UNIVERSIDADE FEDERAL DE PELOTAS
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Tese

Título:
Avaliação do Efeito Antifúngico de Fixadores de Dentaduras Modificados pelo Acréscimo de Derivados Pirazolinicos

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Pelotas, 2015

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Avaliação do Efeito Antifúngico de Fixadores de Dentaduras Modificados Pelo Acréscimo de Derivados Pirazolinicos

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Dedicatória

*A*os meus pais, Maria Helena e José Antônio, por todo o exemplo de integridade,

amor, respeito e dedicação... Amo vocês!

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Notas Preliminares

A presente Tese foi redigida segundo o Manual de Normas para Dissertações, Teses e Trabalhos Científicos da Universidade Federal de Pelotas de 2006, adotando o Nível de Descrição 4 – estruturas em Artigos, que consta no Apêndice D do referido manual. Disponível no endereço eletrônico: (http://www.ufpel.tche.br/prg/sisbi/documentos/Manual_normas_UFPel_2006.pdf).

Resumo

Oliveira, Simone Gomes Dias. Avaliação do efeito antifúngico de Fixadores de Dentaduras modificados pelo acréscimo de derivados pirazolinicos**.** 2015. 142f. Tese de Doutorado – Programa de Pós Graduação em Odontologia. Universidade Federal de Pelotas, Pelotas.

O uso de próteses totais convencionais configura-se como um importante método de reabilitação bucal devido principalmente a vantagem econômica, sendo a principal queixa quanto ao seu uso a falta de retenção e estabilidade. A fim de melhorar essas características existem no mercado produtos com propriedades adesivas chamados de fixadores de dentadura. O principio de ação desse material resume-se na absorção de líquidos do meio, aumento da viscosidade e aumento da adesividade a mucosa. O presente trabalho foi dividido em três artigos. O artigo 1 objetivou avaliar o estado da técnica e o estado da arte através de revisão sistematizada em banco de patentes e de artigos referentes a fixadores de dentaduras. Para o monitoramento do estado da técnica e da arte, foi realizada uma pesquisa bibliográfica de estudos publicados entre 1960 e 2014, em sete bases de dados MEDLINE (PubMed), Web of Science , Lilacs , IBECS , Biblioteca Cochrane , Scielo e Scopus . Além disso, os seguintes bancos de dados de patentes foram rastreados: USPTO , EPO , JPO , INPI , Derwent Innovations Index, Patentscope e Questel Orbit . Os dados foram tabulados e analisados pelo Microsoft Office Excel 2013 software e Questel Orbit. Um total de 54 artigos e patentes 78 foram incluídos na análise referente ao monitoramento bibliográfico e tecnológico. Os estudos mais predominantes foram realizados in vivo (n=30), e os tipos de adesivos mais estudados foram em creme ou pó (n=14). Os avanços recentes de tais materiais têm sido mais relacionados com a apresentação comercial do que com os efeitos adicionais de adesivos para próteses dentárias. O artigo 2 objetivou avaliar uma nova metodologia confiável para o teste de adesividade dos fixadores de dentadura frente às variáveis como: meio (saliva artificial, saliva natural e água destilada) e método (imersão e molhamento). Para a validação da metodologia confiável de adesividade dos fixadores este foi definido usando um planejamento experimental com fatores fixos e variáveis. Foi utilizado para tal proposito uma maquina de ensaios universais operando com célula de carga de 100 N e velocidade de 1 mm/min. O método de ensaio estabelecido mostrou que o meio em que o teste é realizado, saliva artificial, água e saliva natural e o método utilizado, imersão ou

molhamento, foram determinantes para o resultado de adesividade dos fixadores de dentadura. O artigo 3 objetivou caracterizar e avaliar o efeito do acréscimo de um antifúngico alternativo, derivado de pirazois, em fixadores de dentadura comerciais em três diferentes apresentações (pó, pasta e creme). Os fixadores de dentadura comerciais foram modificados com a inclusão de antifúngicos (Nistatina e Pirazol) em três concentrações por porcentagem em massa diferentes (30%; 3% e 0.3%). A ação antifúngica foi determinada através dos testes de disco difusão e contato direto. O teste de contato direto foi mensurado em 1, 4, 8 e 12 horas. A atividade citotóxica foi avaliada em fibroblastos de camundongos (NIH/3T3) pelo ensaio colorimétrico de redução do MTT. Os resultados obtidos demonstraram que as inclusões dos antifúngicos não afetam a adesividade dos fixadores de dentadura ($p>0,05$). O teste de disco difusão e de contato direto demonstraram que a inclusão de Nistatina e de Pirazol nos fixadores de dentadura comercial promoveram nestes ação antifúngica frente a *Candida albicans*. Os fixadores formulados com pirazol se mostraram atividade antifúngica similar aqueles formulados com nistatina. Em relação a citotoxicidade, a concentração mais alta (30%) foi significativamente a mais citotóxica ($p<0,05$), sendo que as concentrações subsequentes se mostraram com baixa citotoxicidade. Os fixadores com pirazol de comportaram semelhantes a nistatina em relação a citotoxidade nas concentrações 2(3%) e 3(0,3%). Considerando-se as limitações do presente estudo, conclui-se que há possibilidade do uso de fixadores de dentadura como sistema de entrega de antifúngicos comerciais (Nistatina) ou não (pirazol) sendo a segunda concentração (3%) a mais eficiente, que independe da apresentação comercial é menos citotóxica.

Palavras-chaves: Fixadores de dentadura; Produtos com ação antimicrobiana; *Candida albicans*; Pirazol; Antifúngicos.

Abstract

Oliveira, Simone Gomes Dias. Avaliação do efeito antifúngico de Fixadores de Dentaduras modificados pelo acréscimo de derivados pirazolinicos**.** 2015. 141f. Tese Doutorado – Programa de Pós Graduação em Odontologia. Universidade Federal de Pelotas, Pelotas.

The use of conventional dentures still appears as the main oral rehabilitation method of total edentulous patients mainly because the economic advantage of this kind of treatment. Despite these prostheses were made for several years, the main complaint about their use remains the lack of retention and stability. In order to improve these characteristics exists in the market products with adhesive properties called denture adhesives. These denture adhesives have a variety of formulations and commercial presentation, but the principle of action summarized in the absorption of the liquid medium, increased viscosity and increased adhesion to the mucosa. There are controversies in the literature about the effect of these denture adhesives in the oral microbiota and the possibility that these promote accumulation of biofilm which in turn cause chronic infections, such as chronic atrophic candidiasis. This work was divided into three articles. Article 1 aimed to evaluate the state of the art and state of the art through systematic review on bank of patents and articles related to denture adhesives. For monitoring the technique and art state, was performed a literature search of studies published between 1960 and 2014 in seven MEDLINE (PubMed), Web of Science, Lilacs, IBECS, Cochrane Library, SciELO and Scopus. In addition, the following patent databases were screened: USPTO, EPO, JPO, PTO, Derwent Innovations Index, Questel Orbit and Patentscope. Data were tabulated and analyzed using Microsoft Office Excel 2013 software and Questel Orbit. A total of 54 papers and patents 78 were included in the analysis related to bibliographic and technological monitoring. The results showed that the highest number of patents (n = 19) was deposited by Procter & Gamble Company (Cincinnati, Ohio). A total of 54 papers and patents 78 were included in the analysis related to bibliographic and technological monitoring. Furthermore, the most prevalent in vivo studies were performed (n = 30), and the types of adhesives have been most studied cream or powder (n = 14). Recent advances of such materials have been related more to the presentation than for the additional effects of denture adhesives. Article 2 aimed to evaluate a new methodology for the reliable adhesive test of front denture adhesives the variables as a (artificial saliva, natural saliva and

distilled water) and method (immersion and wetting). The commercial denture adhesives were modified with the inclusion of antifungals (Pyrazol and nystatin) in three different concentrations in percentages by weight (30%, 3% and 0.3%). To validate the adhesiveness reliable methodology for fixing this was defined using an experimental design with fixed and variable factors. Was used for this purpose universal testing a machine operating with a 100 N load cell and speed of 1 mm / min. The established test method showed that the environment in which the test is performed, artificial saliva, water and natural saliva and the method used, dipping or wetting, was decisive for the outcome of the denture adhesives. It is essential that researchers analyze their methodologies before predicting and say which product is more or less efficient, avoiding misconceptions about them. Article 3 aimed to characterize and evaluate the effect of the addition of an alternative antifungal derived from pyrazoles, in commercial denture adhesives in three different presentations (powder, tape and cream). The antifungal activity was determined by the disk diffusion testing and direct contact. Direct contact test was measured at 1, 4, 8 and 12 hours. The cytotoxic activity was evaluated on mouse fibroblasts (NIH / 3T3) using the colorimetric MTT reduction assay. The results showed that the inclusion of antifungals did not affect the adhesion of the denture adhesives ($p > 0.05$). The disk diffusion test results and demonstrated that direct contact include nystatin and pyrazole in commercial denture adhesives promoted to these antifungal activity against *Candida albicans*. Adhesives dentures formulated with pyrazol proved similar antifungal those formulated with nystatin. With respect to cytotoxicity, the highest concentration was significantly more cytotoxic ($p < 0.05$), and subsequent concentrations showed low cytotoxicity. The dentures adhesives with pyrazole behaved like nystatin relative cytotoxicity at concentrations of 3% and 0.3%. Considering the limitations of this study, it is concluded that there is possibility of using denture adhesives as delivery system of trade antifungal (Nystatin) or not (pyrazole) and the second concentration (3%) to more efficient, which is independent commercial and less cytotoxic presentation.

Keywords: Fixative dentures, drug delivery systems; Oral Cavity; *Candida albicans*; Pirazoles.

Lista de Tabelas e Figuras

Lista de Abreviaturas

CAC	Candidíase Atrófica Crônica
DMSO	Dimetilsulfóxido
CIM	Concentração Inibitória Mínima
MC	Microdiluição em Caldo
CLSI \ NCCLS	*National Committee for Clinical Laboratory Standards*
CFM	Concentração Fungicida Mínima
DEMEM	Meio de Eagle Modificado por Dulbeco
SFB	Soro Fetal Bovino
RPM	Rotações por minuto
MIN	Minuto
MOPS	Ácido Morfilenopropanosulfonico
REYA	*Reduced Egg Yolk Agar*
PZ	Zona de Precipitação
MTT	Sal Tetrazolium [3-(4,5- dimetiltiazol-2-y)-2,5-difeniltetrazoliumbrometo
IR	Infravermelho

Sumário

1 Projeto de Pesquisa

1.1 Introdução

1.1.1 Estado da Arte

Mucoadesivos, adesivo de dentadura ou fixadores protéticos

Quando se deseja o desenvolvimento de um mucoadesivo a mucosa bucal possui alguns atrativos para a alocação desse tipo de dispositivo. As células epiteliais da mucosa bucal estão rodeadas por substância de base intercelular chamada muco com a espessura que varia de 40 mm a 300 mm (Allen & Forte, 1989). Esse muco serve como um veículo de entrega eficaz, agindo como um lubrificante e permitindo que as células se movam (Peppas & Buri, 1985). Ademais, o muco pode formar uma estrutura de gel fortemente coesa que se liga à superfície das células epiteliais como uma camada gelatinosa (Gandhi & Robinson, 1988). Muco e moléculas são capazes de se unir para fazer polímeros ou uma rede tridimensional estendida. Outra característica importante é a permeabilidade da mucosa oral. Em geral, a permeabilidade baseia-se na espessura relativa e grau de queratinização desses tecidos na ordem de sublingual> bucal> palatal. A permeabilidade da mucosa bucal foi estimada em 4-4000 vezes maior do que a da pele (Galey et al 1976).

Para o desenvolvimento de mucoadesivos, deve-se objetivar uma forma segura e eficaz de entrega de drogas. Devem ser fortemente considerados os fatores que influenciam a liberação da droga e a penetração através da mucosa bucal, fatores organolépticos, efeitos dos aditivos utilizados para melhorar o padrão de liberação e absorção da droga, efeitos de irritação local da droga (Sudhakar et al 2006). Em geral são requeridas as características: tamanho 1 a 3 cm^2 e uma dose diária de 25 mg ou menos são preferíveis; duração máxima de administração bucal de cerca de 4-6 h (Alur et al 2001).

Atualmente os mucoadesivos bucais são formados por polímeros ou hidrogéis. Os polímeros compreendem um grupo grande e diversificado de moléculas, incluindo as substâncias de origem natural biodegradáveis, copolímeros e polímeros tiolados. Estas formulações são frequentemente solúveis em água e, quando em uma forma seca atraem água a partir da superfície biológica e esta transferência de água leva a uma forte interação. Estes polímeros também podem formar líquidos viscosos quando hidratados com água. Isso aumenta o tempo de retenção sobre superfícies mucosas e pode levar a interações adesivas. Polímeros bioadesivos devem possuir certas características físico-químicas, incluindo hidrofilicidade, numerosas ligações de hidrogênio, flexibilidade para a interpenetração com muco e tecido epitelial, e propriedades visco-elásticas (Batchelor 2004).

Os hidrogéis são matrizes hidrófilas que absorvem água quando colocado num meio aquoso. Na mucosa bucal, este meio aquoso pode ser fornecido pela saliva, que pode também atuar como meio de dissolução. Os hidrogéis são estruturados de tal maneira que as fibras de reticulação apresentam a sua matriz como uma forma eficaz de impedir sua dissolução e, assim, promovem a retenção de água (Mishra et al 1996).

Em suma, os sistemas mucoadesivos orais oferecem inúmeras vantagens em termos de administração, acessibilidade, retentividade, baixa atividade enzimática, economia e alta adesão do paciente (Sudhakar et al 2006). Porem sabe-se que o sucesso no desenvolvimento dessas novas formulações exige a assimilação de uma grande quantidade de informação emergente sobre a estrutura química e física da natureza destes novos materiais.

Derivados Pirazolinicos : uma alternativa antifúngica

Os compostos heterocíclicos vêm despertando grande interesse devido a sua aplicabilidade nos mais diversos campos da química moderna e a enorme variedade e complexidade estrutural, que possibilita gerar uma vasta série de novas estruturas com propriedades físicas e químicas diversas. Estes

fatores justificam o incremento no número de heterociclos desenvolvidos nos últimos anos.

Dentre os compostos heterocíclicos, merecem destaque os sistemas nitrogenados de cinco membros, especialmente os derivados de pirazóis e pirróis, os quais atraem o interesse de pesquisadores devido a sua importância medicinal e biológica. Embora pirazóis e derivados sejam raramente encontrados na natureza, eles são importantíssimos do ponto de vista biológico. Esta importância é evidenciada pelos exemplos de fármacos comerciais que contêm o anel pirazolínico em sua estrutura. São eles, o Celecoxib (Celebra®)(Fig.1), um dos primeiros representantes da segunda geração de agentes antiinflamatórios não esteroidais e o Rimonabant (Fig.2), popularmente conhecido como “pílula da barriga”. Outro derivado pirazolínico é o Fipronil, de uso veterinário, eficiente no combate a carrapatos. Além desses a literatura relata, entre outros, a atividade promissora do 4,5-diidropirazol BW540C como antiinflamatório .

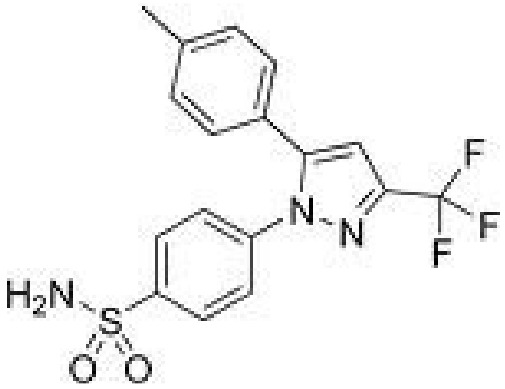


Figura 1. Estrutura química do Celecoxib

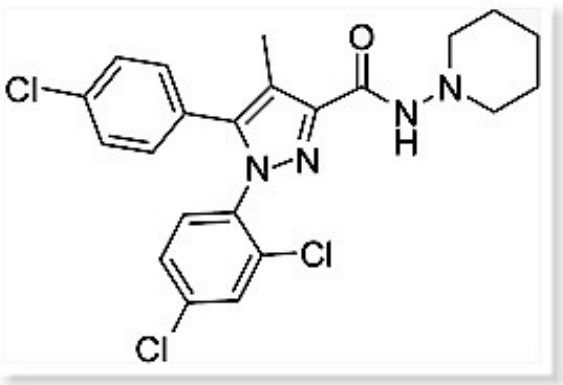
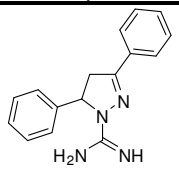
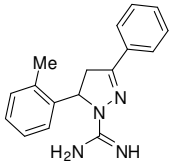
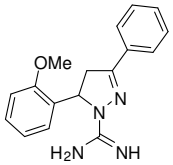
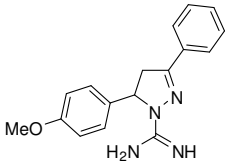
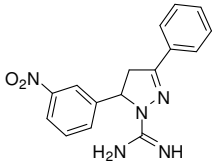
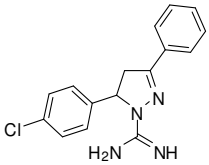
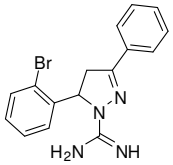
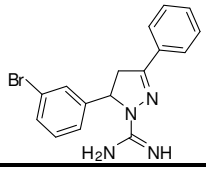


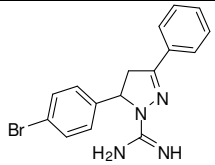
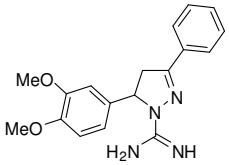
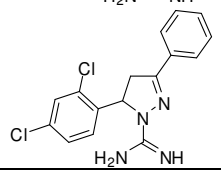
Figura 2. Estrutura química do Rimonabant

Esses compostos heterociclos contendo a unidade pirazol possuem um amplo espectro de atividades biológicas, tais como inibidor da monoamina oxidase, anticonvulsivante, antibacteriano hipotensores, antipiréticos e antinflamatorio.

Os compostos derivados pirazolinicos que serão utilizados no decorrer desse trabalho estão dispostos na tabela abaixo, e sua síntese esta descrita por Pizzuti et al 2009.

Tabela 1 - Nomenclatura dos compostos sintetizados.

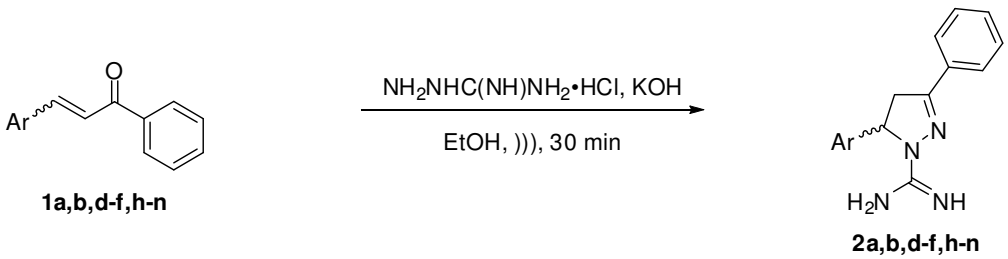
Composto	Numeração	Nomenclatura
	**2a**	3,5-difenil-4,5-diidro-1*H*-amidinopirazol
	**2b**	3-fenil-5-(2-tolil)-4,5-diidro-1*H*-amidinopirazol
	**2d**	3-fenil-5-(3-nitrofenil)-4,5-diidro-1*H*-amidinopirazol
	**2e**	3-fenil-5-(4-nitrofenil)-4,5-diidro-1*H*-amidinopirazol
	**2f**	3-fenil-5-(2-metoxifenil)-4,5-diidro-1*H*-amidinopirazol
	**2h**	5-(4-clorofenil)-3-fenil-4,5-diidro-1*H*-amidinopirazol
	**2i**	5-(2-bromofenil)-3-fenil-4,5-diidro-1*H*-amidinopirazol
	**2j**	5-(3-bromofenil)-3-fenil-4,5-diidro-1*H*-amidinopirazol

Composto	Numeração	Nomenclatura
	**2k**	5-(4-bromofenil)-3-fenil-4,5-diidro-1*H*-amidinopirazol
	**2l**	5-(3,4-dimetoxifenil)-3-fenil-4,5-diidro-1*H*-amidinopirazol
	**2m**	5-(2,4-diclorofenil)-3-fenil-4,5-diidro-1*H*-amidinopirazol

As 3,5-diaril-4,5-diidro-1*H*-amidinopirazol foram sintetizados a partir da reação das 3-diaril-2-propen-1-onas 1a,b,d-f,h-n com 3 equivalentes de cloridrato de aminoguanidina em meio básico de KOH (3 equivalentes) e etanol. Os produtos foram obtidos na forma sólida com rendimentos de 36-99%. A Tabela 2 mostra os pontos de fusão e rendimentos dos compostos.

Os amidinopirazóis não foram sintetizados pela metodologia clássica, portanto, não se tem parâmetros para a comparação e o apontamento das vantagens do método sonocatalisado, mas pode-se dizer que os produtos foram obtidos em espaços de tempo muito curtos, utilizando o etanol, que é um solvente bio-renovável. Estes fatores são importantíssimos do ponto de vista ambiental.

Esquema 1 – Reação das 3-diaril-2-propen-1-onas



1a,b,d-f,h-n **2a,b,d-f,h-n**

	a	**b**	**d**	**e**	**f**	**h**
Ar	C_6H_5	2-MeC_6H_4	2-$MeOC_6H_4$	4-$MeOC_6H_4$	3-$O_2NC_6H_4$	4-ClC_6H_4

	i	**j**	**k**	**l**	**m**	**n**
Ar	2-BrC_6H_4	3-BrC_6H_4	4-BrC_6H_4	3,4-$(MeO)_2C_6H_3$	2,4-$Cl_2C_6H_3$	3,4,5-$(MeO)_3C_6H_2$

Tabela 2 - Pontos de fusão e rendimentos dos compostos 2a,b,d-f,h-n.

Composto	Fórmula Molecular Massa Molar ($g.mol^{-1}$)	Ponto de fusão (°C)[a]	Rendimento (%)[b]
2a	$C_{16}H_{16}N_4$ 264,33	167–169	75
2b	$C_{17}H_{18}N_4$ 278,35	188–190	75
2d	$C_{17}H_{18}N_4O$ 294,35	165–167	98
2e	$C_{17}H_{18}N_4O$ 294,35	147–149	80
2f	$C_{16}H_{15}N_5O_2$ 309,32	176–178	53
2h	$C_{16}H_{15}ClN_4$ 298,77	163–165	98
2i	$C_{16}H_{15}BrN_4$ 343,22	168–170	99
2j	$C_{16}H_{15}BrN_4$ 343,22	262–264	36
2k	$C_{16}H_{15}BrN_4$ 343,22	136–138	80
2l	$C_{18}H_{20}N_4O_2$ 324,38	146–148	99
2m	$C_{16}H_{14}Cl_2N_4$ 333,22	94–96	87
2n	$C_{19}H_{22}N_4O_3$ 354,40	179–181	90

[a] Pontos de fusão não corrigidos.
[b] Rendimentos dos compostos isolados.

A identificação dos compostos foi feita através da análise de dados de RMN ^1H e $^{13}C\{H\}$, espectrometria de massas e espectroscopia de infravermelho. Estes compostos foram avaliados (*in vitro)* referente *a*o potencial antifúngico, anti-enzimático e citotóxico por nosso grupo de pesquisa. Os resultados foram: CIM e CFM>15,6µg\ml para *C. albicans*; CIM e CFM>62,5 µg\ml para *C. parapsilosis;* CIM e CFM=62,5 µg\ml para *C. famata*; CIM e CFM=125 µg\ml para *C. glabrata* e CIM=15,6 µg\ml para *C. lipolytica*. Os valores médios de fosfolipase e proteinase (Pz) de *C. albicans* antes e após a exposição foram respectivamente: 0,2 (±0,022) e 0,6 (±0,024) ; 0,3(±0,04) e 0,9(±0,074) . Estes resultados não foram estatisticamente significantes para proteinase, porém significantes para fosfolipase (p=0,01), sendo a concentração de 15,6 µg\ml a mais efetiva. Não foram observadas diferenças estatísticas entre os grupos testados e o controle quanto à citotoxidade. Com

base nesse resultados, concluimos que os derivados pirazolínicos são promissores agentes antifúngicos, seja através da morte das leveduras ou inibição da sua atividade enzimática, além de apresentarem baixa citotoxidade.

1.1.2 Estado da Técnica

Os mucoadesivos são encontrados de diferentes formas na literatura. Mucoadesivos em forma de géis são utilizados especialmente na cavidade oral. Um exemplo é a U.S.Pat.N. 5,192,802 que descreve um mucoadesivo em gel feito de carboximetil celulose e goma xantana. O gel se propõe a tratar aftas, febre e hemorróidas. No entanto, este tipo de transportador farmacêutico tem um tempo de vida em cavidade oral muito limitado já que os fluidos como a saliva podem removê-los. Géis bioadesivos são também descritos na USPat.Nos. 5.314.915; 5.298.258 e 5.642.749.

Tabletes mucoadesivos são descritos em U.S.Pat.No 4,915,948. O material bioadesivo solúvel em água utilizado neste dispositivo é uma goma xantana ou uma pectina combinada com um material de reforço de adesão tal como um poliol. Embora o tempo de permanência em contato com a mucosa seja melhorado, estes não são de fácil utilização, especialmente quando usada no cavidade oral, dadas as desagradáveis sensações associadas à sua solidez, espessura, e tempo de dissolução lenta. Os tablets mucoadesivos são também descritos na U.S.Pat.No 4,226,848; 4,292,299 e 4,250,163 e são dispositivos de camada única ou bicamada tendo uma espessura média de 0,2 a 2,5 mm. Os tablets descritos nestas patentes utilizam um componente não adesivo como o éter de celulose, um componente bioadesivo como o ácido poliacrílico, carboximetilcelulose de sódio ou polivinilpirrolidona e um ligante para efeitos de prensagem. Os derivados de celulose podem ou não ser solúveis em água.

Outro tipo de mucoadesivos são as laminas ou películas adesivas. Essas laminas ou películas adesivas são finas e flexíveis e, assim, diminuem a sensação de corpo estranho e são utilizados para entregar os fármacos através

da pele ou mucosa. Estas estão descrita em U.S.Pat.N. 3,996,934 e 4,286,592. Estes produtos são utilizados para entregar drogas através da pele ou mucosa.

Além dos sistemas de películas ou laminas adesivas há os filmes mucoadesivos. Estes tipos de sistemas, que são insolúveis em água e se apresentam geralmente sob a forma de laminados são descritos em U.S.Pat.No. 4,517,173, 4,572,832, 4,713,243, 4,900,554 e 5,137,729. O documento U.S.Pat.No 4,517,173 descreve e reivindica uma membrana aderente constituída por camadas, incluindo uma camada farmacêutica, uma camada pouco solúvel em água e uma camada intermédia. A camada farmacêutica inclui o fármaco e um derivado de celulose selecionado a partir de hidroxipropil celulose, metil celulose, metil-celulose e hidroxipropil. A camada pouco solúvel em água é obtido pela combinação de um ou mais derivados de celulose, e a camada intermédia é composta por derivados de celulose. O documento U.S.Pat.No 4,572, 832 refere-se a uma película de gelatina para administração bucal, feita pela utilização combinada de uma proteína solúvel em água, um éster de ácido ou polímero de carboxivinilo, e um álcool poli-hídrico tal como celulose.

As pastas e filmes adesivos para dentaduras são outro tipo de produto bioadesivo. No entanto, estas preparações são utilizadas primariamente pelas suas propriedades adesivas, para aderir dentaduras às gengivas, em vez de para a proteção do tecido ou para entrega tópica de fármacos, embora drogas tais como anestésicos locais possam ser utilizados na pasta para alívio da gengiva. O documento U.S.Pat.No 4.894.232 e o 4.518.721 descrevem pastas adesivas para dentaduras obtidas através de uma combinação de carboximetilcelulose de sódio e óxido de polietileno, em polietileno glicol. Para o nosso grupo de pesquisa esse tipo de bioadesivo, poderiam ter propriedades antimicrobianas adicionais. A sua grande vantagem seria que as desvantagens do uso de mucoadesivos orais, como por exemplo a ação da saliva ou o atrito acometido por algum alimento seria minimizado.

1.2 Justificativa

O uso de próteses totais convencionais configuram-se como o principal método de reabilitação bucal de pacientes edentados totais devido principalmente a vantagem econômica desse tipo de tratamento. Apesar de essas próteses serem confeccionada há diversos anos, a principal queixa quanto ao seu uso continua sendo a falta de retenção e estabilidade. A fim de melhorar essas características existe no mercado produtos com propriedades adesivas chamados de Fixadores de dentadura. Esses fixadores apresentam uma diversidade de formulações e apresentações comerciais, porém o principio de ação resume-se na absorção de líquidos do meio, aumento da viscosidade e aumento das propriedades adesivas. Uma das desvantagens desse material esta no efeito de que esses fixadores causariam na microbiota oral e a possibilidade destes promoverem acumulo de biofilme e episódios de infecções crônicas, como por exemplo, candidíase atrófica crônica. Esses fixadores de dentadura apresentam propriedades adesivas, porém não foram utilizados como veículos de antimicrobianos, mesmo sendo mais favoráveis aos mesmos já que não sofrem atrito de alimentos e ficam protegidos, geralmente, abaixo das próteses dentárias. Ademais, acredita-se que esse possível uso desses fixadores de dentadura como sistema de entrega de fármacos favoreceriam uma parcela especifica da população que sofrem com resistência antifúngica, pacientes imunodeprimidos e debilitados.

O desenvolvimento de um fixador de dentaduras com características de mucoadesivos com função de sistema de entrega de fármacos poderia auxiliar o clinico frente ao tratamento de infecções orais comuns e beneficiar pacientes pela facilidade de utilização dos mesmos.

1.3 Objetivos e metas a serem alcançados

1.3.1 Objetivo Geral

O objetivo deste trabalho foi avaliar o efeito antifúngico de Fixadores de Dentaduras modificados pelo acréscimo de derivados pirazolinicos.

1.3.2 Objetivos específicos

1) Avaliar o estado da técnica e do estado da arte através de revisão sistematizada em banco de patentes e de artigos referente a fixadores de dentaduras;

2) Validar uma metodologia confiável para o teste de adesividade dos fixadores de dentadura frente a variáveis como meio (saliva artificial, saliva natural e água destilada) e método (imersão e molhamento);

3) Caracterizar e avaliar o efeito do acréscimo de um antifúngico alternativo, derivado de pirazois, em fixadores de dentadura comerciais em três diferentes apresentações (pó, pasta e creme).

1.3.3 Metas pretendidas

META FÍSICA 1 – Caracterização, definição dos componentes e desenvolvimento de um Fixador de dentaduras em forma de filme

Atividade – A partir da revisão de literatura serão selecionados os componentes mais utilizados para o desenvolvimento de fixadores de dentadura

Indicador Fisico- Obtenção de um material adesivo com as seguintes propriedades: (1) características de pH neutro ou literalmente básico; (2) retenção e adesividade satisfatórias; (3) odor e gosto agradáveis; (4) fácil aplicação e remoção; (5) viscosidade adequada; (6) toxidade nula ou mínima à cavidade oral; (7) capacidade antifúngica (anti-biofilme) contra isolados de Candida; (8) o produto final não deve ser irritante a mucosa oral e deve ser livre de impurezas lixiviáveis; (9) deve possuir uma vida útil adequada; (10) deve

possuir grupos adesivos ativos e (11) Não deve promover o desenvolvimento infecções secundárias.

META FÍSICA 2 - Caracterização, comparação e definição dos componentes dos fixadores de dentaduras comerciais.

Atividade – Os fixadores de dentaduras serão analisados em relação a sua composição, ph, viscosidade, adesividade, retenção e citoxicidade.

Indicador Físico - Obtenção da caracterização de um material, fixador de dentadura, com as seguintes propriedades: ph neutro ou ligeramente básico, viscosidade média, retenção satisfatória, toxicidade mínima ou nula confirmada por meio de ensaio de cultivo celular.

META FÍSICA 3 – Obtenção e caracterização de substâncias com propriedade antifungicas.

Atividade – A capacidade antifungica das substâncias obtidas será testada por meio de testes de microdiluição, halo de inibição, teste de atividade anti-enzimatica, determinação do efeito antifúngico em biofilme de Candida albicans aderidas em superfície de resina acrílica e em Candida albicans aderidas em superficies celulares.

Indicador Físico – Obtenção de uma substância com ação antiungica comprovada, por meio da realização dos testes acima citados.

META FÍSICA 4- Definição das matérias-primas a serem utilizadas na formulação do fixador de dentaduras.

Atividade – As matérias-primas serão analisadas em relação à característica de viscosidade e solubilidade por meio de técnicas de análise físico-químicas.

Indicador Físico – Obtenção de matéria-prima com viscosidade e solubilidade especificas.

META FÍSICA 5 – Incorporação de substancias antifúngicas nos fixadores de dentaduras

Atividade – As matérias-primas selecionadas na Meta Física 2 e 3 serão misturadas de maneira homogênea apos a definição do método de mistura e quantidade apropriada.

Indicador Físico – Obtenção de fixadores de dentaduras com mínima alteração na viscosidade e adesividade apos incorporação de substancias antifúngicas.

META FÍSICA 6 - Avaliação do desempenho mecânico dos fixadores de dentadura (produto final)

Atividade – O fixador de dentadura será avaliado em relação as seguintes propriedades: ph, viscosidade, adesividade, odor e sabor e facilidade de remoção do produto.

Indicador físico – Obtenção de fixador de dentadura com ph neutro ou básico, com potencial de fixação de 6-8h, odor e sabor agradável, viscosidade satisfatória para aplicação e remoção do produto.

META FÍSICA 7 - Avaliação do desempenho biológico do fixador de dentadura (produto final).

Atividade – O adesivo será avaliado em relação às seguintes propriedades: potencial antifúngico, potencial inibitório de fatores de aderência da Candida albicans, citoxicidade, ação antifúngica em superfície de resina acrílica e em superfície celular.

Indicador Físico - Obtenção de um fixador com as seguintes propriedades: potencial antifúngico e/ou inibidor de fatores de virulência da Candida albicans, desempenho com baixa ou nula citoxicidade, ação positiva em superfícies protéticas e celulares.

META FÍSICA 8 - Documentação formal e completa da solução proposta.

Atividade – Relatórios em formulários de apontamentos.

Indicador: Documentos validados e registrados no sistema de Relatórios do laboratório.

META FÍSICA 9 – Depósito de patente.

Atividade: Requerimento de depósito de patente no INPI via PCT, iniciando dessa forma o depósito no Brasil e posteriormente nos EUA e Comunidade Européia.

Indicador: Protocolos dos depósitos.

1.4 Metodologia de desenvolvimento

1.4.1 Caracterização, definição dos componentes e desenvolvimento dos Fixadores de dentadura Comerciais

1.4.2 Preparação dos fixadores de dentadura com a inclusão de antifungicos

Os espécimes de fixadores serão preparados através de compressão direta. Os materiais utilizados para a composição destes estão dispostos no Quadro.1.

Quadro 1. Formulacão dos Fixadores de dentadura e composição dos grupos testados

Grupo	Apresentação Comercial	Fixador de Dentadura (g)	Nistatina(g)	Pirazol(g)
GPO0%	Pó	0.05	0	0
GPON30%	Pó	0.05	0.015	0
GPON3%	Pó	0.05	0.0015	0
GPON0.3%	Pó	0.05	0.00015	0
GPOP30%	Pó	0.05	0	0.015
GPOP3%	Pó	0.05	0	0.0015
GPOP0.3%	Pó	0.05	0	0.00015
GC0%	Creme	0.05	0	0
GCN30%	Creme	0.05	0.015	0
GCN3%	Creme	0.05	0.0015	0
GCN0.3%	Creme	0.05	0.00015	0
GCP30%	Creme	0.05	0	0.015
GCP3%	Creme	0.05	0	0.0015
GCP0.3%	Creme	0.05	0	0.00015
GF0%	Fita	0.05	0	0
GFN30%	Fita	0.05	0.015	0
GFN3%	Fita	0.05	0.0015	0
GFN0.3%	Fita	0.05	0.00015	0
GFP30%	Fita	0.05	0	0.015
GFP3%	Fita	0.05	0	0.0015
GFP0.3%	Fita	0.05	0	0.00015

1.4.3 Caracterização físico – química dos filmes mucoadesivos

1.4.3.1 pH do filme mucoadesivo

A possível modificação do pH foi aferida para evitar danos na mucosa oral. Três espécimes de cada grupo serão deixados submersos em copos com 50ml de solução tampão de fosfato a

um ph de 6,8 (para mimetizar a condição bucal). Serão feitas medições através de um phametro nos tempos 15, 30, 60, 90 e 120 min.

1.4.3.2 Variação de volume

Três tipos de cada grupo serão pesados e medidos antes de serem imersos em solução tampão fosfato (0,2 Molar – pH 6,8) a 37ºC por 120 min. Após esse período estes serão novamente pesados e medidos.

1.4.4 Teste de mucosadesão

Este teste será realizado com três espécimes de cada grupo experimental e em dois momentos diferentes. Para este teste serão obtidos em um frigorifico local a mucosa bucal de suínos recém abatidos. O tecido será colocado submerso em saliva artificial e armazenado a -20ºC até posterior utilização. No momento da realização do teste o tecido será descongelado e alocado em laminas de vidro ficando esticado e liso. Os fixadores serao colocado e comprimido a uma força constante de 0,4N por 30 segundos contra o suporte com a mucosa oral suína. Esse suporte será imerso em saliva artificial e mantido a 37ºC. Será observado o tempo de total desprendimento dos fixadores da mucosa oral suína.

1.4.5 Caracterização, Comparação e Definição dos componentes dos Fixadores de Dentadura Comerciais

Para a realização desta etapa serão utilizados os grupos conforme o Quadro 2.

Quadro 2. Grupos utilizados nas metodologias envolvidas na Caracterização, Comparação e Definição dos componentes dos Fixadores de Dentaduras Comerciais

Fixadores de Dentaduras Comerciais			
Tipo Pasta (Creme)		Tipo Fita Adesiva	
Algasiv®	N=20	Algasiv®	N=20
Protefix®	N=20	Protefix®	N=20
Fixodent®	N=20	-	-
Corega®	N=20	Corega®	N=20

1.4.6 Seleção dos Fixadores Comerciais

Serão avaliadas as marcas comerciais mais consumidas, disponíveis no mercado, nas suas diferentes formas. Serão testadas as marcas comerciais Protefix ©, Corega ©, Fixodent© e Algasiv ©.

1.4.7 Composição dos adesivos

Os fixadores de dentadura serão analisados por meio de um espectrofotômetro de infravermelho (IR) após a extracção, em primeiro lugar com éter de petróleo, em seguida, com tetrahidrofurano. Gotas dos serão colocadas em placas de brometo de potássio e os solventes evaporados antes da analise ser executada. Os materiais insolúveis serão misturados com brometo de potássio em pó e comprimido para resultar nos discos para as análises. Os espectros resultantes serão comparadas com os espectros conhecidos dos compostos, afim de confirmar a composição dos fixadores comerciais.

1.4.8 Análise de Ph

Uma amostra de cada produto será pesado e misturado com água deionizada (1 g/10 ml, uma solução a 10%). Diluições seriadas serão realizados para estabelecer soluções de 1.0, 2.0, 3.3, 5.0 e 10%. A agua deionizada será utilizada como solução controle. Imediatamente após a preparação das amostras, valores de pH serão obtidos para cada diluição e para a água deionizada.. O pH será medido de forma cega. Após as leituras de pH inicial,a amostra e o controle serão medidos em 1 -, 2 -,3 -, 4 -, 5 -, 6 -, e os intervalos de 8 horas. Toda a amostra diluições e controle serão armazenadas a 25,8 ^{o}C durante o teste. Todas as leituras de pH serão realizadas em triplicata.

1.4.9 Análise de Viscosidade

Para tal propósito será usado o viscosímetro. Serão utilizadas três amostras em datas diferentes, as quais serão submetidas com a utilização de *Spindle* 4, a um aumento crescente das velocidades e posteriormente será feita a mesma avaliação para velocidades decrescentes. Os fixadores de dentaduras comerciais serão utilizados como padrão para comparação.

1.4.10 Análise de Retenção e Adesividade

A medição será realizada entre amostras de resina acrílica. Os fixadores comercializados serão utilizados como controle. Será utilizado o aparelho de

teste universal. Serão confeccionados cilindros de resina acrílica de 25mm de diâmetro e 55mm de altura.

O teste será realizado pela aplicação de 0,3 g de adesivo na superfície polida dos cilindros de resina. Em seguida, mais de 2 kg será aplicado ao cilindro de resina acrílica durante 15 s para garantir uma força de aplicação coerente. O peso será retirado por 30 s. Por fim, os conjuntos serão separados. As forças necessárias para puxar os cilindros de resina será medido e gravado. Cada produto será testado sete vezes, sendo a média e o desvio padrão calculados. Os testes serão realizados em duas diferentes conjuntos de condições experimentais: (1), com uma cruzeta de teste de velocidade de 1 mm / min e (2) com uma tensão aumentar a força de 20 g / s.

1.4.11 Analise de Citotoxidade

1.4.11.1 Técnica de cultivo celular

Este ensaio utilizará como modelo experimental a linhagem celular de fibroblastos de camundongos (NIH/3T3). O protocolo de pesquisa será submetido ao Comitê de Ética em Pesquisa da Faculdade de Odontologia da Universidade Federal de Pelotas (FOUFPel).

Os procedimentos de manutenção da linhagem celular serão realizados em capela de fluxo laminar, seguindo os protocolos para a manutenção de esterilidade dos materiais, suplementos e meios de cultivo utilizados. As células serão mantidas em garrafas de cultivo celular com DMEM (Meio de Eagle Modificado por Dulbecco) e SFB (Soro Fetal Bovino) em estufa de CO_2 a 37ºC. O crescimento celular será monitorado diariamente em microscópio invertido de contraste de fase, e o meio de cultivo trocado a cada 2 ou 3 dias, de acordo com o metabolismo celular.

As células serão subcultivadas após ocuparem no mínimo 80% da área cultivável do frasco, o que se denomina subconfluência. Para o subcultivo, o meio de cultura do frasco será removido, reservado em um tubo de centrifugação, e a monocamada celular será lavada uma vez com solução tampão fosfato-salina, sem cálcio, nem magnésio (PBS). Em seguida, as células serão separadas com 2 ml de solução de tripsina (Sigma) a 0,25% com ácido etilenodiaminotetracético 1mM (EDTA) durante 5 minutos a 37ºC. A

tripsina será inativada com o meio de cultivo contendo SFB reservado anteriormente e as células em suspensão transferidas para o tubo de centrifugação e centrifugadas a 1500 rpm durante 5 minutos, à temperatura ambiente. Após a aspiração do sobrenadante, o precipitado de células será suspenso em 1 ml de meio de cultura. Alíquotas dessa suspensão de células serão distribuídas em frascos de 25 cm^2 contendo 5 ml de meio de cultivo. Os frascos serão mantidos em estufa à temperatura de 37ºC e atmosfera úmida contendo 5% de CO_2. Cada procedimento de subcultura dará origem a uma nova passagem da linhagem celular.

1.4.11.2 Contagem Celular

Será determinado o número de células existentes nos frascos de cultivo. A finalidade dessa contagem é conhecer o número de células para uma divisão igualitária de células por grupo. O número de células semeadas em cada poço será de 2×10^4.

Para a determinação do número de células (contagem celular), elas serão lavadas duas vezes em PBS e suspensas do fundo do frasco utilizando solução de tripsina a 0,25% em PBS e 1% de EDTA. O conteúdo de cada garrafa será removido, colocado em tubo de ensaio contendo 5 ml de DMEM e centrifugado, para a inibição da ação da tripsina. O sobrenadante dos tubos será descartado e os precipitados suspensos em 1 ml de DMEM. Dessa suspensão celular 20 µl serão dispensados em um tubo de ensaio com mais 20 µl de azul de Trypan a 0,4%. Fora do fluxo laminar, uma gota dessa mistura será colocada na câmara de Neubauer (ou hematocitômetro) e levada ao microscópio invertido de fase para realização da contagem do número de células. As células coradas em azul representarão células mortas, enquanto as células não coradas, células viáveis. Serão contados os 4 quadrantes das extremidades. O cálculo será obtido pela fórmula onde o número total de células viáveis contadas será multiplicado por 10^4 (pelo volume contado que é 0,1 mm^3 ou 10^4 ml. Esse valor será dividido pelo número de quadrados contados (nesse caso 4) e multiplicado pelo fator de diluição que é dois. A partir dessa fórmula obteremos a quantidade aproximada de células presentes em cada frasco.De acordo com a quantidade de células existentes será

adicionado DMEM suficiente a essa suspensão para obter-se a quantidade desejada de células por volume.

1.4.11.3 **Preparação das Diluições**

Serão confeccionados 10 diluições em DMEM, para cada grupo experimental.

1.4.11.4 Teste de viabilidade celular (MTT)

A suspensão das células será plaqueada em uma concentração de 2 x 10^4 células por poço e distribuídas em uma placa de cultura celular (ELISA) de 96 poços. Cada poço receberá 200 µl de DMEM completo. A placa será então incubada a 37ºC, em ar a 5% CO_2, por 24 horas. Após este período o meio de cultura será removido dos poços e volumes iguais (200 µl) do material experimental serão adicionados em cada poço. Nos poços controles, 200 µl de DMEM serão adicionados. Após a remoção das substancias testes, 200 µl de PBS e 20 µl de MTT (sal tetrazolium [3-(4,5-dimetltiazol-2-yl)-2,5-difeniltetrazolium brometo] será adicionado em cada poço. A placa será incubada sem luminosidade por 24 horas a 37ºC. Então o MTT será aspirado e 200 µl de dimetilsulfóxido (DMSO) será adicionado a cada poço. Subsequentemente, a absorbância a 570 nm foi medida usando um espectrofotômetro e os resultados analisados estatisticamente

1.4.12 Análise das Propriedades Antimicrobianas

1.4.13 Substancias com Potencial antimicrobiano

As substâncias que serão incorporadas nos fixadores de dentadura comerciais para os testes antimicrobianos serão: (1) Derivados de Pirazóis; (2) Nistatina.

Quadro 3. Grupos utilizados nas metodologias envolvidas na Analise das Propriedades Antimicrobianas dos Fixadores de Dentadura Comerciais

Fixadores de Dentaduras Comerciais			
Tipo Pasta (Creme)		Tipo Fita Adesiva	
GAP1 Algasiv®	N=20	GAF1 Algasiv®	N=20
GAP2 Algasiv®+Nistatina	N=20	GAF2 Algasiv®+Nistatina	N=20
GAP3 Algasiv®+Pirazol	N=20	GAF3 Algasiv®+Pirazol	N=20

GPP1 Protefix®	N=20	GPF1 Protefix®	N=20
GPP2 Protefix®+Nistatina	N=20	GPF2 Protefix®+Nistatina	N=20
GPP3 Protefix®+Pirazol	N=20	GPF3 Protefix®+Pirazol	N=20
GFP1 Fixodent®	N=20	-	-
GFP2 Fixodent®+Nistatina	N=20	-	-
GFP3 Fixodent®+Pirazol	N=20	-	-
GCP1 Corega®	N=20	GCF1 Corega®	N=20
GCP2 Corega®+Nistatina	N=20	GCF2 Corega®+Nistatina	N=20
GCP3 Corega®+Pirazol	N=20	GCF3 Corega®+Pirazol	N=20

1.4.14 Teste de Microdiluição

1.4.14.1 Microrganismos

Os microrganismos utilizados para este projeto serão provenientes do Laboratório de Microbiologia da Faculdade de Odontologia da Universidade Federal de Pelotas.

1.4.14.2 Drogas e Diluição

Os compostos serão dissolvido para teste em álcool etílico 70% e DMSO. Os compostos serão previamente pesados e dissolvidos em 500µg/ml do solvente proposto. A partir da solução mãe, resultante da série, serão preparadas diluições resultando em concentrações seriadas para todos os compostos. As concentrações dos produtos testados terá uma variação de concentração de 0,49 a 250µg/ml.

1.4.14.3 Determinação da CIM (Concentração Inibitória Minima)

O antifungigrama com os compostos serão realizado através da técnica de Microdiluição em Caldo (MC) de acordo com o documento de referência M27A3 (CLSI, 2008), adaptado para o novo composto.

O teste de susceptibilidade a antifúngicos pelo método de MC será realizado em placas plásticas de microtitulação estéreis (Nuclon®[1]) com 96

poços de fundo chato, constituídos em oito séries identificadas de A a H, cada qual com doze poços.

Em cada poço será adicionado 100µl de produto preparado mais 100µl de preparo do inóculo. Para a elaboração do produto será feita a diluição de 20µl de produtos, obedecendo às dez concentrações, em 1980µl de RPMI. As placas serão colocadas em uma estufa a 36 º, e lidas às 24 e 48h, seguindo o protocolo M-27A3 (CLSI, 2008).

Os poços pertencentes à coluna com o número 11 em cada placa serão utilizados como controle positivo, possuindo apenas 100µl de meio de cultivo RPMI/MOPS e 100µl da solução inóculo. Os poços de número 12 serão considerados os controles negativos, contendo apenas 200µl do meio de cultivo líquido RPMI/MOPS. As placas serão incubadas a 37℃ em estufa por até 72 horas.

Para leitura do teste, será realizada comparação visual do crescimento da levedura ocorrido nos poços referentes às diferentes concentrações testadas (poços de 1 a 10) com o seu crescimento no poço-controle positivo. A menor concentração capaz de produzir proeminente inibição (50%) do crescimento da levedura em relação ao poço controle-positivo será identificada como a CIM (Concentração Inibitória Mínima) do fármaco para esta amostra.

1.4.15 **Definição das materiais primas para formulação do novo Fixador de Dentadura**

As matérias primas utilizadas para a formulação de um novo fixador de dentadura serão provenientes da etapa 4.1 e 4.2 Além dessas materiais primas já utilizadas nos fixadores de dentadura comercializados serão feitas substituições dos espessantes por gomas naturais (ex: Goma Xantana e Goma Karaya), vaselina e fibras naturais.

1.4.37. Avaliação do desempenho mecânico e citoxidade do novo Fixador de Dentadura

A partir da etapa 4.2 serão feitas varias formulações e serão testados mecanicamente essas formulações comparando-se com os resultados obtidos

para os fixadores comerciais. Serão executados os testes, já descritos anteriormente, de Ph, viscosidade, adesividade e retenção e citoxidade.

Quadro 3. Grupos utilizados nas metodologias envolvidas na Avaliação do desempenho mecânico e citoxidade do fixador de dentadura experimental	
Tipo Pasta (Creme) N=20	Tipo Fita Adesiva N=20
GCP1 Corega®	GCF1 Corega®
GCP2 Corega®+Nistatina	GCF2 Corega®+Nistatina
GCP3 Corega®+Pirazol	GCF3 Corega®+Pirazol
GEP1 Fixador experimental	GEF1 Fixador experimental
GEP2 Fixador experimental + Nistatina	GEF2 Fixador experimental + Nistatina
GEP3 Fixador experimental + Pirazol	GEF3 Fixador experimental + Pirazol

1.4.38 Avaliação do desempenho biológico do novo Fixador de Dentadura

As formulações confeccionadas e testadas com resultados promissores serão avaliadas com relação ao desempenho biológico. Serão realizados os testes (já descritos anteriormente) de potencial antifúngico, inibição da atividade enzimática, ação antimicrobiana em superfície de resina acrílica e ação antimicrobiana em superfície celular.

Quadro 4. Grupos utilizados nas metodologias envolvidas na Avaliação do desempenho biológico do fixador de dentadura experimental	
Tipo Pasta (Creme) N=20	Tipo Fita Adesiva N=20
GCP1 Corega®	GCF1 Corega®
GCP2 Corega®+Nistatina	GCF2 Corega®+Nistatina
GCP3 Corega®+Pirazol	GCF3 Corega®+Pirazol
GEP1 Fixador experimental	GEF1 Fixador experimental
GEP2 Fixador experimental + Nistatina	GEF2 Fixador experimental + Nistatina
GEP3 Fixador experimental + Pirazol	GEF3 Fixador experimental + Pirazol

1.5 Orçamento

Os materiais e equipamentos a serem utilizados neste trabalho estão detalhados na tabela abaixo:

Tabela 3 – Materiais de Custeio

Produto	Fabricante	Quantidade	Preço Unitário	Preço Total
Agar Sabouraud Dextrose	DIFICO	1	R$ 257,00	R$ 257,00
Álcool Etílico 70%	Archote	5	R$ 4,29	R$ 21,45
Azul de Trypan	Vetec	1	R$ 85,00	R$ 85,00
Agar Egg Youlk	Laborclin	5	R$ 47,30	R$ 236,50
RPMI	Vetec	1	R$ 240,00	R$ 240,00
Solução Salina (NaCl)	ADV	5	R$ 1,95	R$ 9,75
MOPS	GBICO	1	R$ 277,10	R$ 277,10
Eppendorf	Analitica	1 pcte	R$ 100,00	R$ 100,00
Filtro 22mm	Millex	1	R$ 233,20	R$ 233,20
Placas Petri 60X15	Pontovet	100	R$ 27,00	R$ 135,00
Placas Petri 90X15	Pontovet	100	R$ 3,92	R$ 39,20
Placas de Microtitulação 96 poços	LF Equipamentos	500	R$ 847	R$ 1694,00
Fixador Protefix Pó	Queisser Pharma	30g	R$ 33,32	R$ 333,20
Fixador Protefix Pasta	Queisser Pharma	30g	R$ 24,63	R$ 246,30
Fixador Protefix Fita Adesiva	Queisser Pharma	30u	R$ 35,15	R$ 70,30
Fixador Corega Pó	Glaxosmithkline OTC	50g	R$ 30,97	R$ 185,82
Fixador Corega Pasta	Glaxosmithkline OTC	40g	R$ 40,55	R$ 304,12
Fixador Corega fita adesiva	Glaxosmithkline OTC	20u	R$ 29,27	R$ 87,81
Fixador Fixodent Pasta	Procter e Gamble	68g	R$ 55,90	R$ 246,61
Fixador Algasiv Pasta	Combe	30g	R$ 38,22	R$ 382,20
Fixador Algasiv Fita Adesiva	Combe	12u	R$ 32,71	R$ 163,65
Éter de Petroléo	Quimibras	500ml	R$ 16,20	R$ 16,20
Tetrahidrofurano	Quimibras	1000ml	R$ 54,80	R$ 54,80

Polimero Resina Acrilica	Jet	220g	R$ 26,58	R$ 53,16
Monomero Resina Acrilica	Jet	120ml	R$ 11,91	R$ 23,82
XTT	Fluka	1g	R$ 743,42	R$ 743,42
Clorexidina	Sigma	100ml	R$ 477,00	R$ 477,00
Nistatina	Sigma	1g	R$ 556,78	R$ 556,78
Vaselina Sólida	Sigma	1Kg	R$ 110,00	R$ 110,00
Goma Xantana	GastronomyLab	1Kg	R$ 227,90	R$ 227,90
Goma Karaya	Premcem gums private limited	1kg	R$ 437,89	R$ 437,89
			Total:	R$ 8049,18

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1.7 Cronograma

	2012								2013											
	Mai	**Jun**	**Jul**	**Ago**	**Set**	**Out**	**Nov**	**Dez**	**Jan**	**Fev**	**Mar**	**Abr**	**Maio**	**Jun**	**Jul**	**Ago**	**Set**	**Out**	**Nov**	**Dez**
Definição do tema	x																			
Analise do Problema		x	X																	
Qualificação Doutorado				x																
Revisão da Literatura e Tecnológica				x	x	x	x	x	X	X										
Artigo 1 – Revisão Literatura e Revisão Tecnológica									X	X										
Pesquisa de Preço e aquisição da matéria prima						X	x													
Desenvolvimento e Caracterização de Fixadores de Dentadura							X	X	X	X	X	X	X							
Análise da composição dos fixadores de dentadura comerciais									X	X	X	X								
Analise do Ph e Viscosidade dos fixadores comerciais											X	X	X							
Analise da Retenção e Adesividade dos fixadores comerciais													X	X	X					
Analise citotóxica e genotoxica dos fixadores comerciais															X	X	X			
Analise das propriedade antimicrobianas dos fixadores comerciais															x	x	X	x	x	X

	2014												2015			
	Jan	**Fev**	**Mar**	**Abr**	**Mai**	**Jun**	**Jul**	**Ago**	**Set**	**Out**	**Nov**	**Dez**	**Jan**	**Fev**	**Mar**	**Abr**
Artigo 2 - Comparação mecânica entre os fixadores de dentadura comerciais	**x**	**X**	**X**													
Artigo 3 – Comparação biológica e antimicrobiana entre os fixadores de dentadura comerciais		**X**	**X**	**X**												
Desenvolvimento do Novo Fixador de Dentaduras			**X**	**X**	**X**	**X**										
Desempenho Mecanico do Novo Fixador de Dentaduras						**X**	**X**	**X**								
Desempenho Citotoxico e genotoxico do Novo Fixador de Dentadura								**X**	**X**	**X**						
Desempenho antimicrobiano do novo Fixador de dentaduras									**X**	**X**	**X**					
Redação de Relatorios e Patente												**X**				
Artigo 4 – Desenvolvimento de um novo fixador de dentadura com propriedades melhoradas													**X**	**X**	**X**	
Artigo 5 – Efeito Antimicrobiano de um novo fixador de dentaduras														**X**	**X**	**X**
Defesa do Doutorado																**X**

2 Relatório do Trabalho de Campo

O projeto inicialmente submetido a qualificação junto ao Programa de Pós- Graduação em Odontologia consistiu no trabalho intitulado: “Desenvolvimento de sistema de entrega de fármacos através de filmes e adesivos para mucosa oral”. Foram aceitas as sugestões da banca de qualificação do projeto e as modificações foram realizadas.

Algumas modificações no cronograma e no delineamento do projeto foram necessárias durante o desenvolvimento do presente projeto de pesquisa.

As dificuldades na obtenção de reagentes e insumos importados além de métodos e processos que acabaram por inviabilizar o processo de desenvolvimento e caracterização de novos materiais fixadores de dentaduras. Assim as metas físicas referentes ao desenvolvimento de um novo produto, de um novo fixador de dentaduras com a utilização de gomas naturais, não puderam ser alcançadas neste primeiro momento. Neste contexto, a decisão do comitê de orientação foi utilizar os adesivos orais já comercializados (fixadores de dentaduras) e sobre os mesmos realizar modificação pela inclusão de um fármaco antifúngico experimental na composição desses materiais.

As metas referentes à obtenção e caracterização de substâncias com propriedade antifúngicas foram todas realizadas e alcançadas. Alguns testes foram modificados por causa dos reagentes necessários e tempo de execução. As metas físicas referentes a incorporação de substancias antifúngicas nos fixadores de dentaduras, também foram alcançadas.

Artigo 1 – Revisão Literatura e Revisão Tecnológica (*)

Title Page

Current trends and future perspectives in the development of denture adhesives: an overview based on technological monitoring process and systematic review

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Abstract

Denture adhesives are materials used to enhance denture retention, stability and function. The aim of this study was to systematically review the articles and patents with regard to denture adhesives in order to obtain a scientific and technological overview of this material. A literature search of studies published between 1960 and 2014 was conducted in seven databases: MedLine (PubMed), Web of Science, Lilacs, Ibecs, Cochrane Library, Scielo and Scopus. Additionally, the following patent databases were screened: USPTO, EPO, JPO, INPI, Derwent Innovations Index, Patentscope and Questel Orbit. Data was tabulated and analyzed by Microsoft Office Excel 2013 software and Questel Orbit. A total of 54 articles and 78 patents were included in the analysis. The largest number of patents (n=19) was deposited by Procter & Gamble Company (Cincinnati, Ohio, USA). Furthermore, the most prevalent studies were conducted *in vivo* (n=30), and the types of adhesive most studied were cream or powder (n=14). It was possible to identify the current scientific and technological scenario of denture adhesives, in which patents filed in many underdeveloped countries were mostly foreign-owned. Moreover, the recent advances of such materials have been more related to presentation than to the additional effects of denture adhesives.

Keywords*:* Dental prosthesis, Dentures, Denture adhesives, Dental materials, Systematic review.

Introduction

Denture adhesives are commercially available dental materials that have long been recognized as a useful adjunct to improve denture retention, stability and function [1-3]. The value of retention obtained with adhesives can be more than twice as high when compared with that of dentures used without these products [4]. Although some dentists are reluctant to indicate these materials, because it could imply that the dentures were incorrectly fabricated and present lack of retention and stability, the use of these maiomterials may be a therapeutic and efficient procedure for the preparation and subsequent use of total dental prosthesis [5-7]. Denture adhesives can be useful to perform clinical procedures that demand more stability of the denture base, such as at the time of obtaining maxillo-mandibular relationship and during setting up of the teeth. They can be used in patients with sensitive mucosa, acting as a cushion to reduce tissue irritation resulting from compression such as ulcers and inflammation. Moreover, they also help to increase the stability of temporary and/or immediate dentures [5, 8, 9]**.**

The American Dental Association first reported the use of denture adhesives in 1935, and the first patent related to this material was issued in the United States in 1913 [5, 6]. The materials used in the 19th century were a mixture of vegetable gums forming a material capable of absorbing moisture from saliva. After hydration, the mixture increased in volume, and became a viscous material that enabled the prosthesis to adhere to the buccal mucosa. Usually, their mechanism of action results from increased contact between the tissues and denture, which creates a retentive force between the oral mucosa and the prosthesis [10, 11, 3]. The main components in these materials are compounds responsible for adhesive properties (karaya gum, tragacanth, acacia, pectin, gelatin, methyl cellulose, polyethylene oxide, acrylamide, polyvinyl chloride), antimicrobial agents (sodium borate, hexachlorophene, methyl paraben), additives, wetting and plasticizing agents [5]. Nowadays these materials are available in formulations such as powders, pastes or creams for soluble adhesives, and strips or cushions for insoluble materials [10, 12, 13, 8, 14, 9] .

The use of methods capable of mapping scientific and technological developments may enable opportunities to be identified for current and future technological scenarios [15, 16]. According to the American Dental Industry, it is estimated that between 15% and 33% of patients with total prosthesis are using denture adhesives [17, 11]. There is a large market for this technological sector, and institutions

and companies should conduct research and development (R&D) activities in areas presenting market demand [18-21]. However, it is not easy to orient their strategies and use them to their own benefit [19, 22]. Technological information contained in patents can be a fundamental instrument for strategic planning, since they contain a detailed description of the invention, which is often not available in another document [18, 23, 24]. It is estimated that almost 80% of all technological information can be found only in patent publications [15, 25]. Technology management is a set of management disciplines that allows organizations to make a better use of their capital to maintain their competitive edge [26]. Patent data analysis can also be used to help to analyze industry trends, to identify business opportunities and encourage innovation [27, 28]. Other important applications include the possibility of conception of scientific-technological projects to obtain financing, the development of new materials, and establishment of partnerships between universities and companies [27].

In competitive business environments, patent intelligence, the transformation of content found in patents into technical, business, and legal insight, is getting attention as a tool to aid efforts to secure competitive advantages [21]. Furthermore, the analysis of scientific and technological information can allow the identification of opportunities for new product, process or device development [29]. Thus, the aim of this study was to systematically review articles and patents related to denture adhesives in order to use as a strategic tool for prospecting in this technological sector.

Methods

Data sources and search strategy

This systematic review was performed according to the PRISMA statement [30]. The search strategy included the following seven databases: MedLine (PubMed), Web of Science, Lilacs, Ibecs, Cochrane Library, Scielo and Scopus. In addition, the search and analysis of patent applications was conducted by the online system Questel Orbit (Paris, France), which contains patent data on over 90 authorities. The following addition patent databases were screened: USPTO (United States Patents and Trademark Office), EPO (European Patent Office), JPO (Japan Patent Office), INPI (National Institute of Intellectual Property of Brazil), Derwent Innovations Index and Patentscope. The descriptors used in the fields “Title” and “Abstract” were used in the search

strategy described in Table 1. The references cited in the papers included were also checked; and the cited articles were also tracked using SCOPUS citation tools.

Furthermore, a patent search was also made using International Patent Classification (IPC) with the following codes: A61K-6/00 (preparations to dentistry), A61C-8/00 (means to fix dentures), A61C-13/00 (dentures), A61C-13/23 (adhesive films). Just as in most applications, each patent may submit more than one CIP. This technology and its applications are related to different areas of science and technology, such as dentistry, chemistry or pharmacology, and it can interfere with them. The aim of identifying these codes is to create a specific tool for search and retrieval of documents.

Afterwards the duplicates were removed using the program EndNote x7 (Thompson Reuters, Philadelphia, PA, USA). Reviewers selected documents of interest independently after reading the title and abstract. The eligibility criteria consisted of selecting only patents and studies related to denture adhesives published between January 1960 and April 2014. English, Spanish and Portuguese documents were screened. Literature reviews and patents related to dental adhesives with applications differing from assisting the retention and stability of dentures were not included.

Data extraction

The title and abstract of each article identified were screened by two independent reviewers (WLOR and CHR) to determine whether the article and patent should be further considered for inclusion. After the screening, the articles included were read in full in order to extract the relevant data. Two reviewers, who were not blinded to the publication authors or inventors, independently performed the data extractions. Disagreements were resolved by discussion to reach a consensus, or through a third reviewer (EP).

Data were extracted by the Microsoft Office Excel 2013 software program (Microsoft Corporation, Redmond, Washington, USA) using a standardized form. Reviewers tabulated the data of interest to compose a spreadsheet in Excel format with all the trial documents containing: the title of patents and articles, the applicant or author's names, the inventor's name, the priority date of patents, the publication date of articles, the document status, the International Patent Classification, the types of denture adhesives used, priority countries and the type of study conducted.

Data analysis

Scientific and technological information was analyzed by the Microsoft Office Excel 2013 software program (Microsoft Corporation, Redmond, Washington, USA). In addition, the on-line system Questel Orbit (Paris, France) was used to analyze technological data obtained from patents. Two reviewers (WLOR e CHR), who received training in these software programs, conducted the analysis independently.

Results

The search initially retrieved 156 patents, with 59 being excluded after reading the title and abstract (Figure 1). Out of 97 patents selected, 15 were excluded because they did not fit the eligibility criteria, since they were not related to denture adhesives. A total of 82 patents were included in the analysis. Moreover, in the search of scientific articles, 9,494 were identified initially. After removing the duplicates and reading the title and abstract, 81 articles remained and 9 were excluded (4 involved a literature review and 5 were not related to denture adhesives). A total of 72 articles fulfilled all criteria and were included in the analysis.

Out of the studies included, 56% were *in vivo* (clinical trials or animal experimentations). There was more presence of the combined or individual use of the type cream/paste and powder (Figure 2). As regards priority patents, 63% and 33% of published articles were from the United States, the country with the most patents deposited in the sector (Figure 3). The American company Procter & Gamble (Cincinnati, Ohio, USA) had the largest number of patents related to denture adhesives (Figure 4). Moreover, there was a remarkable increase in scientific and technological production in the 1990s, as demonstrated in Figure 5.

Discussion

This technological monitoring allowed the knowledge available in the denture adhesive sector to be mapped, and it was possible to obtain a scientific and technological overview of these materials. Furthermore, this type of analysis can allow technological prospecting by institutions and companies, which is imperative for scientific and technological innovation [19, 31-33]. As demonstrated in Figure 5, there was a gradual increase in studies related to denture adhesives, with a predominance of patents deposited in the 1990s. Considering that the first patent related to the material

dates back to 1913 [5, 6]**,** it seems that the interest in the market has intensified over the past 25 years. Furthermore, in the denture adhesive field there is a larger body of technological than scientific information available, which means that only the search and analysis of articles might show a different and limited overview of this technology.

An ideal denture adhesive is described in the literature as being nontoxic, nonirritating, biocompatible with the oral mucosa and not promoting microbial growth. Furthermore, the product should be odorless, tasteless, and easy to apply and to remove from the tissue-bearing surface of dentures [5, 6]. In an endeavor to develop products that satisfied these criteria, the composition of denture adhesives continues to change as the manufacturers try to improve the efficacy of their products. In the 1970s calcium salts were added, and in the 1980s the effectiveness of denture adhesives was improved by adding zinc to the previous formulations. Active ingredients in current formulations can include combined polymethyl vinyl ether-maleic anhydride (PVM-MA) zinc, which are high molecular weight copolymers with adhesive and cohesive properties, and calcium salts with carboxymethylcellulose, a viscosity modifier [34-36].

Denture adhesives that contain zinc were recently withdrawn from the market by companies in some countries (i.e. Super Poligrip “Original”; Super Poligrip “Ultra Fresh"; Super Poligrip “Extra Care”, GlaxoSmithKline, London, UK). The presentation in powder form could be more dangerous to patients, since it would be more easily aspirated and could jeopardize the denture wearer’s health. Chronic, excessive ingestion of zinc may result in copper deficiency, which is an established and increasingly recognized cause of neurologic disease [37]. It also may cause bone marrow suppression and polyneuropathy that can result in numbness and paresthesia of the extremities, loss of balance, and walking problems [37, 38]. The current trend is the development of zinc-free products (i.e., Super Poligrip “Free”, GlaxoSmithKline, London, UK), in order to try to avoid the side effects of this compound [38].

In the future, the use of additives such as antimicrobial or antifungal agents could gain popularity, especially for use in edentulous patients who are susceptible to diseases such as candidiasis [14]. Strategically, the material can be used as a delivery system for various types of medicines, containing components to help to reduce irritation and inflammation of the oral mucosa adjacent to the denture. Furthermore, a current challenge is the development of denture adhesives with properties that do not allow the adhesion of food or even bacterial plaque, so that they can be used for longer

periods of time without the necessity of daily exchanges. Moreover, it was found that the manufacturers of current materials have not yet found a way to deal with the challenge of maintaining their properties for longer periods without jeopardizing their odorless and tasteless qualities, or their inhibition of bacterial growth.

According to the American Dental Industry, between 15% and 33% of patients with dentures are users of these adhesives [17]. In the USA alone, the use of complete dentures was projected to increase to 61.0 million in 2020 [17]. With regard to the technological development of the sector, the majority of scientific articles (33%) and patent documents (63% of priority) came from the United States. Furthermore, according to National Survey of Oral Health (Projeto SB Brasil, 2010), almost 25% of Brazilian population wear dentures. There is a large market value for the development of these materials, which is not being properly exploited by many countries. For example, Brazil showed no patent or article published in this sector. The patents filed in these underdeveloped countries are mostly foreign-owned. Moreover, countries can use patented technologies to obtain competitive advantages, since the strategic use of intellectual property has become a vital tool of corporate strategy in the global economy [27]. The country with the highest number of deposited patents related to denture adhesives was the United States (Figure 3). This information can be of great value to companies and research centers that aim to protect new adhesives, since it allows them to identify important markets for this technology [39].

Furthermore, cooperation can be decisive for companies or institutions that need to use limited resources in the most efficient way [27, 31, 32]. The analysis of the main companies that have worked on developing this material (Figure 4) indicates that Procter & Gamble (Cincinnati, Ohio, USA) and GlaxoSmithKline (London, UK) had the highest number of patent deposits observed, with 18 and 13 respectively. This type of information can be useful to universities, research centers or independent researchers seeking partnerships to develop new adhesives. The benefits of this type of cooperation includes the division of costs for developing new products; shortened lead times; and that each company can contribute with its own competence [27, 39].

The patent document is an essential source of information for technological analysis, considering the wide variety of content available only in this type of document [40, 41]. However, there are limitations to using patent data as an indicator of technological development: not all inventions meet the patentability standards and

inventors can rely on secrecy or other appropriate means to protect their inventions [19, 42]. Furthermore, there is a time lag of at least 18 months between the first patent filing and the patent publication [19]. Because of this, the most recent patents included in this study were deposited up to October 2012. Moreover, each patent office uses a different tool that allows the recovery of documents, which makes it very difficult to collect and find interesting information [18, 39]. Therefore, there is a need for obtaining licenses to software programs that facilitate technological monitoring by institutions and companies, as Questel Orbit (Paris, France) or VantagePoint (Search Technology, Inc., Norcross, GA, USA).

From this review it was possible to obtain a scientific and technological overview of the field of denture adhesives. By combining and analyzing scientific and technological information, the design of this study can provide strategic information to drive new projects and consider changes in legislation or public policies to encourage support for the scientific and technological development. Furthermore, the development of new denture adhesives has a great international market potential that may reflect in an increase in R&D activities and patent applications in this area.

Conclusions

Based on this systematic review and technological monitoring it was possible to obtain a scientific-technological overview of denture adhesives. It was found that health safety requirements and regulamentation issues may represent a favorable scenario for new R&D projects, especially in many underdeveloped countries.

Competing interests

The authors declare that they have no competing interests.

Author's contributions

WLOR searched the literature, contributed in data extraction and analysis, and wrote the initial draft of manuscript; SDGO have been involved in drafting the manuscript and revising it critically for important intellectual content; CHR searched the literature, contributed in data extraction and analysis; AFS have been involved in drafting the manuscript and revising it critically; RGL revised the manuscript critically

and finished the final version; EP have been involved in drafting the manuscript and revising it critically. All authors read and approved the final manuscript.

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Figures and tables

Table 1. Search strategy in PubMed (*MedLine*)

Search	Terms
#4	Search #1 AND #2 AND #3
#3	Search Dental Materials OR Materials, Dental OR Dental Material OR Material, Dental OR Oral medicine OR Stomatology OR Medicine, Oral OR Dentistry OR Odontology
#2	Search (Dental prosthesis retention OR retention, dental prosthesis OR prosthesis retention, dental OR denture detention OR retention, denture OR denture stability OR stability, denture OR fixative denture OR denture, fixative OR dental prosthesis fixative OR fixative, dental prosthesis OR adhesive denture OR denture, adhesive OR dental prosthesis adhesive OR adhesive, dental prosthesis[Title/Abstract])
#1	Search (Dental Prosthesis OR Prostheses, Dental OR Dental Prostheses OR Prosthesis, Dental OR Dentures[Title/Abstract])

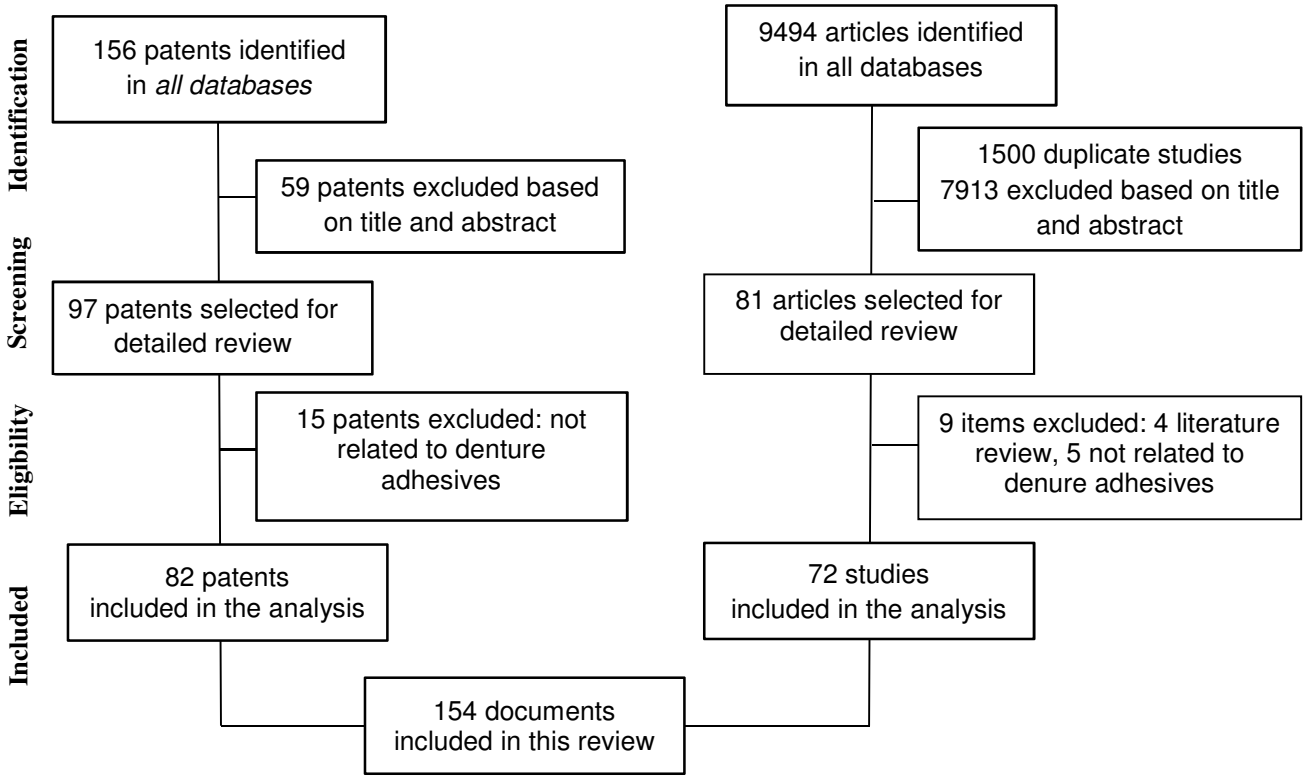

FIGURE 1: Flowcharts of the selection process of patents and articles according Prisma Statement [29]

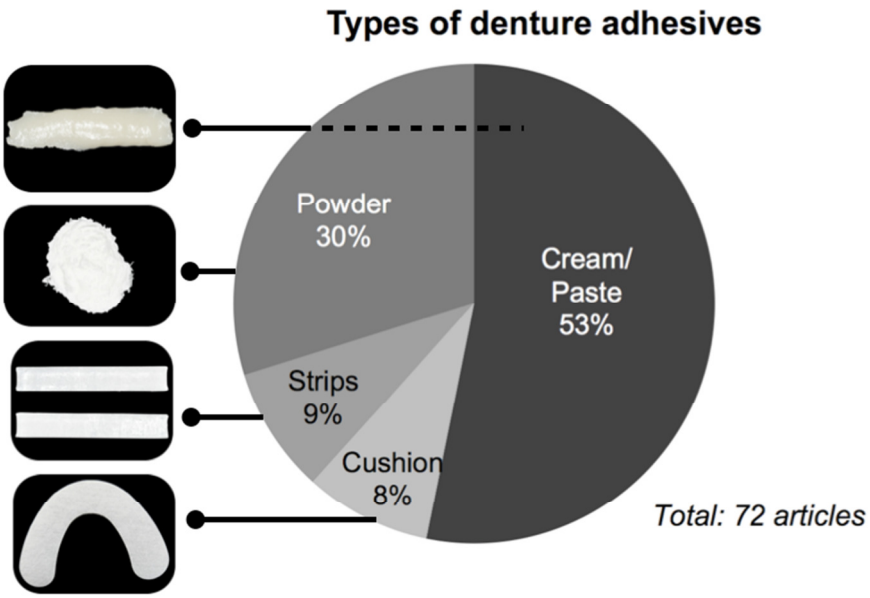

FIGURE 2: Types of denture adhesives tested according to the selected articles

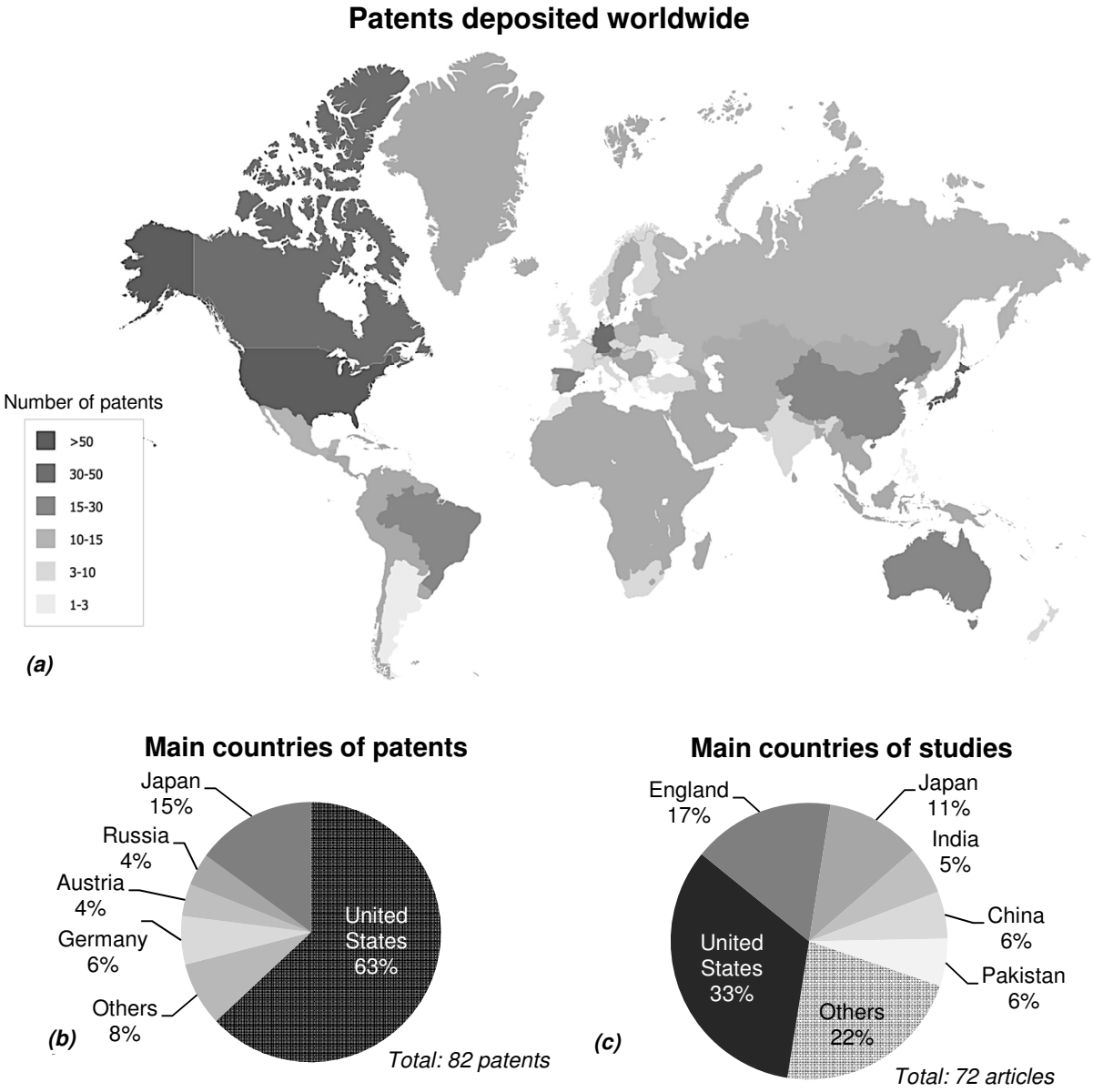

FIGURE 3: Scientific and technological production of denture adhesives in the world: (a) patents deposited worldwide; (b) main countries of patents deposited and (c) published papers related do denture adhesives (1960-2014).

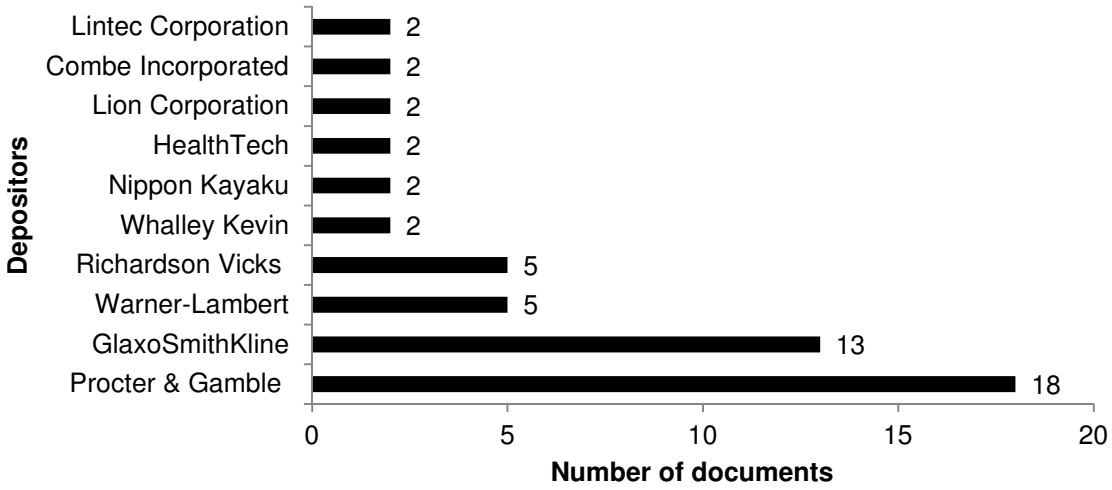

FIGURE 4: Main institutions and companies with patents deposited, related to denture adhesives (1960-2014).

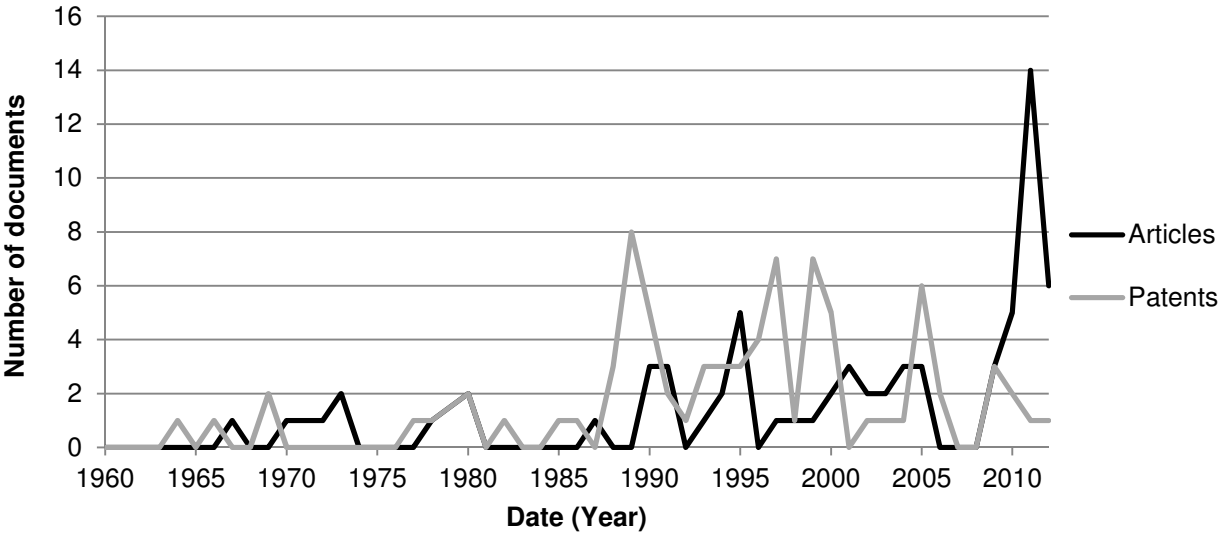

FIGURE 5: Number of patent applications deposited and papers published related to denture adhesives (1960-2012).

Artigo 2 - Avaliação de uma nova metodologia para o teste de adesividade de fixadores de dentadura (*)

Title Page

A new method for evaluation of retention effectiveness of dentures adhesives

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Abstract

One of the predisposing factors for the success of oral rehabilitation with conventional dentures is withheld prosthetic devices providing comfort and security to its users. The denture adhesives are products traded on a large scale that promise an additional adherence to conventional dentures. In general, three types of commercial form, powder, cream and tape, act as means of absorbing liquids and thereby increase its stickiness and provide better peripheral seal and cohesiveness between the prosthesis and mucosa. Many studies have been conducted to evaluate the adhesion and efficiency of these denture adhesives, but it is clear the lack of standardization and adaptation of methodologies used. The objective of this study was to establish evaluate and validate a test method to determine the effectiveness of this bond strength. The method was defined using an experimental design with fixed and variable factors. The established test method showed that the environment in which the test is performed, artificial saliva, water and natural saliva and the method used, dipping or wetting, was decisive for the outcome of the denture adhesive. Thus, it is essential that researchers analyze their methodologies before predicting and say which product is more or less efficient, avoiding misconceptions about them. You need standardization of a stable methodology, accurate, robust and replicable.

Keywords*:* Bond strength; fixative denture; adhesive denture.

Introduction

The prevalence of complete tooth loss has decreased over the last decade, but tooth loss remains a major disease worldwide, especially among older adults [1]. Currently the use of implants are several suitable alternatives can be explored for oral rehabilitation of these patients, however conventional dentures are the most important treatment option, mainly in developing countries, such as Brazil, due to affordability of treatment [2]. Among the objectives to be achieved when making a conventional denture, retention and stability issues of great interest on the part of its, since they are directly related to your comfort and safety [3]. Regarding this retention and stability, it is known that the use of a suitable technique for making a prosthesis can not be a predisposing factor isolated from patient satisfaction [4]. The denture adhesives have been recognized by denture wearers, as an auxiliary agent retention, stability and function of your dentures. The use of adhesives can optimize the retention of dentures increasing the adhesive properties, cohesive and viscosity between the prosthesis and the oral mucosa, eliminating gaps between them [5]. Moreover, the adhesives may also be indicated for patients with low saliva secretion [6], poor muscle tone, neurological deficiencies, or those who have undergone surgical trauma modifications of ridges [7].

Currently, considering the widespread use [1,8] and the wide dissemination of commercial denture adhesives is great interest to investigate factors related to its use linked to retention of ownership and / or adhesion of complete dentures. Studies have shown different retention values according to the commercial presentation: powder, cream or tape. Generally, the tape is seen as more retentive, then the form of paste and finally the dust [9, 10, 11, 12].

Fact is that there are no rules that establish the minimum quality of denture adhesives, contributing to the lack of a validated test method and therefore the lack of reference membership values. Thus, the objective was to establish evaluate and validate a reliable and reproductible test method capable of to measure and to compare the retention effectiveness of adhesive dentures. The hypothesis to be tested is that measuring the adhesion strength of total denture adhesives against different variables.

Material and methods

Three different forms of adhesive dentures, powder, tape and cream were used in present study (Table 1). These were tested with distilled water, natural saliva and artificial saliva. The formulation of the artificial saliva is described by Wong & Sissons [13].

Two cylindrical apparatus of acrylic resin with flattened surfaces were building up in hourglass shape and cutted in middle to assure the good contact between the two half where adhesive dentures were applied before test. Aparatus were attached in a mechanical test machine (EMIC, São José dos Pinhais, Brazil) with load cell of 100N and crosshead speed of 1,0 mm/min.

Several factors may affect the determination of bond strength of denture fixatives. Based on the literature and preliminary tests the most relevant factors were selected, which were divided into two groups, trade dress and medium, and in the latter the most striking factors in the results were concentrated. Table 2 details the factors and specific levels.

The measurement of adhesion was carried out in three stages, for each time were repeated twenty test and each of these times was done in a couple of acrylic resin specimens. For this purpose, we used the universal testing machine. The acrylic resin

cylinders were made so as to be stable in the universal testing machine and had 25mm in diameter and 55mm in height. The test was performed by applying 0.3 g of adhesive on the polished surface of the resin cylinders. Then 2 kgF was applied to the cylinder of acrylic resin for 15 s to ensure consistent application of force. The weight was withdrawn for 30 sec. Finally, the sets were separated. The forces required to pull the resin cylinders were measured and recorded.

For the test called "wetting" the fixer was weighed and placed in the polished surface of the test bodies and pre-wetted with 0.5 ml of distilled water or artificial saliva or natural saliva. This amount of liquid was measured by automatic pipette previously calibrated. For the test called "soaking" the set of specimens over denture fixative was immersed in a device coupled to testing machine with 20 ml of artificial saliva so that the whole stay submerged.

Statistical comparisons were performed using SigmaPlot 12.0 software (Systat Software Inc, San Jose, CA, USA INC). Comparisons between groups for tensile bond strength was performed using Kruskal Wallis at $p<0.05$.

Results and Discussion

The resulting variability of the interaction between the factors with the greatest influence in determining the adhesion strength was analyzed in pairs, in order to facilitate the interpretation of results, which are presented in Table 3.

From the statistical analysis, some results should be discussed further in understanding the results of some scientific papers. The medium in which the test was carried out proved to be definitive for the results obtained.

When distilled water was used for carrying out the adhesion tests, the results showed that the tape had higher adhesion to the cream and the powder. The powder was the commercial presentation that was less retentive. Several studies using distilled water as the wetting medium have obtained similar results with the powder being the worst behavior of the material [9, 10, 11, 12]. But this worst adhesion of powder denture adhesives are debatable when there is no standardization of the methodology employed . A difference in the formulation of the products and the methodology used already put the powder as most effective immediately after application , compared to other types of denture fixatives, they would need 3 hours to achieve maximum levels of retention [11]. Note that the powder type denture adhesives tends to have you a faster elimination of the active ingredients by saliva , due to the absence of an oil based on its composition[11].

The dentures adhesives can be divided into insoluble and soluble, which vary in their composition. Within the insoluble group include the tape and soluble group include cream, paste and powder. The soluble group presents synthetic agents, which depend on the chemical properties of one or more active ingredients that increase its volume from 50% to 150% and become viscous and sticky in the presence of water or saliva, filling the spaces between the base of prosthesis and the supporting tissues. The active ingredients are a mixture of salts of polymers with different degrees of solubility in water, which are designed to produce short and long acting adhesive. The carboxymethylcellulose (CMC) and methyl cellulose the Polyvinylether (PVM-MA) are examples of short and long-acting salts, respectively. The CMC salts provide a strong initial retention, however due to its high solubility dissolve rapidly, losing its effectiveness within a short period. The PVM-MA salts, however, have a low solubility which takes longer to activate themselves but have a longer period of action.

Subsequently to improve their effectiveness was added calcium and zinc salts in its formulation. Despite the fact that the tape composition varies between trademarks, they all include essentially a manufactured blade impregnated with an active component water based. Examples of adhesive ingredients include sodium alginate or polymer of ethylene oxide, which become sticky when activated by the saliva, and the difference between them is the thickness of the blade [7]. Denture adhesives provide adhesion via carboxyl groups by electrovalent linkages, which increase the viscosity of the medium, through its ingredients such as polymethylvinylether (PVM-MA) a synthetic polymer, sodium carboxymethylcellulose and (CMC) ingredients of natural origin [14].

In our study, when it was used artificial saliva, the cream was the most adherent, followed by tape and, again, the powder had the poorest results. In vitro study evaluated the efficacy of denture adhesives, was observed greater retention when artificial saliva was used due to an increase in viscosity[12]. The adhesives in paste form had a greater retention than the adhesive powder when biasing forces are applied [12]. This can be attributed, the increased viscosity of the adhesive in paste form compared to powder adhesives [12,15]. In other studies, using different methodologies, the adhesive cream type showed the highest incisal bite force values in all groups when compared to other types of adhesives (dust and tape), showing a greater improvement in groups with clinically regular prostheses and bad [16].

When the natural saliva was used as a medium, the tape was demonstrated with superior adhesion, then the cream behaved similarly powder presentation. In general, the powder behaved with less adhesiveness in most test media with only exception immersion in artificial saliva. This can be attributed mainly to the rapid elimination of the active ingredients of the adhesive by saliva in the absence of an oil base in the composition which is present in the cream type adhesives, for example [10]. Have the

tape behaved in a superior manner in most tests. Adhesive tape type behave as a reliner material aids with poor retention and regular, with its ability to adapt in its thickness and maintain its new shape by the side of the fabric and the base of the prosthesis [18]. Denture adhesives tape improves retention , chewing ability and assigns confidence in social activities , especially in those with poor tissue support and those who reported poor retention of his old prosthesis [19]. In controversy , with the use of another methodology , studies indicate that denture adhesive tape was not acceptable for prostheses with good adaptation , due to the fact that increases the vertical dimension , and when small forces are applied in various areas produce an uneven distribution adhesives in the prosthesis [20].

If we compare the means only, we see that the results show a higher adhesion when natural saliva was used and a similar tack with the use of distilled water and artificial saliva. The retentive feature of the denture adhesive increases significantly when used in combination with saliva, assuming that the primary condition for the retention of dentures is the presence of saliva and in the absence thereof may negatively influence the retention of dentures [11] .

One detail to note when the artificial saliva was used in the form of immersion there was no statistical difference between commercial presentations. We believe that the immersion method is the most appropriate method for the adhesion method of denture fixatives. Moreover, this seems to be the most similar to what happens in the oral cavity. No this study in the literature used the immersion as a method for testing. Would a more interesting investigation of this method in order to have a more appropriate position and no mistake as the stickiness of the commercial applications of denture fixatives.

The adhesive mechanism of action, which depend on the combination of physical and chemical forces. Physical forces based on Stefan's principle state that the force required to separate two discs is directly proportional to viscosity of fluid between them. The adhesive increases the viscosity of saliva thus increases the force required to separate the prosthesis surface of the oral mucosa. Despite their properties, the use of adhesives is limited due to lack of knowledge on the part of the professional, and a negative attitude may reflect the lack of clinical experience. However, if used properly, the adhesive can be a good resource for the professional [21].

Conclusion

Within the limitations of this study, it can be concluded that the environment in which the tests of adhesiveness of denture fixatives are performed are essential to their results, and that researchers and clinicians should have parsimony to predict their results. You need to study more detailed this type of material, but for that there is the need for more homogeneous studies and more similar and standardized methodologies.

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Table 1. Formulation of different types of commercial denture adhesives

Commercial Presentations (Corega ®,GlaxoSmithKline, London, UK)	Formulation
Powder	Partial Sodium salt - Calcic of Poly (Metilviniléter / Maleic Acid 49.80%, 49.80% carboxymethylcellulose; mint flavor.
Cream	Sodium salts / calcium poly (Metilviniléter / maleic acid), carboxymethyl cellulose, mineral oil and petrolatum
Tape	Sodium carboxymethylcellulose, microcrystalline wax, polyethylene glycol and polyethylene glycol

Table 2. Factors and specific levels for the adhesive test of denture adhesives

	Distilled Water	Artificial Saliva *	Natural Saliva
Powder	X	X	X
Cream	X	X	X
Tape	X	X	X
* For artificial saliva was used the immersion method and wetting			

Table 3. Confidence interval for standard deviation level 5%

Confidence interval for standard deviation				
Medium	Commercial Presentations	Lower Limit	Standard Deviation	Upper Limit
Distilled Water	Powder	0,7	2,08	7,7
	Cream	1,3	2,37	9,1
	Tape	1,6	2,65	12,9
Artificial Saliva	Powder	1,3	1,68	8,7
	Cream	1,8	2,67	11,9
	Tape	0,6	2,44	8,9
Artificial Saliva with immersion	Powder	1,4	2,06	10
	Cream	0,9	2,00	9,5
	Tape	0,8	2,50	10,8
Natural Saliva	Powder	1,6	2,33	12,4
	Cream	2	2,17	9,6
	Tape	1,6	2,10	10,3

Artigo 3 – Avaliação dos efeitos do acréscimo de antifúngicos em fixadores de dentadura (*)

Artigo 3

Title page

Antifungal effect of Dentures adhesives modified by the addition of pirazolinicos derivatives

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Abstract

The aim of this study was to test the adhesion, cytotoxicity and effective inclusion of nystatin or an alternative antifungal derived from pyrazoles in different commercial presentations in front denture fixatives *Candida albicans*. Denture fixatives commercial formulations were used for inclusion of antifungal compound (pyrazole or nystatin) in three concentrations: 0.3%; 3% and 30%.The retention of 21 groups (n=60) denture fixatives was tested between acrylic resin apparatus attached in a universal testing machine. The antifungal activity was determined by the disk diffusion and direct contact method. Direct contact test was measured after 1, 4, 8 and 12 hours. The cytotoxic activity was evaluated on cell culture of mouse fibroblasts (NIH/3T3) using the colorimetric MTT reduction assay. The results showed that denture fixatives were not affected by inclusion of antifungals did not affect the adhesion of the . The disk diffusion test results and demonstrated that direct contact include nystatin and pyrazole in commercial denture fixatives promoted to these antifungal activity against *Candida albicans*. Denture adhesives formulated with pyrazol behaved similarly those formulated with nystatin .With respect to cytotoxicity, the highest concentration (3%) was the statistically higher cytotoxic effect ($p<0.05$), and subsequent low concentrations showed cytotoxicity. The dentures adhesives with pyrazole behaved like nystatin relative cytotoxicity at concentrations of 2 (3%) and 3 (0.3%) ($p> 0.05$). Within the limitations of the methodologies used, it is concluded that there is possibility of using denture fixatives as delivery system of trade antifungal (Nystatin) or not (pyrazole) and

the second concentration (3%) more efficient, which is independent of the presentation Commercial and less cytotoxic.

Keywords: Products with antimicrobial action; Denture Fixative; chronic atrophic candidiasis.

Introduction

The use of conventional dentures is still a quite usual alternative to oral rehabilitation in total edentulous [1]. The use of this type of rehabilitation devices is still quite prevalent in underdeveloped and developing countries. A primary factor for the successful rehabilitation of dentures is stability and retention of the same [2]. This sometimes independent of dentists and clinical steps for making the oral dentures [3,4]. Seen it, denture fixatives the would configure an option to increase the retention and stability of dentures and these are being marketed and commercially disseminated widely. [5] These denture adhesives have several commercial applications, powder, tape and cream, and its action is by absorption through the liquid viscosity and promoting adhesion [6].

Dentures adhesives produtcts have been prescribed based on functional advantages [5, 7] for total and psychological oral prostheses [8,9,10,11],. On the other hand, mismatches [12], bone resorption [12], increased vertical dimension [13] and biofilm accumulation are some of drawbacks pointed to the use of these types of denture adhesives. The biofilm accumulation brings the discussion of the possibility of the use of these fasteners as predisposing to common diseases users of oral dentures, such as chronic atrophic candidiasis [14, 15, 16, 17].

Recent decades have seen a significant increase in the incidence of all forms of candidiasis [18]. Candidiasis chronic atrophic is a fungal infection that affects up to 67% of users of dental prostheses [19]. This disease is mainly caused by *Candida*

albicans, but other species of Candida may be associated [20]. This is a multifactorial disease involving factors that exceed only the existence and presence of *Candida albicans* [21, 22]. However, the accumulation of fungal biofilm in a favorable environment, wet, rough and dark, greatly increase the chance of the establishment of this pathology. The treatment of chronic atrophic candidiasis is extensive and includes the use of topical antifungals, exchange of prostheses and oral hygiene [23, 24]. Immunocompromised patients, including those with HIV infection or cancer, are at enhanced risk of Candidiasis chronic atrophic [25]. In addition, of Candidiasis chronic atrophic can be triggered in healthy patients by transient risk factors such as antibiotic or corticosteroid treatment [26]. Oral candidiasis is the most common fungal infection in patients with AIDS and it usually indicates the progression of HIV infection [27]. Treatment of oral candidiasis in HIV-positive patients is difficult because of recurring episodes, intermittent exposure and continual selection of antifungal therapy-resistant strains. Candida carriage was reported common in cancer patients, with C. albicans being the predominant species in patients who undergo radiotherapy for Head and neck [28]. Oral colonization (up to 93%) and infection (up to 30%) are frequently noted in the patients [29]. The main reason is that the irradiation-induced histological changes leading to oral mucositis, together with salivary quantitative and qualitative changes, have been reported to facilitate yeast growth [28]. Beside that a possible explanation for the higher predisposition of irradiated patients to candidosis is due to reduced phagocytic activity of salivary granulocytes against these micro-organisms [30].

There is a visible increase in the incidence of oral candidiasis and it raise the concerns of the resistance of fungi front to antifungal agents currently available on the market. This have been attracted attention of research groups for search of new drug alternatives [23, 24] more effective, less toxic and cheaper [31] than traditional drugs.

One of these alternatives are the new pirazoles compounds, recently described for our research group that have shown action against these microorganisms, low cytotoxicity and high performance enabling its large-scale production [32].

There use of antifungical compounds in fixative dentures are still unexplored on literature. This potential antifungal could be beneficial to as they confirm the increased chance of oral fungal infections with the use of these adhesives; and the use of an oral biofilm with adhesiveness and consolidated characterized in dentistry as a potential delivery system for drugs which utilize the benefits of the oral mucosa as safer and more bioavailable via antifungal active ingredients.

So the aim of this study was to test the cytotoxicity and effective inclusion of nystatin and an alternative antifungal derived from pyrazoles in different commercial presentations on denture fixatives against *Candida albicans*.

Material and methods

Synthesis of Pyrazoles

The synthesis and initial testing antifungal activity were previously described [33]. From these initial tests was the initial concentration of antifungal to be tested (0.00015µg).

Formulation of denture Fixatives

For the tests, only a trademark (Corega® - Glaxosmithkline OTC) in the three commercial presentations: powder, tape and cream. The composition of these products are described in Table 1.

Table 1. Formulation of different types of commercial denture adhesives used in present study.

Commercial Presentations	Batch Number	Manufacturer	Composition(*)
Powder	SH01724	Corega ®, GlaxoSmithKline, London, UK	Partial Sodium salt - Calcic of Poly (Metilviniléter / Maleic Acid 49.80%, 49.80% carboxymethylcellulose; mint flavor.
Cream	X13295		Sodium salts / calcium poly (Metilviniléter / maleic acid), carboxymethyl cellulose, mineral oil and petrolatum
Tape	3S0110V		Sodium carboxymethylcellulose, microcrystalline wax, polyethylene glycol and polyethylene glycol

(*) The composition as provided by manufacturer.

Groups and the proposed formulations are described in Table 2.

Table 2. Formulation of denture Fixatives and composition of the groups tested

Commercial Presentations	Groups	Nystatin (g)/ wt%	Pirazoles (g)/ wt%
Powder	GP0%	0	0
	GPN30%	0.015/ 30%	0
	GPN3%	0.0015/ 3%	0
	GPN0.3%	0.00015/ 0.03%	0
	GPP30%	0	0.015/ 30%
	GPP3%	0	0.0015/ 3%
	GPP0.3%	0	0.00015/ 0.03%
Cream	GC0%	0	0
	GCN30%	0.015/ 30%	0
	GCN3%	0.0015/ 3%	0
	GCN0.3%	0.00015/ 0.03%	0
	GCP30%	0	0.015/ 30%
	GCP3%	0	0.0015/ 3%
	GCP0.3%	0	0.00015/ 0.03%
Tape	GT0%	0	0
	GTN30%	0.015/ 30%	0
	GTN3%	0.0015/ 3%	0
	GTN0.3%	0.00015/ 0.03%	0
	GTP30%	0	0.015/ 30%
	GTP3%	0	0.0015/ 3%
	GTP0.3%	0	0.00015/ 0.03%

Characterization of adhesion

The measurement of adhesion was carried out in three stages, for each time were repeated twenty test and each of these times was done in a couple of acrylic resin specimens. For this purpose, we used the universal testing machine. The acrylic resin cylinders were made so as to be stable in the universal testing machine and had 25mm in diameter and 55mm in height. The test was performed by applying 0.3 g of adhesive already formulated in the polished surface of the resin cylinders. Then 2 kg was applied to the cylinder of acrylic resin for 15 s to ensure consistent application of force. The weight was withdrawn for 30 sec. Finally, the sets were separated. The forces required to pull the resin cylinders were measured and recorded. The measures of bond strength were made in a universal testing machine, operating at 100 N load cell and speed of 1 mm / min.

For the test called "wetting" the fixer was weighed and placed in the polished surface of the test bodies and pre-wetted with 0.5 ml of distilled water or artificial saliva or natural saliva. This amount of liquid was measured by automatic pipette previously calibrated. For the test called "soaking" the set of specimens over denture fixative was immersed in a device coupled to testing machine with 20 ml of artificial saliva so that the whole stay submerged.

Disk Diffusion test

For the realization of the agar diffusion test was used as parameter the methodology used in the study of Sassone et al. [33]. The strain of isolated oral of *Candida albicans* (ATCC 62342) was acquired in the Microbiology Laboratory of the

School of Dentistry, Federal University of Pelotas. These strains were pre-cultivated test on Mueller-Hinton broth (BD, Sparks, MD, USA) at 37 ° C for 48h. After culture was made a collection and a spike with sterile swabs to test tubes containing phosphate-buffered saline (PBS). These tubes were calibrated at 0.5 Mcfarland scale. Plates Mueller-Hinton broth (BD, Sparks, MD, USA) were incoculadas and the material to be tested was disposed on the plate so as to be distant 15 mm from the other material. The zone of inhibition was measured starting from the disk to the rim where circuferencia was microorganism growth [35].

Additionally we used the classification proposed by Karaman et al [36], where: sensitive products are those that promote inhibition zone ≥ 3 mm or ≥ that the positive control; moderately sensitive products are those that promote inhibition zone ≥ 2 mm but less than the positive control; resistant products are those that promote inhibition zone ≤ 2 mm. The positive control was the denture fixative without accretion of antifungal and the negative control was the fastener with nystatin.

Contact test direct

For direct contact test were used as reference the study Damlar et al [37]. The selected strains were the same used for the diffusion test in agar. For this, 24 hours before the challenge strains were subcultured in BHI agar and incubated at 37 ° C. The inoculum was prepared by dissolving an aliquot of the microorganism in BHI broth ((BD, Sparks, MD, USA) obeying to 0.5 Macfarland scale ($1x10^8$ CFU). In this inoculum were placed 100 l in each well of a microtiter 96-well plate. In these wells was added the fasteners manipulated according to Table 2. The plates were incubated for 1/4/8/12 hours. After this was added 240 µl challenge BHI broth in each well, the

plates were taken for 5 minutes to a plate shaker. 100 l of each well were then passed into 900 µl BHI broth (BD, Sparks, MD, USA), and serial 1:10 dilutions made in 4 aliquots successive. This dilution were plated in 25 Petri dishes of 9 cm containing Mueller-Hinton broth (BD, Sparks, MD, USA) and incubated at 37 ° C for 48 hours.

After this period the numbers of colonies were previously made by two examiners trained and calibrated. Positive controls (inoculum without the presence of any product) and negative (only culture medium) was administered in each group. Starting from the positive control for each group being accepted as the 100% growth inhibition percentages were calculated for each test adhesives dentures.

Cytotoxicity assay

This test used as an experimental model cell line of mouse fibroblasts (NIH / 3T3). Cell line maintenance procedures were performed in a laminar flow hood, following the protocols for maintaining sterility of the materials, culture media and supplements used. The cells were maintained in tissue culture flasks with DMEM (Eagle Medium Dulbecco's Modified) and FBS (Fetal Bovine Serum) in CO2 incubator at 37 ° C. Cell growth was monitored daily under an inverted phase contrast microscope, and the culture medium changed every 2 or 3 days according to cell metabolism.

Cells were subcultured after occupy at least 80% of the cropland in the bottle, which is called subconfluent. For subcultivation, the culture medium vial is removed reserved in a centrifuge tube and the cell monolayer is washed once with phosphate-buffered saline without calcium or magnesium (PBS). Then, the cells were separated

with 2 ml of trypsin solution (Sigma) 0.25% to 1 mM ethylenediaminetetraacetic acid (EDTA) for 5 minutes at 37 ° C. Trypsin was inactivated with FCS containing culture medium and the previously booked cells in suspension transferred to centrifugation tube and centrifuged at 1500 rpm for 5 minutes at room temperature. After aspiration of the supernatant, the cell pellet was suspended in 1 ml of culture medium. Aliquots of this cell suspension were distributed in 25 cm^2 flasks containing 5 ml of culture medium. The flasks were kept in an oven at 37 ° C and a humid atmosphere containing 5% CO2. Each subculture procedure gave rise to a new passage of the cell line.

It was determined the number of cells in the culture flasks. The purpose of this was to count the number of cells to an equal division of cells per group. The number of cells seeded in each well was 2×10^4.

To determine the number of cells (cell count), they were washed twice in PBS and suspended bottom flask using trypsin solution in PBS and 0.25% EDTA 1%. The contents of each bottle was removed, placed in a test tube containing 5 ml DMEM and centrifuged for inhibiting the trypsin. The supernatant was discarded and the tubes precipitates suspended in 1 ml of DMEM. This cell suspension 20 µl were dispensed in a test tube with 20 µl of Trypan blue 0.4%. Outside the laminar flow and a drop of this mixture was placed in a Neubauer chamber (or hemacytometer) and brought to the stage of an inverted microscope to perform the cell number count. The blue stained cells represented dead cells, while unstained cells are viable cells. Were counted in 4 quadrants of the extremities. The calculation was obtained by the formula wherein the total number of viable cells counted was multiplied by 10^4 (the counting volume is 10^4 mm^3, or 0.1 ml. This value was divided by the number of squares counted (in this case 4) multiplied by the factor dilution is two. The formula was obtained from this the approximate number of cells present in each. The according to the number of existing

cells DMEM was added to this suspension sufficient to obtain the desired amount of cell volume.

The denture fixatives were formulated as shown in table 2, and placed in DMEM. The cell suspension was plated at a concentration of 2 x 10^4 cells per well and spread on a cell culture plate (ELISA) 96-well. Each well received 200 µl of complete DMEM. The plate was then incubated at 37 ° C in air with 5% CO_2 for 24 hours. After this period the culture medium was removed from wells, and equal volumes (200 µl) of experimental material will be added to each well. In the control wells, 200µl of DMEM was added. After removal of the test extracts, 200µl of PBS and 20 µl of MTT (tetrazolium [3- (4,5-dimetltiazol-2-yl) -2,5-diphenyltetrazolium bromide] Salt was added to each well. The plate was incubated without light at 37 ° C for 24 hours. Then MTT was aspirated and 200 µl of dimethylsulfoxide (DMSO) was added to each well. Thereafter, absorbance at 570 nm was measured using a spectrophotometer and the results statistically analyzed with GraphPad Prism 5.

Statistical comparisons were performed using SigmaPlot 12.0 software (Systat Software Inc, San Jose, CA, USA INC). Comparisons between groups was performed using Kruskal Wallis and One way ANOVA at $p<0.05$.

Results and Discusion

Since the XVIII century adhesives have been used to improve retention of the prosthesis, and the first scientific report of the adhesive occurred in 1935 by the Council of Dental Materials of the American Dental Association [7]. It is known that 30% of users use dentures or have already made use of adhesives, and the adhesives user number varies from 15 to 33% of prosthesis users and that the period of a year were

sold in the United States about 55 million units denture adhesives, representing a value exceeding $ 220 million. [38]

The denture fixatives have been used to optimize the retention of dentures to increase the adhesive and cohesive properties of the saliva and the viscosity of the medium between the prosthesis and its seating area, obstructing the space between the denture base and the oral mucosa [5]. Thus, compliance is a key factor for the success of this material.

Regarding the adhesion, previous tests were carried out to obtain the best method and the best way to test. Commercial and experimental fastener means using distilled water, artificial saliva and salivary natural and wetting and soaking methods were tested. Results varied across the different means and methods, and so the authors decided to elect the artificial saliva as a means and the immersion method, as we believe this to be the closest to what occurs in the oral cavity and thus there was no statistical difference between the three commercial presentations (Figure 1). This result is interesting as they wanted to test the bonding strength with the inclusion of indifferent antifungal commercial presentation. As a result, it was observed that the inclusion of the antifungals did not affect the adhesion of the fasteners ($p > 0.05$).

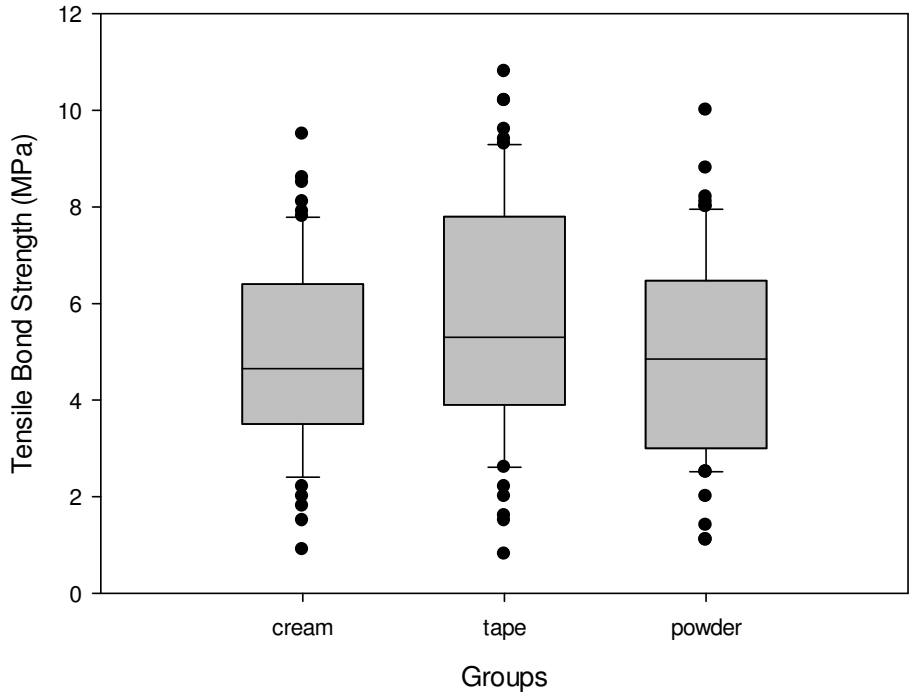

Figure 1. Comparison of the different presentations of denture fixatives for adhesion.

Grasso [7] in a review of the literature mentions that the denture fixatives can be divided into insoluble and soluble, which vary in their composition. Within the insoluble group include the tape and soluble group include cream, tape and powder. The soluble group presents synthetic agents, which depend on the chemical properties of one or more active ingredients that increase its volume from 50% to 150% and become viscous and sticky in the presence of water or saliva, filling the spaces between the base of prosthesis and the supporting tissues. The active ingredients are a mixture of salts of polymers with different degrees of solubility in water, which are designed to produce short and long acting adhesive. The carboxymethylcellulose (CMC) and methyl cellulose the Polyvinylether (PVM-MA) are examples of short and long-acting salts, respectively. The CMC salts provide a strong initial retention, however due to its high solubility dissolve rapidly, losing its effectiveness within a short period. The PVM-MA salts, however, have a low solubility which takes longer to activate themselves but have

a longer period of action. Subsequently to improve their effectiveness was added calcium and zinc salts in its formulation. Despite the fact that the tape composition varies between trademarks, they all include essentially a manufactured blade impregnated with an active component water based. Examples of adhesive ingredients include sodium alginate or polymer of ethylene oxide, which become sticky when activated by the saliva, and the difference between them is the thickness of the blade.

The effect of denture fixatives in the oral microbiota is an interesting aspect because conventional dentures are seen as a favorable environment for proliferation and survival of bacteria and biofilm formation. The microbial colonization, especially *Candida* species, can induce a chronic inflammatory response, called chronic atrophic candidiasis [39, 40]. Some studies found that the use of stickers for prosthesis did not significantly alter the oral microbiota [41,42]. However, other studies suggest the denture fixatives as stimulators of proliferation of Candida species and that this could be a predisposing factor for chronic denture stomatitis [14, 15, 16, 17]. Regardless of the adhesive used, the user must be aware that these products must be completely removed from the prosthesis and mucosa, as can harbor harmful microorganisms for bucal. So health, care dentures and oral tissues need special attention and patients should be educated about it [43].

The disk diffusion assay was chosen to test the antifungal efficiency of commercial and experimental fasteners. Some authors point out that this test is a reliable alternative to the microdilution broth method, reference NCCLS M27-A2 [44]. The results of this test are shown in Table 3.

Table 3. Median values (mm) of Disk Diffusion

Groups	Fixative Denture		
	Powder	Cream	Tape
Pirazoles			

0.015 g	4	3	5
0.0015g	3	1	4
0.00015g	2	1	0
Nistatin			
0.015 g	4	3	4
0.0015g	3	3	3
0.00015g	0	2	0

Many analyzes were made to take advantage of the results obtained. According to the data obtained, it can be seen that comparing with the experimental nystatin and pyrazol these promoted inhibition halo and behaved similarly ($p > 0.05$). This result confirms and opens a potential use for this new pyrazole, as an alternative antifungal compound. Using this new pyrazole has been discussed in another study and its effectiveness had been proven against different Candida species and this also showed low cytotoxicity [32]. Among antifungal agents, the therapeutic agents employed for the most topical treatment of oral candidiasis are polyenes (Nystatin and amphotericin B) and azoles (itraconazole, miconazole and clotrimazole) [45,46]. Despite being widely used, They have Certain limitations due to side effects such as toxicity and the emergence of resistant strains [47] Thus reaffirming the need for the development of other compounds with low cytotoxicity and the potential for treatment of mycoses [48].

In general, commercial presentations were not decisive in the action of antifungal for both nystatin as to the pyrazole ($p > 0.005$). However, the use of the pyrazole as antifungal, there was an improvement in the action against species of *Candida albicans* when the business outlook was on tape ($P = 0.031$), and this action was over cream and powder presentation. This can occur by insoluble ribbon feature, contrary soluble characteristics of cream and powder. Thus, for this insolubility may have a greater availability of active principle and thus an increased antifungal action. Some studies of the antifungal action of commercial denture fixatives without the

inclusion of antfungicos, but these do not compare the action of the different commercial types and the influence thereof. In our study, the denture fixatives which did not contain the antifungal not had the inhibition zone production and consequent inhibitory action on the growth of Candida species. Other in vitro study noted, trademarks of ten, only two induced an inhibitory effect on the growth of *C. albicans*, but most products induced changes in the macro and microscopic morphology of the colonies of this fungus and yeast [49].

For testing by direct contact with similar results presented by the disc diffusion and the pyrazole tested had similar results antifungal nystatin ($p > 0.05$). The results were observed for 12 hours and this time was determined from the commercial appeal of duration of action of the product for that period. The results are expressed in figures 2, 3 and 4 are measured as a percentage inhibition.

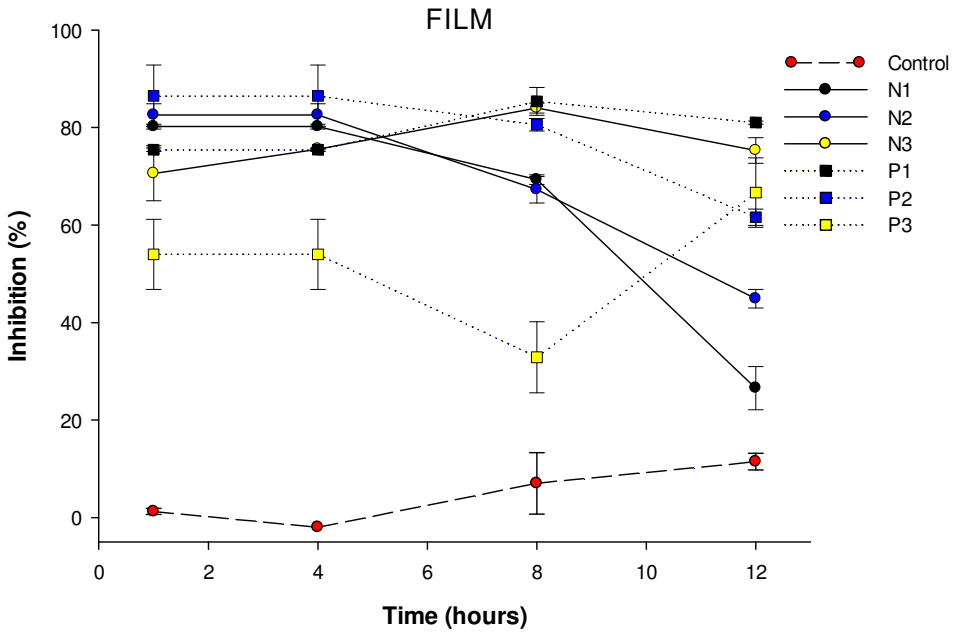

Figure 2 - Antifungal behavior of denture fixatives, tape type, commercial and experimental in different times

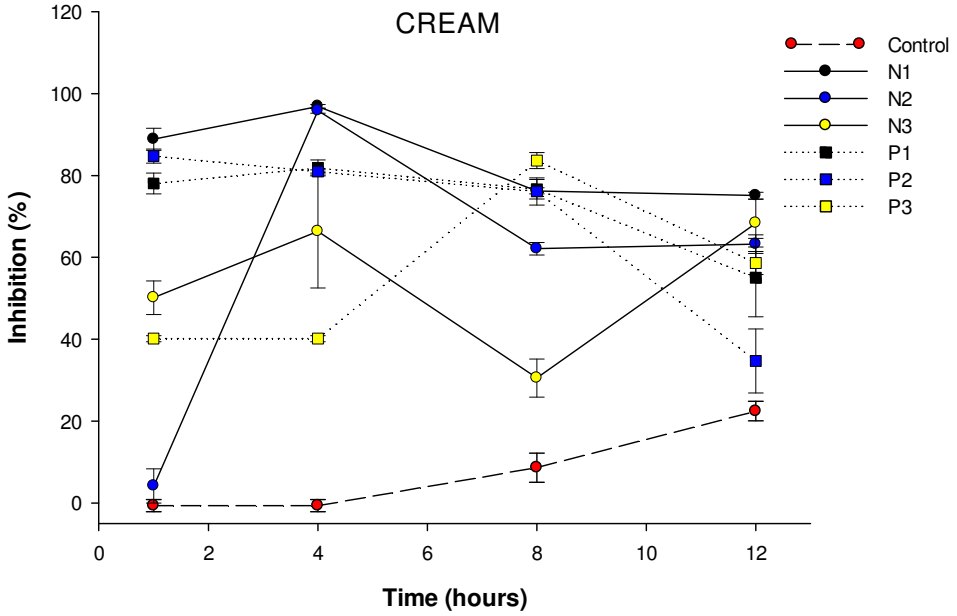

Figure 3 - Antifungal behavior of denture fixatives, cream type, commercial and experimental in different times

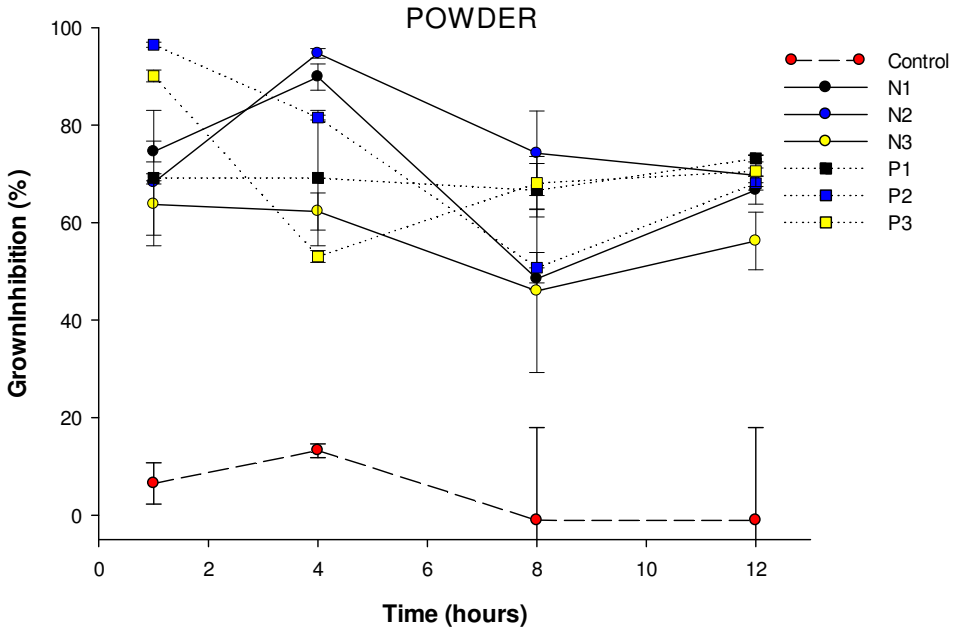

Figure 4 - Antifungal behavior of denture fixatives, powder type, commercial and experimental in different times

All results confirm the possibility of the use of pyrazoles as an alternative antifungal and the possibility of action of denture fixatives as a possible means of delivery of antifungals.

The cytotoxicity (Figure 5) was also evaluated, as only the antifungal action would not be enough for positive characterization of the material. The objective of Ekskrand et al. [5] in 1993, was to evaluate the cytotoxic effects, microbial contamination in 19 commercial brands bumper prosthesis. All stickers for prosthesis were evaluated cytotoxic and some had microbial contamination, and the most remarkable contamination in adhesives raw materials base "natural".

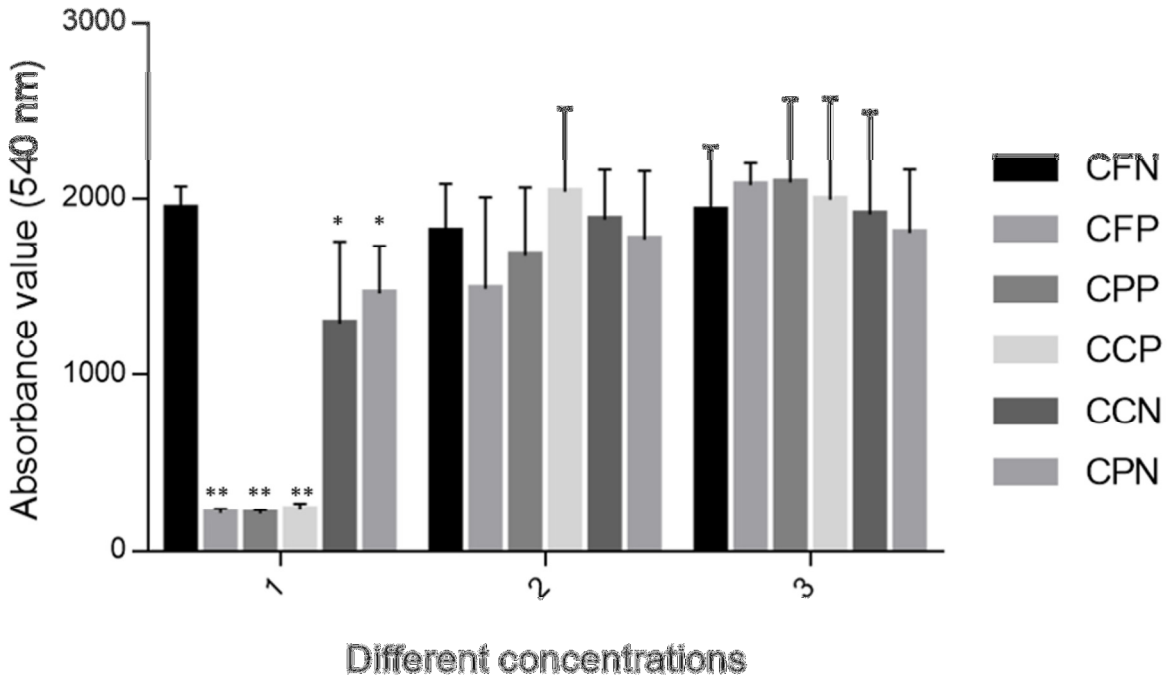

Figure 5. Analysis of cytotoxic different commercial presentations of commercial and experimental denture fixatives

Starting from the analysis of cytotoxicity can be seen that the only difference was statistically significant at the highest concentration ($P < 0.005$), where the denture fixatives, independent of the commercial form, containing nystatin had to be less

cytotoxic. However, in other concentrations (2 and 3) both the fastener with nystatin as with pyrazole, independent of commercial presentation, showed low cytotoxicity.

In view of these results, our group indicates the possibility affirmed the use of denture fixatives as delivery system of trade antifungal (Nystatin) or not (pyrazole) and the second concentration (1.560 μg) more efficient, which is independent of the commercial presentation and less cytotoxic.

Conclusion

Within the limitations of the methodologies used, it is concluded that there is possibility of using denture fixatives as delivery system of trade antifungal (Nystatin) or not (pyrazole) and the second concentration (1.560 μg) more efficient, which is independent of the presentation commercial and less cytotoxic.

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3 Considerações Finais

O estudo de novas alternativas para o tratamento da candidíase atrófica crônica tornou-se imprescindível à medida que os casos de resistência antifúngica vêm demonstrando-se como um desafio na clinica.

Atualmente o estudo sobre os sistemas de entrega de fármacos vem despertando o crescente interesse das indústrias farmacológicas e dos grupos de pesquisa. As principais linhas de pesquisa envolvem a descoberta de novas alternativas capazes de dar especificidade ao fármaco e/ou o controle da liberação e absorção dos mesmos. Há um bioadesivo já utilizado em odontologia mas que ainda não é utilizado como sistema de entrega de fármacos, os fixadores de dentadura. Esses fixadores de dentadura já apresentam as propriedades adesivas consolidadas porém não foram utilizados como veículos de fármacos, mesmo sendo mais favoráveis aos mesmos já que não sofrem atrito de alimentos e ficam protegidos, geralmente, abaixo das próteses dentárias.

Os derivados pirazolinicos testados nesse trabalho, além de apresentarem efeito contra cepas de *Candida albicans* e não *albicans*, tem como vantagens o uso de matéria prima sintética, síntese por via limpa e alto rendimento. Isso viabilizaria a produção desse antifúngico em larga escala.

Este estudo abre a possibilidade do uso de fixadores de dentadura como um veiculo para a utilização de antifúngicos comerciais e alternativos.

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ANEXO I

Author guidelines - International Archives of Medicine

Contents

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The title should be specific to the study yet concise, and should allow sensitive and specific electronic retrieval of the article. It should be comprehensible to readers outside your field. Avoid specialist abbreviations if possible. Titles should be presented in title case, meaning that all words except for prepositions, articles, and conjunctions should be capitalized. If the paper is a randomized controlled trial or a meta-analysis, this description should be in the title.

Examples:

Climate Change and Increased Spread of Malaria in Sub-Saharan Africa A Cluster-Randomized Controlled Trial of a Nurse-Led Intervention after Stroke Please also provide a brief "running head" of approximately 40 characters.

Authors and Affiliations

Provide the first names or initials (if used), middle names or initials (if used), surnames, and affiliations—department, university or organization, city, state/province (if applicable), and country—for all authors. One of the authors should be designated as the corresponding author. It is the corresponding author’s responsibility to ensure that the author list, and the summary of the author contributions to the study are accurate and complete. If the article has been submitted on behalf of a consortium, all consortium members and affiliations should be listed after the Acknowledgments.

Abstract

The abstract is divided into the following four sections with these headings: Title, Background, Methods and Findings, and Conclusions. It should contain the all following elements, except for items in square brackets, which are only needed for some study types. Please use the same format for abstracts submitted as presubmission inquiries.

Background

This section should describe clearly the rationale for the study being done. It should end with a statement of the specific study hypotheses and/or study objectives.

Methods and Findings

Describe the participants or what was studied (eg cell lines, patient group; be as specific as possible, including numbers studied). Describe the study design/intervention/main methods used/What was primarily being assessed eg primary outcome measure and, if appropriate, over what period.

[If appropriate, include how many participants were assessed out of those enrolled eg what was the response rate for a survey.]

[If critical to the understanding of the paper, describe how results were analysed, ie which specific statistical tests were used.]

For the main outcomes provide a numerical result if appropriate (it nearly always is) and a measure of its precision (e.g. 95% confidence interval). Describe any adverse events or side effects.

Describe the main limitations of the study.

Conclusions

Provide a general interpretation of the results with any important recomendations for future research.

[For a clinical trial provide any trial identification numbers and names (e.g. trial registration number, protocol number or acronym).]

Introduction
The introduction should discuss the purpose of the study in the broader context. As you compose the introduction, think of readers who are not experts in this field. Include a brief review of the key literature. If there are relevant controversies or disagreements in the field, they should be mentioned so that a non-expert reader can delve into these issues further. The introduction should conclude with a brief statement of the overall aim of the experiments and a comment about whether that aim was achieved.

Methods
This section should provide enough detail for reproduction of the findings. Protocols for new methods should be included, but well-established protocols may simply be referenced. Detailed methodology or supporting information relevant to the methodology can be published on our Web site.

This section should also include a section with descriptions of any statistical methods employed. These should conform to the criteria outlined by the Uniform Requirements, as follows: "Describe statistical methods with enough detail to enable a knowledgeable reader with access

to the original data to verify the reported results. When possible, quantify findings and present them with appropriate indicators of measurement error or uncertainty (such as confidence intervals). Avoid relying solely on statistical hypothesis testing, such as the use of P values, which fails to convey important quantitative information. Discuss the eligibility of research participants. Give details about randomization. Describe the methods for and success of any blinding of observations. Report complications of treatment. Give numbers of observations. Report losses to observation (such as dropouts from a clinical trial). References for the design of the study and statistical methods should be to standard works when possible (with pages stated) rather than to papers in which the designs or methods were originally reported. Specify any general-use computer programs used."

Results
The results section should include all relevant positive and negative findings. The section may be divided into subsections, each with a concise subheading. Large datasets, including raw data, should be submitted as supporting files; these are published online alongside the accepted article. The results section should be written in past tense.

As outlined in the Uniform requirements, authors that present statistical data in the Results section, should "...specify the statistical methods used to analyze them. Restrict tables and figures to those needed to explain the argument of the paper and to assess its support. Use graphs as an alternative to tables with many entries; do not duplicate data in graphs and tables. Avoid nontechnical uses of technical terms in statistics, such as "random" (which implies a randomizing device), "normal," "significant," "correlations," and "sample." Define statistical terms, abbreviations, and most symbols."

Discussion
The discussion should be concise and tightly argued. It should start with a brief summary of the main findings. It should include paragraphs on the generalisability, clinical relevance, strengths, and, most importantly, the limitations of your study. You may wish to discuss the following points also. How do the conclusions affect the existing knowledge in the field? How can future research build on these observations? What are the key experiments that must be done?

References
The International Committee of Medical Journal Editors offers guidance to authors in its Uniform Requirements for Manuscripts Submitted to Biomedical Journals publication. The recommended style for references is based on the National Information Standards Organization NISO Z39.29-2005 (R2010) Bibliographic References as adapted by the National Library of Medicine for its databases. Details are in Citing Medicine. (Note Appendix F which covers how citations in MEDLINE/PubMed differ from the advice in Citing Medicine.) Sample references typically used by authors of journal articles are provided below.

Articles in Journals
Standard journal article
List the first six authors followed by et al. (Note: NLM now lists all authors.)
Halpern SD, Ubel PA, Caplan AL. Solid-organ transplantation in HIV-infected patients. N Engl J Med. 2002 Jul 25;347(4):284-7.
As an option, if a journal carries continuous pagination throughout a volume (as many medical journals do) the month and issue number may be omitted.
Halpern SD, Ubel PA, Caplan AL. Solid-organ transplantation in HIV-infected patients. N Engl J Med. 2002;347:284-7.
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Rose ME, Huerbin MB, Melick J, Marion DW, Palmer AM, Schiding JK, et al. Regulation of interstitial excitatory amino acid concentrations after cortical contusion injury. Brain Res. 2002;935(1-2):40-6.

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People who contributed to the work, but do not fit the criteria for authors should be listed in the Acknowledgments, along with their contributions. You must also ensure that anyone named in the acknowledgments agrees to being so named.

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Acknowledgements

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Standard journal article

[1] Flink H, Tegelberg A, Thörn M, Lagerlöf F. Effect of oral iron supplementation on unstimulated salivary flow rate: A randomized, double-blind, placebo-controlled trial. J Oral Pathol Med 2006;35:540-7.

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More information about other reference types is available at www.nlm.nih.gov/bsd/uniform_requirements.html, but observe some minor deviations (no full stop after journal title, no issue or date after volume, etc).

Abbreviations

Use only standard abbreviations. Consult Scientific Style and Format. The CBE Manual for Authors, Editors, and Publishers, 7th ed. ISBN 0-9779665-0-X, 2006. Explain any non-standard abbreviations (to be avoided if possible) in the text at first mention. Avoid abbreviations in the title of the article. Give tooth designations in accordance with the two digit system (ISO 3950-1977).

ANEXO III

Author guidelines – Journal Biomedical Materials Research

Aims and Scope

The Journal of Biomedical Materials Research Part A is an international, interdisciplinary, English-language publication of original contributions concerning studies of the preparation, performance, and evaluation of biomaterials; the chemical, physical, toxicological, and mechanical behavior of materials in physiological environments; and the response of blood and tissues to biomaterials. The Journal publishes peer-reviewed articles on all relevant biomaterial topics including the science and technology of alloys,polymers, ceramics, and reprocessed animal and human tissues in surgery,dentistry, artificial organs, and other medical devices. The Journal also publishes articles in interdisciplinary areas such as tissue engineering and controlled release technology where biomaterials play a significant role in the performance of the medical device.

The Journal of Biomedical Materials Research is the official journal of the Society for Biomaterials (USA), the Japanese Society for Biomaterials, the Australasian Society for Biomaterials, and the Korean Society for Biomaterials.

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2. Voet D, Voet JG. Biochemistry. New York: John Wiley & Sons; 1990. 1223 p.

3. Gilmor ML, Rouse ST, Heilman CJ, Nash NR, Levey AI. Receptor fusion proteins and analysis. In: Ariano MA, editor. Receptor localization. New York: Wiley-Liss; 1998. p 75-90. Please note that journal title abbreviations should conform to the practices of Chemical Abstracts.

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