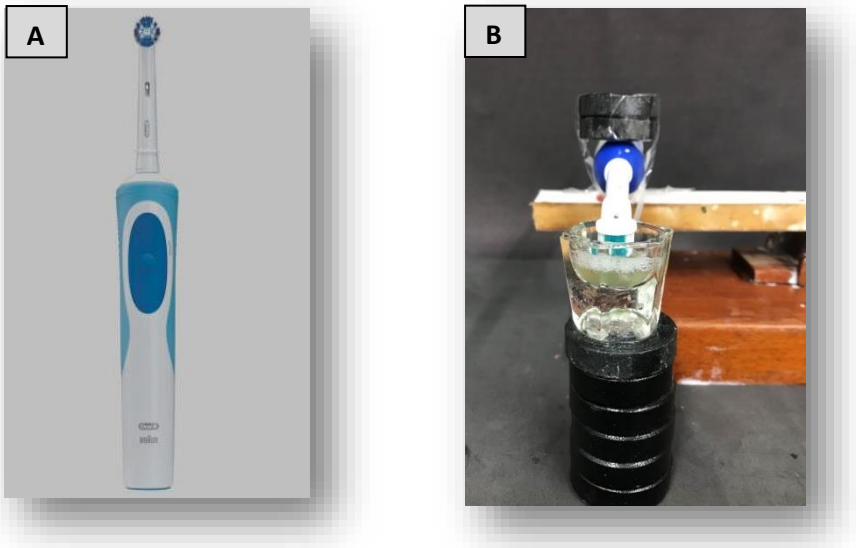


Figura 8. A- Escova Oral B; B- Escovação dos Espécimes, com peso 190 g acoplado.

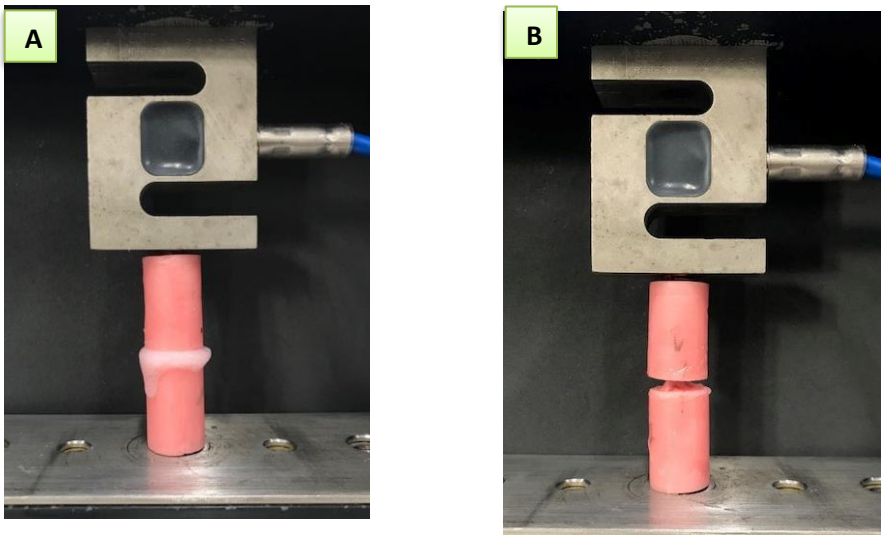


Figura 9. A. Compressão Prévia na Máquina de Ensaios Universal; B- Teste de Tração na Máquina de Ensaios Universal.

13. ANEXO

Anexo 1 – Revista selecionada para submissão do artigo - The Journal of Prosthetic Dentistry

DESCRIPTION The Journal of Prosthetic Dentistry is the leading professional journal devoted exclusively to prosthetic and restorative dentistry. The Journal is the official publication for 24 leading U.S. international prosthodontic organizations. The monthly publication features timely, original peerreviewed articles on the newest techniques, dental materials, and research findings. The Journal serves prosthodontists and dentists in advanced practice, and features color photos that illustrate many step-by-step procedures. The Journal of Prosthetic Dentistry is included in Index Medicus and CINAHL. The Journal of Prosthetic Dentistry is one of the highest ranked Prosthodontics title by number of citations and impact factor on the 2016 Journal Citation Reports®, published by Thomson Reuters. The Journal has a five year impact factor of 2.201.

IMPACT FACTOR 2018: 2.787 © Clarivate Analytics Journal Citation Reports 2019.

ABSTRACTING AND INDEXING: Scopus

GUIDE FOR AUTHORS Instructions in Other languages Spanish: 2013 Guía para la Preparación de ManuscritosTurkish: 2013 Makale Hazırlama RehberiPortuguese: 2013 Guia para a Preparação de Manuscritos Now in its 65th year, The Journal of Prosthetic Dentistry is the leading professional journal devoted exclusively to prosthetic and restorative dentistry. The Journal is the official publication of 24 leading U.S. and international prosthodontic organizations, serving prosthodontists and dentists in aadvanced practice. It features timely, original peer-reviewed articles on the newest techniques, dental materials, and research findings, with color photographs that illustrate step-by-step procedures. The Journal of Prosthetic Dentistry is included in Index Medicus and CINAHL, and is the highest ranked Prosthodontics title by number of citations according to the 2014 Journal Citation Reports.®

Article Types Articles are classified as one of the following: research/clinical science article, clinical report, technique article, systematic review, or tip from our readers. Required sections for each type of article are listed in the order in which they should be presented.

Research and Education/Clinical Research The research report should be no longer than 10-12 double-spaced, typed pages and be accompanied by no more than 12 high-quality

illustrations. Avoid the use of outline form (numbered and/or bulleted sentences or paragraphs).

The text should be written in complete sentences and paragraph form. Abstract (approximately 400 words): Create a structured abstract with the following subsections: Statement of Problem, Purpose, Material and Methods, Results, and Conclusions. The abstract should contain enough detail to describe the experimental design and variables. Sample size, controls, method of measurement, standardization, examiner reliability, and statistical method used with associated level of significance should be described in the Material and Methods section. Actual values should be provided in the Results section.

Clinical Implications: In 2-4 sentences, describe the impact of the study results on clinical practice. Introduction: Explain the problem completely and accurately. Summarize relevant literature, and identify any bias in previous studies. Clearly state the objective of the study and the research hypothesis at the end of the Introduction. Please note that, for a thorough review of the literature, most (if not all references) should first be cited in the Introduction and/or Material and Methods section.

Material and Methods: In the initial paragraph, provide an overview of the experiment. Provide complete manufacturing information for all products and instruments used, either in parentheses or in a table. Describe what was measured, how it was measured, and the units of measure. List criteria for quantitative judgment. Describe the experimental design and variables, including defined criteria to control variables, standardization of testing, allocation of specimens/subjects to groups (specify method of randomization), total sample size, controls, calibration of examiners, and reliability of instruments and examiners.

State how sample sizes were determined (such as with power analysis). Avoid the use of group numbers to indicate groups. Instead, use codes or abbreviations that will more clearly indicate the characteristics of the groups and will therefore be more meaningful for the reader. Statistical tests and associated significance levels should be described at the end of this section.

Results: Report the results accurately and briefly, in the same order as the testing was described in the Material and Methods section. For extensive listings, present data in tabular or graphic form to help the reader.

For a 1-way ANOVA report of, F and P values in the appropriate location in the text. For all other ANOVAs, per guidelines, provide the ANOVA table(s). Describe the most

significant findings and trends. Text, tables, and figures should not repeat each other. Results noted as significantmust be validated by actual data and P values.

Discussion: Discuss the results of the study in relation to the hypothesis and to relevant literature. The Discussion section should begin by stating whether or not the data support rejecting the stated null hypothesis. If the results do not agree with other studies and/or with accepted opinions, state how and why the results differ. Agreement with other studies should also be stated. Identify the limitations of the present study and suggest areas for future research.

Conclusions: Concisely list conclusions that may be drawn from the research; do not simply restate the results. The conclusions must be pertinent to the objectives and justified by the data. In most situations, the conclusions are true for only the population of the experiment. All statements reported as conclusions should be accompanied by statistical analyses. References: See Reference Guidelines and Sample References page. Tables: See Table Guidelines. Illustrations: See Figure Submission and Sample Figures page.

Clinical Report: The clinical report describes the author's methods for meeting a patient treatment challenge. It should be no longer than 4 to 5 double-spaced, pages and be accompanied by no more than 8 high-quality illustrations. In some situations, the Editor may approve the publication of additional figures if they contribute significantly to the manuscript.

Abstract: Provide a short, nonstructured, 1-paragraph abstract that briefly summarizes the problem encountered and treatment administered.

Introduction: Summarize literature relevant to the problem encountered. Include references to standard treatments and protocols. Please note that most, if not all, references should first be cited in the Introduction and/or Clinical Report section.

Clinical Report: Describe the patient, the problem with which he/she presented, and any relevant medical or dental background. Describe the various treatment options and the reasons for selection of the chosen treatment. Fully describe the treatment rendered, the length of the follow-up period, and any improvements noted as a result of treatment. This section should be written in past tense and in paragraph form.

Discussion: Comment on the advantages and disadvantages of the chosen treatment and describe any contraindications for it. If the text will only be repetitive of previous sections, omit the Discussion.

Summary: Briefly summarize the patient treatment.

References: See Reference Guidelines and Sample References page.

Illustrations: See Figure Submission and Sample Figures page.

Dental Technique: The dental technique article presents, in a step-by-step format, a unique procedure helpful to dental professionals. It should be no longer than 4 to 5 double-spaced, typed pages and be accompanied by no more than 8 high-quality illustrations. In some situations, the Editor may approve the publication of additional figures if they contribute significantly to the manuscript.

Abstract: Provide a short, nonstructured, 1-paragraph abstract that briefly summarizes the technique.

Introduction: Summarize relevant literature. Include references to standard methods and protocols.

Please note that most, if not all, references should first be cited in the Introduction and/or Technique section. Technique: In a numbered, step-by-step format, describe each step of the technique. The text should be written in command rather than descriptive form ("Survey the diagnostic cast" rather than "The diagnostic cast is surveyed.") Include citations for the accompanying illustrations.

Discussion: Comment on the advantages and disadvantages of the technique, indicate the situations to which it may be applied, and describe any contraindications for its use. Avoid excessive claims of effectiveness. If the text will only be repetitive of previous sections, omit the Discussion.

Summary: Briefly summarize the technique presented and its chief advantages.

References: See Reference Guidelines and Sample References page

Illustrations: See Figure Submission and Sample Figures page.

Systematic Review The author is advised to develop a systematic review in the Cochrane style and format. The Journal has transitioned away from literature reviews to systematic reviews. For more information on systematic reviews, please see www.cochrane.org. An example of a Journal systematic review: Torabinejad M, Anderson P, Bader J, Brown LJ, Chen LH, Goodacre CJ, Kattadiyil MT, Kutsenko D, Lozada J, Patel R, Petersen F, Puterman I, White SN. Outcomes of root canal treatment and restoration, implantsupported single crowns, fixed partial dentures, and extraction without replacement: a systematic review. J Prosthet Dent 2007;98:285-311.

The systematic review consists of:

An Abstract using a structured format (Statement of Problem, Purpose, Material and Methods, Results, Conclusions).

Text of the review consisting of an introduction (background and objective), methods (selection criteria, search methods, data collection and data analysis), results (description of studies, methodological quality, and results of analyses), discussion, authors' conclusions, acknowledgments, and conflicts of interest. References should be peer reviewed and follow JPD format.

Tables and figures, if necessary, showing characteristics of the included studies, specification of the interventions that were compared, the results of the included studies, a log of the studies that were excluded, and additional tables and figures relevant to the review.

Tips From Our Readers

Tips are brief reports on helpful or timesaving procedures. They should be limited to 2 authors, no longer than 250 words, and include no more than 2 high quality illustrations. Describe the procedure in a numbered, step-by-step format; write the text in command rather than descriptive or passive form ("Survey the diagnostic cast" rather than "The diagnostic cast is surveyed").

Submission Guidelines:

Thank you for your interest in writing an article for The Journal of Prosthetic Dentistry. In publishing, as in dentistry, precise procedures are essential. Your attention to and compliance with the following policies will help ensure the timely processing of your submission.

Length of Manuscripts:

Manuscript length depends on manuscript type. In general, research and clinical science articles should not exceed 10 to 12 double-spaced, typed pages (excluding references, legends, and tables).

Clinical Reports and Technique articles should not exceed 4 to 5 pages, and Tips articles should not exceed 1 to 2 pages. The length of systematic reviews varies.

Number of Authors:

The number of authors is limited to 4; the inclusion of more than 4 must be justified in the letter of submission. (Each author's contribution must be listed.) Otherwise, contributing authors in excess of 4 will be listed in the Acknowledgments. There can only be one corresponding author.

General Formatting:

All submissions must be submitted via the EES system in Microsoft Word with an 8.5×11 inch page size. The following specifications should also be followed: Times Roman, 12

pt Double-spacedLeft-justified. No space between paragraphs1-inch margins on all sidesHalf-inch paragraph indentsHeaders/Footers should be clear of page numbers or other information.

Headings are upper case bold, and subheads are upper/lower case bold. No italics are used. References should not be automatically numbered. Endnote or other reference-generating programs should be turned off.Set the Language feature in MS Word to English (US). Also change the language to English (US) in the style named Balloon Text.